Doctor of Environmental Science

in *Graduate School of Environmental Science*

The University of Shiga Prefecture

Physiological responses of a freshwater copepod *Eodiaptomus japonicus* on different temperature and food conditions, evaluating anthropogenic impacts in Lake Biwa

Defended by **Xin LIU**

on February 2016, in Hikone (Japan)

Supervisor: **Professor Syuhei BAN**

Graduate School of Environment Science

The University of Shiga Prefecture

"*Human subtlety will never devise an invention more beautiful, simpler or more direct than dose nature because in her inventions nothing is lacking, and nothing is superfluous*"

Leonardo da Vinci

Acknowledgments

The researches were conducted in the Laboratory of Aquatic Ecosystems in **Department of Ecosystem Studies** (The University of Shiga Prefecture, Japan). All experiments complied with the current laws regarding the treatment of animals of the country in which they were performed.

I am extremely thankful to my co-advisors **Drs. Delphine Beyrend** and **Gaël Dur** for giving me helpful advices and support throughout this study and their precious help in literature review, data analysis, and English editing throughout this study. I specially thank my supervisor, **Prof. Syuhei Ban** for his valuable helpful instructions, advices, comments, publishing and financial supports on this thesis. This thesis would never have been completed without them.

I wish to thank **Dr. Kohei Yoshiyama** (The University of Shiga Prefecture), **Mr. Masao Asano** (BAS Ltd., Japan) and **Dr. Jan Fischer** (Pyroscience Sensor Technology Ltd., Germany) for them sincere suggestions and technical supports that contributed to solve all issues during this study. I thank The University of Shiga Prefecture under graduated student **Natsuki Kitami** for her sincerely technical teaching in algal and zooplankton culturing, and **Yusuke Nakamoto** for his continued efforts to maintain the stock laboratory cultures. Thanks to secretary staff **Emi Doi** (The University of Shiga Prefecture) for her seriously support on clerical works on material order, conferences and travel financial reimbursements.

I should like to thank to **Dr. Naoshige Goto** and **Mr. Bun-ichiro Kaigai**, the captain of the research vessel ***Hassaka***, The University of Shiga Prefecture, for them kind cooperation during field sampling.

The last one but far, far from being the least to my beloved family, especially my wife **Mingxin Yu**, who have always been believed in me, supported my daily life and accompany along the way. This study could never been finished without her because she gives rise to my determination in succeeding.

Funding

This study was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries, Japan to **Prof. Syuhei Ban** through a research project entitled *Development of technologies for mitigation and adaptation to climate change in Agriculture, Forestry and Fisheries*, and private scholarship (2013−2014) from Shiga Intercultural Association for Globalization (SIA) and Japanese government scholarship (2014−2016) from Japan Student Services Organization (JASSO) provided to **Xin Liu**. A part of this work was a contribution to the project *French Asian Study on Global Change Effects through Inter-Site Comparisons of Limnic Ecosystems (FASCICLE)*, funded by the Bio-Asia Program of the French Government (2011−2014) under the scientific coordination of **Profs. Sami Souissi** and **Syuhei Ban**. Two post-doctoral research grants to **Drs. Delphine Beyrend** and **Gaël Dur** from the Japan Society for the Promotion of Science (JSPS) also supported a part of this work.

Abstract

Global climate change is affecting a variety of aquatic organisms including warming and eutrophication in the ecosystems. Copepod is one of the most widely distributed micro-crustaceans in the aquatic ecosystems. They are important components of zooplankton community and play an important role in the trophic dynamics as a good biological indicator of production status. Therefore, understanding the processes that control the abundance and population dynamics of copepods is a major objective in aquatic ecology and limnology.

Calanoid copepod *Eodiaptomus japonicus* is an endemic species in Japan, and reported to be dominant species in the largest and oldest freshwater ecosystem — Lake Biwa. In this study, I studied the effect of temperature and food conditions on the life history traits of *E. japonicus* collected from the lake in the laboratory, to clarify responses of somatic growth, reproduction and metabolic rates to the different temperature and food conditions, and consequently evaluated the impacts of eutrophication and global warming on its production during the last 4 decades in this lake.

The experimental studies for *E. japonicus* highlighted the importance of temperature and food conditions on its life cycle strategy. The results showed that this warm-adapted copepod was able to develop and reproduce in a wide range of temperature and food conditions, and the food effects depended on temperature. Somatic and population growths were depressed under food-limited condition especially at high temperatures due to lowering the net growth efficiency by high metabolic costs. Finally, the long-term analyses showed that production of the copepod tended to increase after 1990, and predicted that the warming would be beneficial for *E. japonicus* population in the lake under current nutritional status, while disadvantageous under 6% lower food conditions assuming lowering the primary production due to global warming.

Keywords

Copepoda, *Eodiaptomus japonicus*, anthropogenic impacts, global warming, eutrophication, temperature and food conditions, life history traits, long-term trends, metabolic plasticity, secondary production, freshwater ecosystem, Lake Biwa

Host laboratory

Laboratory of Aquatic Ecosystems
Department of Ecosystem Studies
School of Environmental Science
The University of Shiga Prefecture
2500 Hassaka-cho, Hikone, Shiga, Japan

CONTENTS

GENERAL INTRODUCTION

1. Background

As a consequence of modern industrialization, anthropogenic impacts including the global warming and eutrophication are affecting a variety of organisms on the Earth. The different climate scenarios predict the increase of global average temperature between about 1.1 and 6.4 ºC, with average rise of 3 ºC by the end of 21st century (IPCC 2007, 2014). , the global warming and eutrophication never likely end due to keeping on development of global economy, increasing consumer demand, and consequently increasing industrial productions. Raising the surface water temperature and changing the nutritional status in an individual water body due to global warming and eutrophication, respectively, would influence aquatic organisms living there. Ambient temperature and food conditions are crucial factors in ectotherms through the metabolism and nutritional intake, and consequently their life history traits and population dynamics in the fields (Mauchline 1998, Dam 2013).

Copepod is one of the most widely distributed micro-crustaceans in the aquatic ecosystems (Mauchline 1998). It is important component of zooplankton and plays an important role in the trophic dynamics of marine, brackish and freshwater ecosystems (Hirst et al. 1999, Uye et al. 2000, Hsieh et al. 2011). Therefore, understanding the processes that control the abundance and production of copepods is a major objective in aquatic ecology and limnology (Jiménez-Melero et al. 2005).

Despite the large amount of studies on calanoid copepods and on other planktonic organisms in general, the most species studied were marine and brackish species (Koski and Kuosa 1999, Bonnet and Carlotti 2001, Lee et al. 2003, Bonnet et al. 2009, Beyrend-Dur et al. 2011). Some studies focused on the freshwater copepods also (Munro 1974, Herzig 1983, Zeller et al. 2004, Boxshall and Defaye 2008). The objective of this study is to understand the responses of life history traits and metabolic rates to changing temperature and nutritional

status in a freshwater copepod, in order to clarify the effects of anthropogenic impacts, such as eutrophication and global warming, on the *in situ* copepod population in a lake ecosystem.

2. The freshwater Copepod: *Eodiaptomus japonicus*

The best candidate that I found for studying effects of temperature and food conditions on life history traits and metabolic rates is calanoid copepod *Eodiaptomus japonicus* (Burckhardt). This copepod is endemic species in Japan, and widely distributed in the temperate and subtropical regions, and has been shown to be dominant species in Lake Biwa (Kawabata 1987a, Yoshida et al. 2001b) and Lake Ikeda (Baloch et al. 1998).

E. japonicus is a key copepod species in mesozooplankton in Lake Biwa. Abundance and distribution of *E. japonicus* in the lake have been already studied (Kawabata 1987a), because of its high abundance and an importance as one of the food resources for commercially important fishes (Kawabata et al. 2002). To make a better insight of the inter-annual variations of this key species population in the lake and its responses to eutrophication and warming within the global climate change context (Hsieh et al. 2011), I conducted several experiments on life history traits and metabolic rates in this species under different controlled conditions in the laboratory. The laboratory experiments allow us to study the life history traits, physiology and their responses under controlled experimental conditions at organism level, as well as the different aspect of the reproductive biology (Beyrend-Dur 2010).

Freshwater calanoid copepods are found in a variety of habitats ranging from freshwater lakes and ponds, to streams or rivers, occasionally in ditches, while some euryhaline species are found in either brackish-, salt- or freshwater (Williamson and Reid 2001). Thus this wide distribution allowed the comparison of the climate changes impact between species inhabiting fresh- and brackish waters.

3. Zooplankton in Lake Biwa

Lake Biwa (35.1°N, 136.1°E), located on a central part of Honshu Island, is the largest lake in Japan. It has a surface area of 670 km^2 and a maximum depth of 104 m. It is a principal water resource in Kansai region of Japan, supplying drinking water for 14 million peoples. In its watershed and downstream areas, the lake also attracts peoples with its scenic beauty. Every year some 30 million tourists visit the lake.

Since zooplankton plays an important role in channeling organic matter fixed by primary producers into higher trophic levels, a number of studies have been also examined it in Lake Biwa. These studies have clarified various aspects of zooplankton ecology in the lake, including feeding habits (Kawabata 1987b), feeding rates (Okamoto 1984b, Nagata and Okamoto 1988, Urabe et al. 1996), population dynamics (Kawabata 1987a, 1989b, 1993) and recycling nutrients (Haga et al. 1995, Urabe et al. 1995). The zooplankton species composition in Lake Biwa changed in the mid-1960s, due to eutrophication caused by human activities (Tsugeki et al. 2003). Study of the long-term variation of zooplankton in this lake showed that the lake ecosystem has experienced a dramatic change in trophic status and thermo regime in the past half century (Hsieh et al. 2011).

Crustaceans, rotifers and ciliates are the most common zooplankton in Lake Biwa. Crustaceans contributed to 86% of the total zooplankton biomass on average, while rotifers and ciliates contributed to 11% and 3%, respectively (Yoshida et al. 2001b). *Daphnia galeata*, *Bosmina longirostris* and *Eodiaptomus japonicus* dominated the crustacean zooplankton all the year round. Among the crustaceans, *E. japonicus* is the most dominant species followed by *D. galeata* (Yoshida et al. 2001b).

4. Ecology and life cycle of *Eodiaptomus japonicus*

The small freshwater calanoid copepod, *E. japonicus*, is an endemic species in Japan

and widely distributed in lakes, ponds and brackish-water (Mizuno 1984). They are mainly distributed above the thermocline (5.5−20.4 m) in Lake Biwa (Kawabata 1987a).

The abundance and distribution of *E. japonicus* have been studied in Lake Biwa previously (Kawabata 1987a). The horizontal distribution of *E. japonicus* was almost uniform. The vertical distribution was closely related to the water structure, but no diel vertical migration was observed. However, ontogenic distribution has been observed; nauplii were distributed in shallower depth layers compared to those of copepodites. At the end of the stagnation period (late spring to early autumn), adults were distributed in the same layer as that of nauplii. Small adult females and low egg production were observed in summer and autumn. In October, *E. japonicus* was dispersed into deeper layers than stagnation period due to autumnal turnover. Individuals that achieved adulthood in November and December overwintered and laid eggs during the following year. The maximum abundance of *E. japonicus* has been observed in June, when water temperature ranged between 13 and 18 ºC, with density reaching up to 19,000 ind m^{-3} (Liu et al. 2014).

E. japonicus presents a typical calanoid life cycle with 6 naupliar stages followed by 5 copepodid and adult stages. The life cycle of this copepod was observed in the laboratory throughout this study.

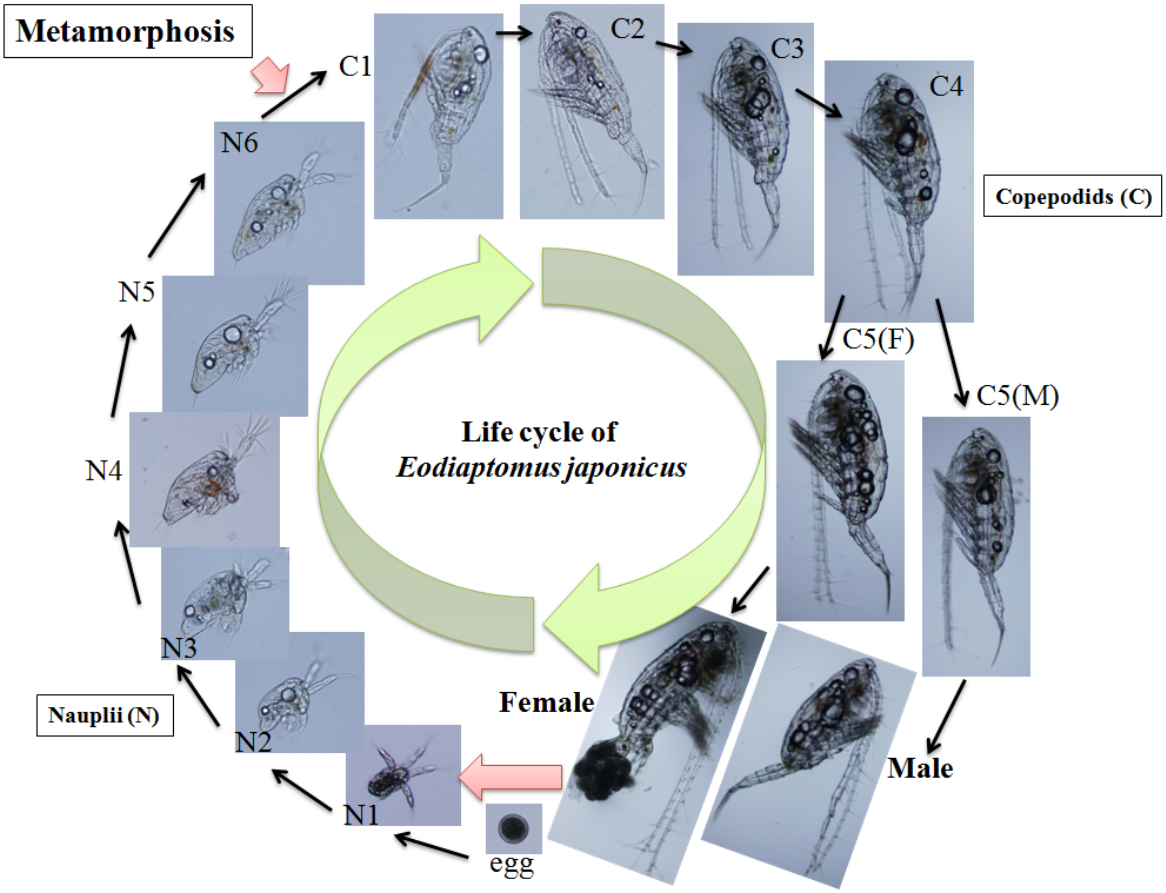

Illustration. 1. Life cycle of *Eodiaptomus japonicus*. Successive developmental stages observed during the development are presented. Pictures are not at the same scale.

The first stage nauplius (N1) hatches from the egg and grows through 6 naupliar stages, after which it changes its body shape during metamorphosis to the first copepodid stage (C1). Then it grows through the copepodid stages until the fifth one (C5), in which sexual distinction can be easily made. The last copepodid stage is the sixth copepodid stage C6 commonly called adult stage (i.e., adult female and male). The adult female carries eggs in an egg sac until egg hatching (Illustration. 1).

5. The scientific approach: Thesis contents

All of the experiments in this PhD thesis were conducted under controlled temperature and food conditions in the laboratory. We newly designed a water-bath fiber-optic oxygen sensor system in this study to determine oxygen consumption rates. It is possible to study physiology in median sized copepod in more precise and less procedures than before.

This thesis is structured as follow:

Chapter 1: Effects of temperature on life history traits of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan).

Chapter 2: Combined effects of temperature and food concentration on growth and reproduction of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan).

Chapter 3: Effects of long-term acclimatization on metabolic plasticity of *Eodiaptomus japonicus* (Copepoda: Calanoida) using optical oxygen meter.

Chapter 4: Long-term trends in biomass and production of *Eodiaptomus japonicus* (Copepoda: Calanoida) in Lake Biwa, related to eutrophication and global warming.

to discuss the impact of global warming and eutrophication on this dominant copepod, and predict their production ecology in Lake Biwa.

CHAPTER 1:

Effects of temperature on life history traits of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan)

This section is mainly based on the manuscript:

- "Effects of temperature on life history traits of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan)" by Xin Liu, Delphine Beyrend-Dur, Gaël Dur and Syuhei Ban published in ***Limnology***.

1. Introduction

Copepods are important components of zooplankton populations and play a critical role in the dynamics of freshwater ecosystems, serving as links between phytoplankton and higher trophic levels in food webs. Understanding the processes that control copepod abundance and production is a major objective in lake ecosystem research (Jiménez-Melero et al. 2005). Growth and productivity are mainly governed by temperature (Halsband-Lenk et al. 2002, Bonnet et al. 2009) and food concentration (Ban 1994, Klein Breteler et al. 1995). Both these factors may affect the life history traits of copepods, consequently affecting community dynamics in aquatic ecosystems.

Lake Biwa is the largest and oldest lake in Japan. Because of its economic importance, it has been subjected to many human activities, with strong impacts on its environment, including eutrophication from the early 1960s to mid 1980s and increasing temperature after 1990 (Kawabata 1987a, Yoshida et al. 2001b, Hsieh et al. 2010). Despite those variations in the environment, the calanoid copepod Eodiaptomus japonicus has dominated the zooplankton for over 50 years (Hsieh et al. 2011). This egg-carrying copepod is an endemic species widely distributed in freshwater bodies in Japan. It has also been shown to dominate zooplankton communities in Lake Ikeda (Japan) (Baloch et al. 1998). In Lake Biwa, maximum abundances have been observed in June, when water temperature ranged between 13 and 18 ºC, with densities reaching 19,000 ind. m^{-3} during the last four decades (Liu et al. 2014). Because of its high abundance and importance as a food resource for fish (Kawabata et al. 2002), *E. japonicus* plays a key role in the food web of Lake Biwa. However, studies on *E. japonicus* population dynamics are scare compared to those on counterpart species in European lakes: e.g., *Eudiaptomus gracilis*, *Eudiaptomus graciloides* (Munro 1974, Herzig 1983, Jiménez-Melero et al. 2005). To obtain an idea of how zooplankton populations in Lake Biwa may respond to increasing water temperatures resulting from global warming, it is

crucial to study the population dynamics of this most important component in the lake.

Development time, survival, and reproduction are basic life history traits of a copepod, and knowledge of these traits is essential for understanding population dynamics (Jiménez-Melero et al. 2005). Development times (i.e., embryonic and post-embryonic) and survival are known to be strongly affected by temperature (Herzig 1983, Chinnery and Williams 2004, Jiménez-Melero et al. 2005, Cook et al. 2007, Devreker et al. 2007, Jiménez-Melero et al. 2007, Bonnet et al. 2009, Jiménez-Melero et al. 2012). Many studies have also shown that temperature controls copepod egg production (Ban 1994, Halsband-Lenk et al. 2002, Lee et al. 2003, Beyrend-Dur et al. 2009, Bonnet et al. 2009, Devreker et al. 2009, Beyrend-Dur et al. 2011, Jiménez-Melero et al. 2012). Under sufficient food supply, temperature is the main parameter that controls seasonal copepod population dynamics (Uye 2000, Halsband-Lenk et al. 2004). Decrease in development time with increasing temperature has been recognized as a general rule for many organisms (Gillooly 2000, Gillooly et al. 2001), including calanoid copepods (Ban 1994, Devreker et al. 2004, Devreker et al. 2007, Beyrend-Dur et al. 2011). Earlier investigation of the development time of *E. japonicus in situ* in Lake Biwa revealed the importance of food limitation (Kawabata 1989a). Whereas Kawabata (1989a) observed the development of selected developmental stages under natural conditions, here we studied the effect of temperature under controlled environments on all life stages: development, growth, survival, and reproduction. We subsequently computed individual and population growth rates to evaluate adaptation to temperature. The experimental protocol used was based on individual observations, allowing for quantification of individual variability and fitting of moulting rate distributions (Souissi and Ban 2001, Devreker et al. 2004, Jiménez-Melero et al. 2005, Devreker et al. 2007, Beyrend-Dur et al. 2011). Individual observations can further be used for calibration of individual-based models (Dur et al. 2009).

2. Methods

2.1 Field collection and stock cultures

E. japonicus females with egg-sacs were sorted from zooplankton samples collected with vertical plankton net hauls (ring diameter, 45 cm; mesh size, 200 μm) from 30 m to the surface at a sampling site situated in the north basin of Lake Biwa (35°19′27″N, 136°11′4″E) on 12 September 2011 and 17 August 2012. Copepods were then cultivated in four 1-L jars filled with autoclaved filtered (Whatman GF/F; porosity, 0.47 μm) tap water as stock cultures. The cultures were maintained at 15 ºC under a photoperiod of 12L:12D with a light intensity of 15.4 μmol m^{-2} s^{-1}. Such conditions were found to be optimal for development. Copepods were fed with ~10^5 cells ml^{-1} of a 1:1 fresh algal mixture of *Chlamydomonas reinhardtii* (IAM, C-9) and *Cryptomonas tetrapyrenoidosa* (NIES, 282). Fresh food suspensions were provided every 2 days. Algal cultures were grown in 1-L flask under a photoperiod of 12L:12D with a light intensity of 125 μmol m^{-2} s^{-1}. For the experiments, we used individuals cultivated in the laboratory for at least two or three generations to avoid biases due to inherent wild population variability (Laabir et al. 1995).

2.2 Experimental conditions

In Lake Biwa, the temperature range encountered by *E. japonicus* populations varies between 5 and 25 ºC [Shiga Prefectural Fisheries Experiment Station (SPFES), unpublished data]. Consequently, we used four representative temperatures: i.e., 10, 15, 20, and 25 ºC. For the experiments, individuals were acclimatized at each of these temperatures, initially in sterilized filtered tap water, and then fed in excess (5×10^4 cells ml^{-1}) with the same algal mixture used for the stock cultures. Food was provided once a day. Water was changed every 2 days at 10 and 15 ºC and once a day at 20 and 25 ºC. Frequency of water renewal was adapted to limit bacterial growth and keep water clean from waste matter produced by the

copepods themselves. All experiments were conducted under the same light conditions as those of the stock cultures.

2.3 Post-embryonic development time experiment

For each experimental condition, we sorted 36–86 newly hatched nauplii (within 12 h) from more than six females acclimatized at each temperature and placed them individually in a 10-mL well of a culture plate at the same temperature as those of the mother. Subsequently, development was followed from first naupliar (N1) to adult stage. Each individual was observed under a dissecting microscope (Olympus, SZX12) twice a day at 10 and 15 ºC, and four times a day at 20 and 25 ºC, to check for exuviae or dead animals. Time zero was defined as the time when the N1 stage hatched from the egg. Stage duration represents the inter-moult duration calculated from the age of moulting from the developmental stage *i* to *i* + 1 for each individual.

2.4 Reproduction experiments

In experiments to determine reproductive traits, we followed the same protocol used by Beyrend-Dur et al. (2011). At each of the four temperatures, more than 500 N1s isolated from the stock cultures were reared in 1-L jars with the same food supply and under the same photoperiod and light intensity as those of the stock culture. When individuals reached the fifth copepodid (C5) stage, a female and male pair was transferred to a 30-mL vial filled with the same food suspension as that of the post-embryonic development experiments. Reproductive parameters were recorded daily until the death of the females. Duration from moulting to adult female until death was expressed as female longevity. Dead males were removed and replaced by a male from the stock culture acclimatized at the same temperature. The culture medium and algal food were exchanged in the same manner as in the experiments on post-embryonic development time.

We determined the following reproductive parameters: clutch size (CS, eggs per clutch); hatching success (HS, percentages of number of nauplii hatched to number of eggs in a clutch); embryonic development time (EDT, time taken from egg laying to hatching of the nauplius); inter- clutch 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 egg production rate (EPR, number of eggs produced by a female per day) calculated from CS/ICD in each clutch. A few clutches including unfertilized eggs, i.e., presenting no delimitation of the egg membranes (<<3.9% of total clutches produced), were not taken into account for the estimation of hatching success.

2.5 Measuring body size

The prosome length was measured with an eyepiece micrometer under a dissecting microscope as body size of the exuviae during development from first copepodid (C1) to pre-adult C5 stages. Using exuviae is a very convenient method to distinguish individual stages and to measure the body size of these live small animals (Twombly and Burns 1996, Lee et al. 2003). The adult prosome lengths were determined after death on individuals preserved in neutral 5% formalin.

2.6 Data and statistical analysis

2.6.1 *Relationship between development time and temperature*

The relationship between development time (*DT*, days) and temperature (*T*, ºC) was described by the most frequently used equation in the literature, Bělehrádek's function:

$$DT = \lambda(T - a)^b \qquad (1\text{-}1)$$

where λ, a and b are fitted constants. The values of λ and a were estimated with a non-linear

analysis using least squares as a loss function in the curve fitting tool box of MATLAB software (The MathWorks Inc. 2009). According to McLaren et al. (1969) *b* was fixed at −2.05.

2.6.2 *Individual variability and moulting probabilities*

To predict the moulting probability from one developmental stage group to the next, the cumulative proportion of individuals moulting from one stage group to the next was plotted against individual development time. We constructed four additional groups of post-embryonic developmental stages for the copepod, as suggested by Souissi and Ban (2001): early (N1−N3) and late (N4−N6) naupliar stages, and early (C1−C3) and late (C4−C5) copepodid stages to avoid the irregular distribution of developmental stages. We fitted a gamma density function (GDF) (Souissi and Ban 2001) to the data, using the *gamcdf* [x/α, β] function included in the curve fitting tool box of MATLAB software (The MathWorks Inc. 2009) to obtain estimates of the maximum likelihood and confidence bounds of the GDF parameters: i.e., α the shape parameter and β the scale parameter.

2.6.3 *Somatic growth*

The effect of temperature on somatic growth was determined from the C1 to adult stages. The body dry weight (W, μg) was calculated from the prosome length (PL, mm) using the following exponential equation (Kawabata and Urabe 1998):

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

Body dry weight was then used to estimate the growth rate of *E. japonicus* at each experimental temperature. The log-transformed body weight was plotted against cumulative development time. Then the natural log-transformed growth rate was fitted to a linear function of temperature. The slope is the instantaneous growth rate (g, day^{-1}) at each

temperature.

2.6.4 *Life table analysis of population growth*

The population growth rate (r, day^{-1}) was derived from Euler-Lotka's equation and calculated iteratively:

$$\sum_{x=ia}^{\omega} l_x m_x \mathrm{e}^{-rx} = 1$$

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.

2.6.5 *Individual variability of fecundity*

Individual fecundity variability (cumulative number of eggs produced by a female in its lifetime) was estimated with the coefficient of variation (CV) at each temperature tested. CV was calculated as follows:

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

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

2.6.6 *Comparison between temperature treatments*

Differences between four temperature conditions in growth and reproductive parameters were tested using the non-parametric Kruskal−Wallis test for independent data with the significance level set at $p < 0.05$. When the test resulted in a significant difference, the post hoc Tukey−Kramer test was conducted. All statistical analyses were performed with MATLAB software (The MathWorks Inc. 2009).

To test the differences of stage duration between temperatures, generalized linear models (GLM) are commonly used (Jiménez-Melero et al. 2007). Here, a log-linear model was

employed for testing the main effects and different interactions among temperature, stage and survival. This log-linear model included three main effects (stage, temperature and survival), three two-variable interactions (stage × temperature, stage × survival, and temperature × survival), and three-variable interactions (stage × temperature × survival). The variable 'stage' had 11 categories (N1 to C5), the variable 'temperature' had four categories (10, 15, 20 and 25°C), and the variable 'survival' had two categories (alive and dead). The saturated (full) model included all main effects, all two-way interactions, and the three-way interactions. The fit of each model was based on observed data and fitted cell frequencies. SPSS software (IBM Inc. 2011) was used for log-linear model analysis.

3. Results

3.1 Post-embryonic development time (post-EDT) *E. japonicus*

Results for the effect of temperature on post-EDTs in *E. japonicus* are summarized in Table 1-1. Among all developmental stages, the first naupliar (N1) stage and the fifth copepodid (C5) stage always showed the shortest and longest durations, respectively. The mean stage duration of C5 females was longer than that of males for all four temperatures tested, though the differences were not significant (Kruskal−Wallis test, $P > 0.05$). Mean post-EDT from N1 to adult took 62−68 days at 10 ºC, while it did not exceed 33 days at the higher temperatures tested. On average, males reached adulthood 6 days earlier than females at 10 ºC, and 1−2 days earlier at higher temperatures, but the differences were not significant (Kruskal−Wallis test, $P > 0.05$). Male and female data were then combined to test the effect of temperature on post-EDT. Post-EDTs were significantly different among the temperatures tested (Kruskal−Wallis test, $P < 0.05$). The sex ratios, i.e., female to male ratios at adult stage, were < 0.9 at lower temperatures (10 and 15 ºC) and > 1.1 at 20 and 25 ºC.

Table 1-1 Mean stage durations (*D*, days), standard deviations (SD), and stage specific survival rates (%) of *Eodiaptomus japonicus* from Lake Biwa reared at 10, 15, 20 and 25 ºC. Sex ratio (females to males) and the survival rate of the adult stage are also indicated.

Stage and sex	10 °C				15 °C				20 °C				25 °C			
	D	SD	%	*n*	*D*	SD	%	*n*	*D*	SD	%	*n*	*D*	SD	%	*n*
No. of eggs				86				36				36				36
N1	2.21	0.68	100	86	1.36	0.54	100	36	0.54	0.20	97	35	0.50	0.13	94	34
N2	4.40	1.39	81	70	2.16	0.72	81	29	1.66	0.38	69	24	1.11	0.31	85	29
N3	4.50	1.34	80	56	2.45	0.69	76	22	1.65	0.38	83	20	1.07	0.40	86	25
N4	5.19	2.35	91	51	2.01	0.66	95	21	1.60	0.64	75	15	1.12	0.41	84	21
N5	6.00	2.55	76	39	2.52	1.00	90	19	1.56	0.26	100	15	1.24	0.39	90	19
N6	6.57	1.65	74	29	2.44	0.49	95	18	1.91	0.37	87	13	1.24	0.31	79	15
C1	6.80	2.08	83	24	3.60	1.58	94	17	2.03	0.49	100	13	1.46	0.24	100	15
C2	7.58	1.26	75	18	4.36	1.34	94	16	2.48	0.66	100	13	1.63	0.25	100	15
C3	7.26	1.74	89	16	3.25	0.80	100	16	2.56	1.13	100	13	1.49	0.57	100	15
C4	7.50	0.85	100	15	3.68	0.66	100	16	2.96	1.03	100	13	1.63	0.43	100	15
C5 M	8.73	1.94	88	7	4.74	1.04	91	10	3.42	0.46	100	6	2.14	0.22	100	6
C5 F	10.50	4.14	86	6	4.80	0.96	80	4	3.62	0.63	100	7	2.38	0.68	100	9
N1-adult M	61.98	5.55		7	30.95	3.13		10	20.55	2.88		6	14.08	0.92		6
N1-adult F	67.88	7.36		6	33.06	3.80		4	23.79	3.08		7	15.09	2.83		9
Sex ratio	0.86				0.40				1.17				1.50			
Survival (adult)	15 %				39 %				36 %				42 %			

N1–N6 naupliar stages, *C1–C5* copepodid stages, *M* male, *F* female, *n* number of individuals in the stage, *N1-adult* the post-embryonic development time from hatching to adulthood

At 20 and 25 ºC, none of the individuals died during copepodid stages. Only 15 % of individuals survived until the adult stage at 10 ºC, while around 40 % survived at higher temperatures. Our log-linear model (Table 1-2) showed that stage and temperature affected the frequency of dead and live individuals. The best model (number 8, Table 1-2) also included the interaction stage × temperature. Such effect should not be considered since temperature is fixed by the researcher and does not determine the stage. This is nevertheless a common error for log-linear models associated with the fact that such models do not distinguish between response and predictor variables (as in Jimenez-Melero et al. 2007). Specific survival rates were lower for naupliar stages compared to copepodid stages (Kruskal−Wallis test, $P < 0.05$).

Table 1-2 Results of the log linear model for a three-way table: effect of temperature and stage on frequency of dead and live individuals of *Eodiaptomus japonicus*.

Model		Goodness of fit tests		
		G^2	*df*	*P* value
1	Stage + temp + survival	155.470	73	<0.001
2	Stage × temp	119.558	43	<0.001
3	Stage × survival	101.820	63	<0.001
4	Temp × survival	150.078	70	<0.001
5	Stage × temp + stage × survival	65.908	33	<0.001
6	Stage × temp + temp × survival	114.166	40	<0.001
7	Stage × survival + temp × survival	96.428	60	<0.002
8	Stage × temp + stage × survival + temp × survival	60.428	30	<0.001
9	Saturated (full) model	0	0	

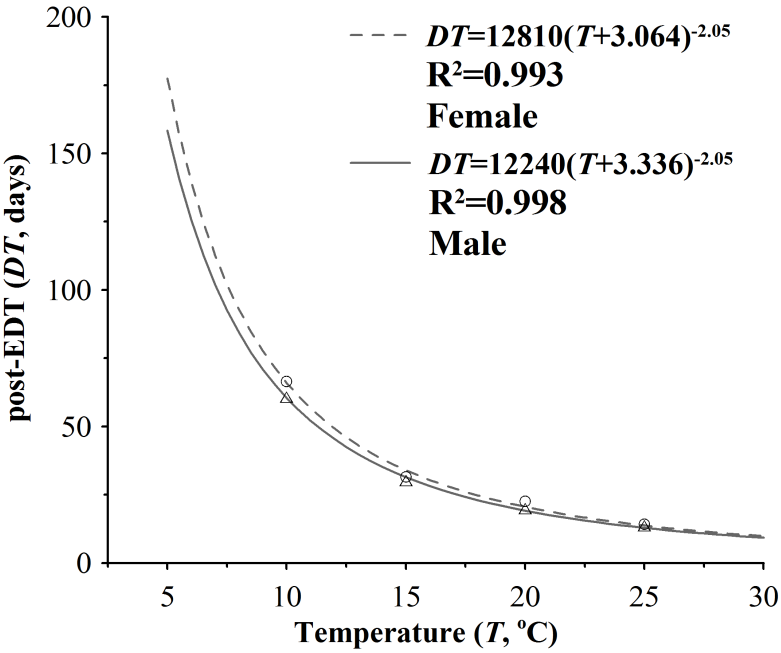

Fig. 1-1 Relationship between temperature and median post-embryonic development time (post-EDT, days) of *Eodiaptomus japonicus* males (*Open triangles*, *solid line*) and females (*open circles*, *dotted line*) reared at four temperature conditions.

The relationship between temperature (T, ºC) and median post-EDT (DT, days) exponentially decreased with increasing temperature from 10 to 25 ºC (Fig. 1-1) and was well described by the Bělehrádek temperature functions as equation (1-1):

$$DT = 12810\,(T + 3.064)^{-2.05}\ (n = 4,\ R^2 = 0.993,\ P < 0.05)\ \text{for females, and}$$

$$DT = 12240\,(T + 3.336)^{-2.05}\ (n = 4,\ R^2 = 0.998,\ P < 0.05)\ \text{for males.}$$

Bělehrádek's functions fit the duration data well for each stage, with good coefficients of determination (Table 1-3).

Table 1-3 Parameters for Bělehrádek's temperature function of stage duration (days) in *Eodiaptomus japonicus*.

Stage and sex	λ	a	R^2
Egg	1249	−2.004	0.999
N1	328.9	−1.427	0.980
N2	898.4	−3.758	0.979
N3	1073	−4.699	0.994
N4	715.5	−1.873	0.989
N5	676.2	−0.551	0.987
N6	620.8	0.600	0.975
C1	1029	−1.807	0.998
C2	1582	−3.773	0.999
C3	978.1	−1.104	0.993
C4	1414	−2.896	0.992
C5 M	2664	−7.096	0.989
C5 F	2144	−4.651	0.987

λ and a are constants of the function described in Eq. (1) in the text. M and F are male and female, respectively

3.2 Individual variability and moulting probabilities fitted by GDF

Variability of post-EDT at certain stage groups can be evaluated by the distribution of moulting rates from one stage group to the next. The fitted GDF showed the distribution of moulting rates depended on temperature and the individual variability of development time was highest at 10 ºC (Fig. 1-2).

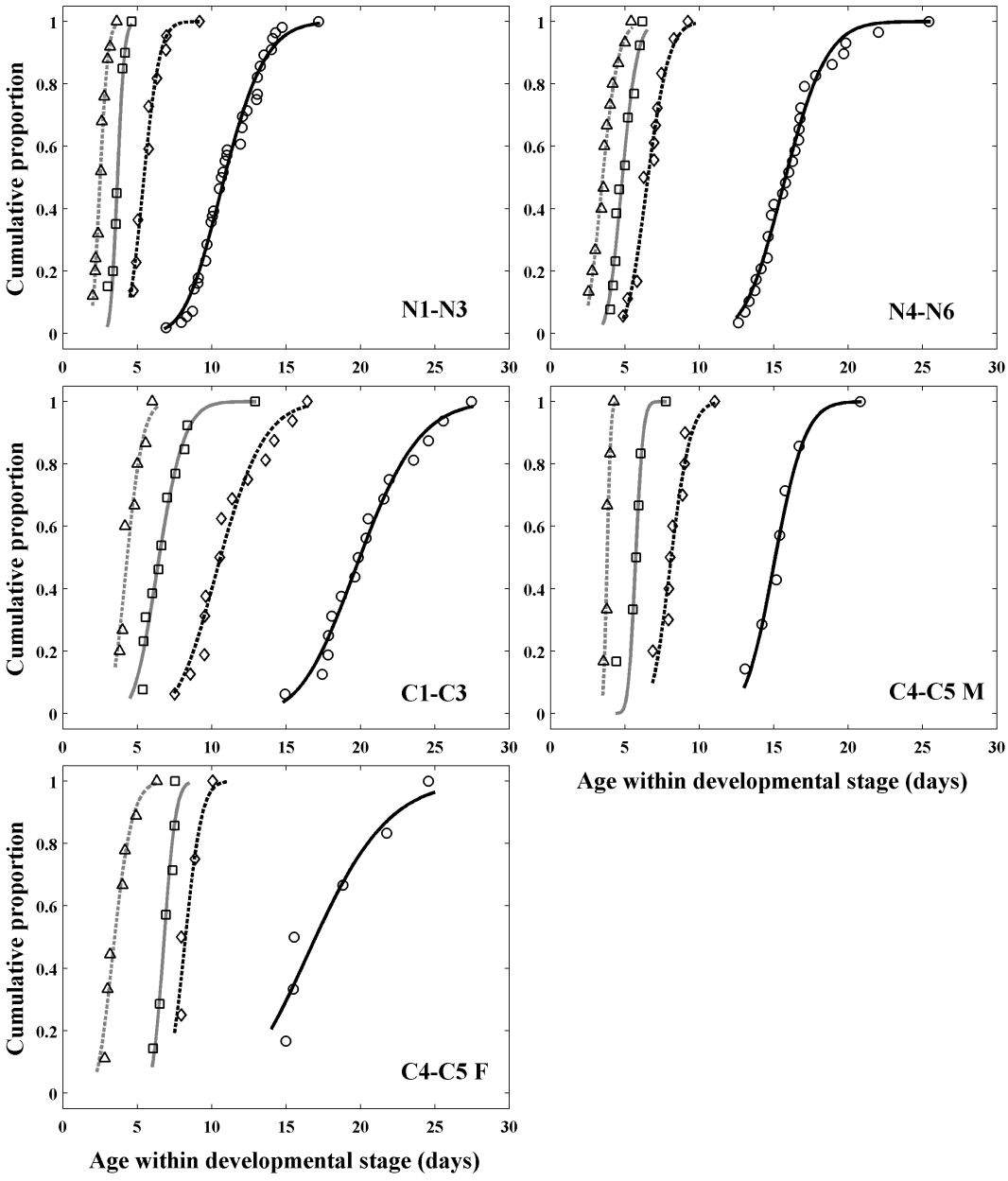

Fig. 1-2 Cumulative proportion of *Eodiaptomus japonicus* individuals moulting from one group of stages to the next as a function of age within developmental stages. *Dots* represent observed data at 10 ºC (*circles*), 15 ºC (*diamonds*), 20 ºC (*squares*), and 25 ºC (*triangles*). Naupliar stages were aggregated into two groups (N1−N3 and N4−N6) as were copepodid stages (C1−C3 and C4−C5) while male (M) and females (F) were shown separately. The *lines* represent the expected data from the gamma density functions at 10 ºC (*solid black*), 15 ºC (*dotted black*), 20 ºC (*solid grey*), and 25 ºC (*dotted grey*).

The values of the parameters of cumulative GDF *gamcdf* [x/α, β] are given in Table 1-4. The cumulative proportion for each group of individuals against development time was strongly fitted ($R^2 > 0.9$) to the gamma function at all experimental temperatures.

Table 1-4 Parameters (α, β and R^2) with confidence bounds at 95 % of the gamma density function (gamcdf [x/α, β]) fit to the median development time for four groups of stages of *Eodiaptomus japonicus* at 10, 15, 20 and 25 ºC.

T (°C)	Stage group	GDF parameter (confidence bounds)		R^2
		α	β	
10	N1–N3	25.83 (21.79, 29.86)	0.426 (0.3587, 0.4933)	0.984
10	N4–N6	53 (44.04, 61.97)	0.2999 (0.2488, 0.351)	0.987
10	C1–C3	40.28 (30.48, 50.09)	0.4995 (0.3765, 0.6225)	0.984
10	C4–C5 M	90.89 (30.21, 151.6)	0.1668 (0.05548, 0.2781)	0.975
10	C4–C5 F	19.53 (−6.141, 45.21)	0.8852 (−0.3089, 2.079)	0.913
15	N1–N3	43.74 (24.6, 62.87)	0.1256 (0.07025, 0.1809)	0.980
15	N4–N6	35.62 (18.82, 52.43)	0.1858 (0.09867, 0.273)	0.972
15	C1–C3	21.49 (11.53, 31.45)	0.5007 (0.2636, 0.7377)	0.963
15	C4–C5M	62.67 (11.62, 113.7)	0.1302 (0.02441, 0.2361)	0.927
15	C4–C5 F	90.75 (−229.6, 411.1)	0.09117 (−0.2327, 0.415)	0.902
20	N1–N3	102.5 (25.26, 179.7)	0.03597 (0.00871, 0.06323)	0.973
20	N4–N6	38.91 (21.02, 56.79)	0.1253 (0.0668, 0.1838)	0.968
20	C1–C3	23.94 (14.91, 32.97)	0.2739 (0.1689, 0.3789)	0.975
20	C4–C5 M	208.4 (−100.1, 516.9)	0.02754 (−0.01309, 0.06816)	0.940
20	C4–C5 F	123.8 (9.036, 238.6)	0.05517 (0.004315, 0.106)	0.950
25	N1–N3	37.86 (26.35, 49.38)	0.06677 (0.0463, 0.08725)	0.988
25	N4–N6	18.63 (13.47, 23.79)	0.1917 (0.1382, 0.2451)	0.985
25	C1–C3	27.36 (−1.942, 56.67)	0.16 (−0.01266, 0.3326)	0.909
25	C4–C5 M	374.7 (−397.4, 1147)	0.01018 (−0.01078, 0.03113)	0.920
25	C4–C5 F	14.42 (3.909, 24.93)	0.2472 (0.06234, 0.4321)	0.960

3.3 Somatic growth

The prosome length of adult *E. japonicus* decreased with increasing temperature (Fig. 1-3). Females were always larger than males at each temperature (Kruskal−Wallis test, $P < 0.05$). The mean adult prosome length was 0.868, 0.839, 0.817 and 0.816 mm for males, and 0.947, 0.922, 0.924 and 0.904 mm for females at 10, 15, 20 and 25 ºC, respectively. The relationship between the mean prosome length of adults (*PL*, mm) and temperature (*T*, ºC) was expressed as:

$PL = 1.077T^{-0.0547}$ $(n = 4, R^2 = 0.854, p < 0.05)$ for females, and

$PL = 1.044T^{-0.0798}$ $(n = 4, R^2 = 0.946, p < 0.05)$ for males.

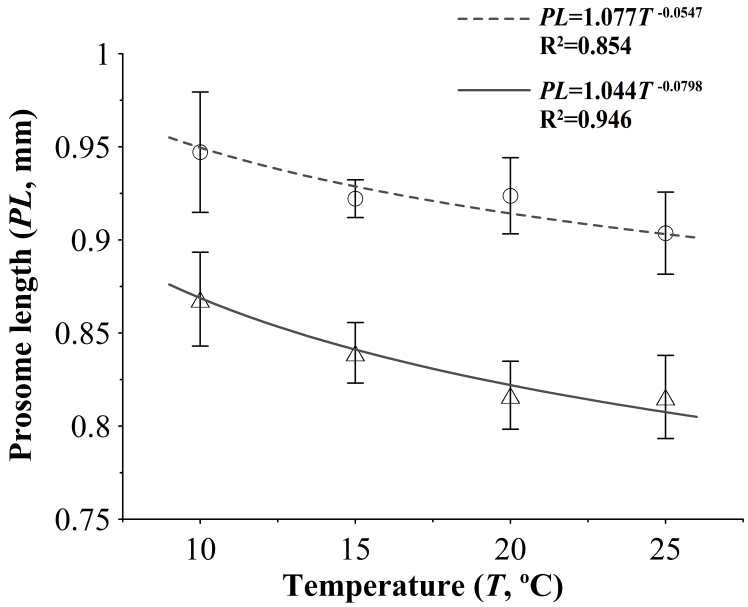

Fig. 1-3 Mean prosome length of adult males (*open triangles* and *solid line*) and females (*open circles* and *dotted line*) of *Eodiaptomus japonicus* reared at four temperature conditions under food satiation. *Vertical bars* indicate standard deviations. Regression equations and coefficients of determination are indicated.

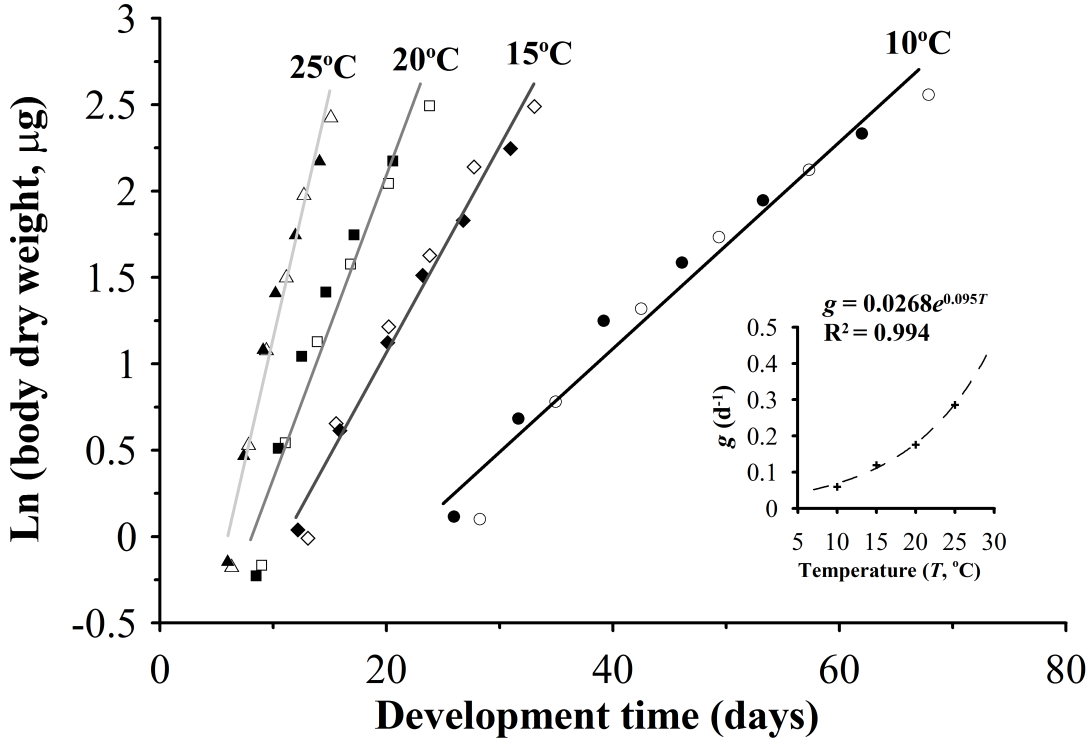

Fig. 1-4 Growth rates of male (*solid symbols*) and female (*open symbols*) copepodids of *Eodiaptomus japonicus* reared at four temperatures under food satiation. The regression lines were expressed as: $\ln W = 0.05982DT - 1.305$ ($R^2 = 0.969$) at 10 ºC, $\ln W = 0.1195DT - 1.324$ ($R^2 = 0.977$) at 15 ºC, $\ln W = 0.1758DT - 1.424$ ($R^2 = 0.947$) at 20 ºC, and $\ln W = 0.286DT - 1.711$ ($R^2 = 0.971$) at 25 ºC, with W the dry weight, and DT the cumulative development time of the copepod. The *inset* presents the relationship between instantaneous growth rates (g, day^{-1}) and tested temperatures (T, ºC) with an exponential regression (*dotted line*). See details in the text.

We calculated the instantaneous growth rate for *E. japonicus* at each of the four temperatures tested. The natural log-transformed body weight of copepodites linearly increased against the cumulative development time at all tested temperatures (Fig. 1-4). The instantaneous growth rate (g, day^{-1}), i.e., the steepness of the slope, exponentially increased with increasing temperature (T, ºC), being 0.06, 0.12, 0.18 and 0.29 day^{-1} at 10, 15, 20 and 25 ºC, respectively (Fig. 1-4). The temperature function was expressed as:

$$g = 0.0268\ e^{0.095T} \qquad (n = 4,\ R^2 = 0.994,\ P < 0.05).$$

3.4 Reproduction

Reproductive parameters of *E. japonicus* were significantly affected by temperature, with the exception of hatching success, which was high at all tested temperatures (i.e., 98−100%, Table 1-5). CS exhibited the lowest and highest values at 10 and 15 ºC, respectively. CS between 20 and 25 ºC did not vary significantly (post hoc Tukey−Kramer test, $df = 3$, $p > 0.05$). EPR increased with increasing temperature, increasing 4.4-fold from 10 to 25 ºC. Inversely, EDT, ICD, and LT decreased with increasing temperature; EDT, ICD and LT decreased from 10 to 25 ºC by 4.7-fold, 3.3-fold and 5.8-fold, respectively. Mean adult longevity at 15 ºC was the longest, being 69 days, with the next being 45 days at 20 ºC (Table 1-5). A similar value of around 30 days was observed at 10 and 25 ºC.

Table 1-5 Mean values and standard deviation (SD) of reproduction of Eodiaptomus japonicus from Lake Biwa reared at 10, 15, 20 and 25 ºC.

Parameters	10 °C			15 °C			20 °C			25 °C			*P*
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	
No. of pairs			20			10			14			15	
HS (%)	98.01			97.50			99.62			97.51			
CS	11.62	2.66	14	16.84	4.64	57	14.54^{a}	3.71	90	14.98^{a}	3.52	99	<0.05
EPR	1.31^{a}	0.32	3	3.20^{a}	1.70	8	4.09,^{a,b}	0.95	10	5.75^{b}	1.89	13	<0.05
EDT	7.77^{a}	0.80	14	3.87^{a}	0.54	57	2.24	0.36	90	1.65	0.45	99	<0.05
ICD	9.38^{a}	1.61	6	5.91^{a}	2.50	48	3.51	1.18	80	2.85	1.81	84	<0.05
LT	8.05	6.28	14	2.52^{a}	2.43	57	1.74^{a,b}	2.58	90	1.39^{b}	1.64	99	<0.05
Longevity	37.53^{a,b}	12.33	8	68.83^{a}	21.43	9	44.97^{a,b}	8.79	10	30.48^{b}	18.85	15	<0.05

Values in the lines with the same superscript are not significantly different as determined with the Kruskal–Wallis and Tukey–Kramer post hoc tests; $p > 0.05$

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

Cumulative egg production increased with increasing temperature (Fig. 1-5). At the end of life, the total number of eggs produced by a female ranged from 20 to 190, 60 to 210 and 20 to 230 eggs female^{-1} at 15, 20 and 25 ºC, respectively. At 10 ºC few females were able to produce more than two clutches during their lifetime. The maximum number of clutches produced was observed at 25 ºC, with 17 clutches produced through the reproductive life of a female. Individual variability of cumulative egg production was high and CV values were over 40 % at all temperatures.

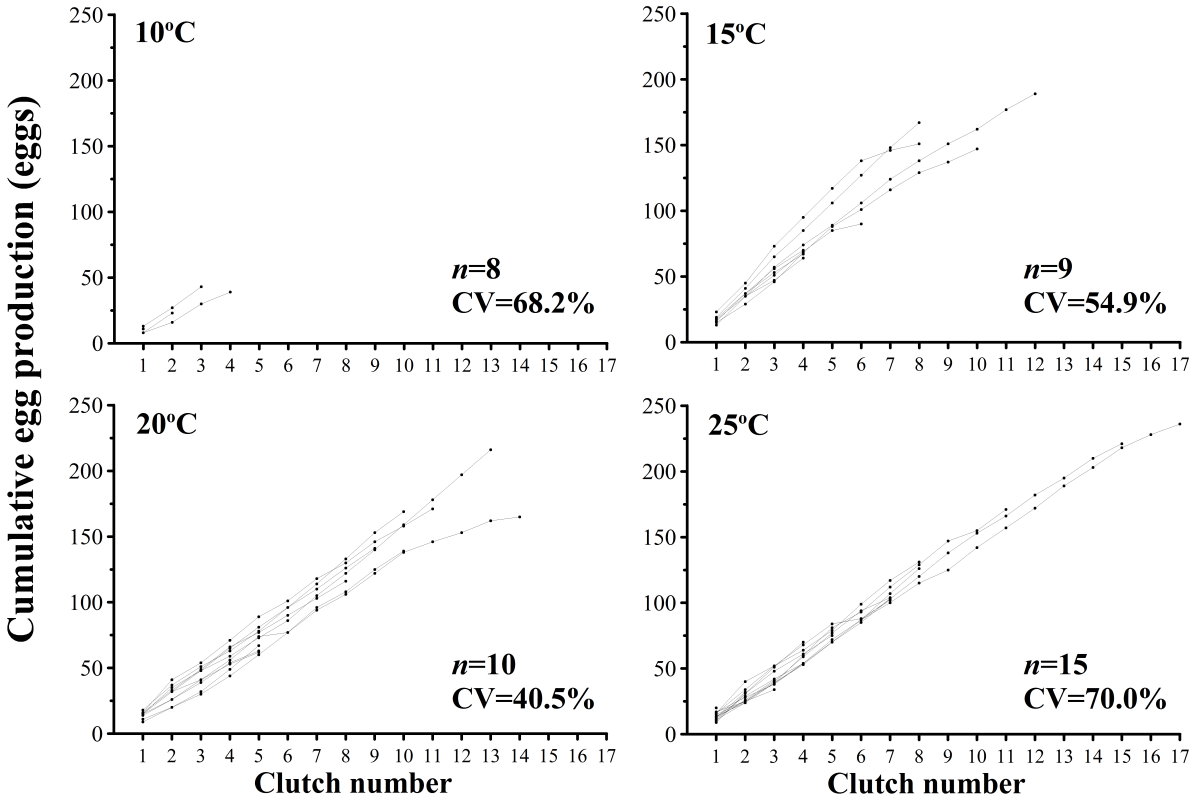

Fig. 1-5 Cumulative egg production for each *Eodiaptomus japonicus* female as a function of clutch number at each of the four temperatures tested. *Each point* represents a clutch of an individual female. *n* is the number of reproductive females observed. CV is the coefficient of variation for cumulative number of eggs produced by a female during her entire lifetime (see details in the text).

Female PL and CS were not significantly correlated (n = 260, R^2 = 0.028, P = 0.76, Fig. 1-6). The largest CS of 28 eggs clutch^{-1} was observed for a female whose PL was 1.0 mm at 15 ºC. Despite large body sizes at 10 ºC, the females produced small clutches—11−16 eggs clutch^{-1}.

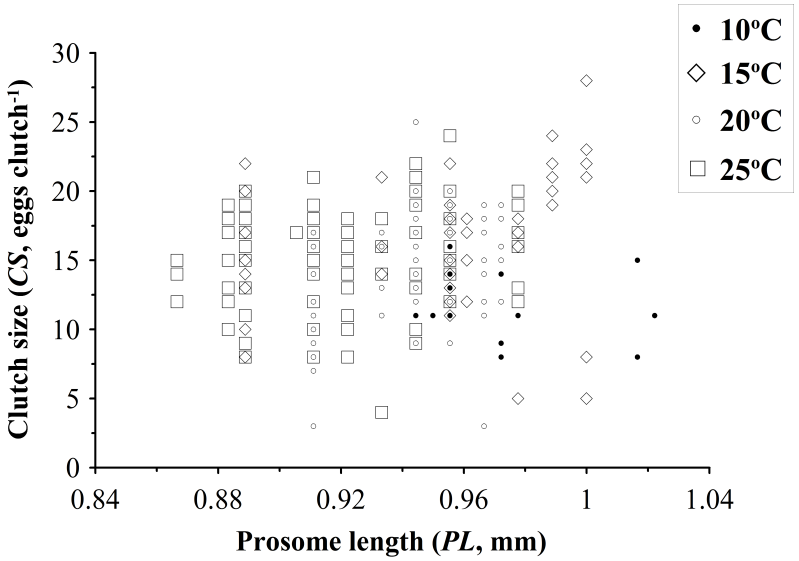

Fig. 1-6 Relationship between clutch size (CS, egg clutch^{-1}) and prosome length (PL, mm) of *Eodiaptomus japonicus* females at four temperature conditions.

3.5 Potential population growth

The population growth rate (r, day^{-1}) of *E. japonicus* calculated from the laboratory experiments increased linearly with increasing temperature (Fig. 1-7). The linear regression equation was expressed as:

$$r = 0.0112T - 0.096 \qquad (n = 4,\ R^2 = 0.956,\ P < 0.05).$$

This equation predicted that this copepod may cease population growth below 8.6 ºC.

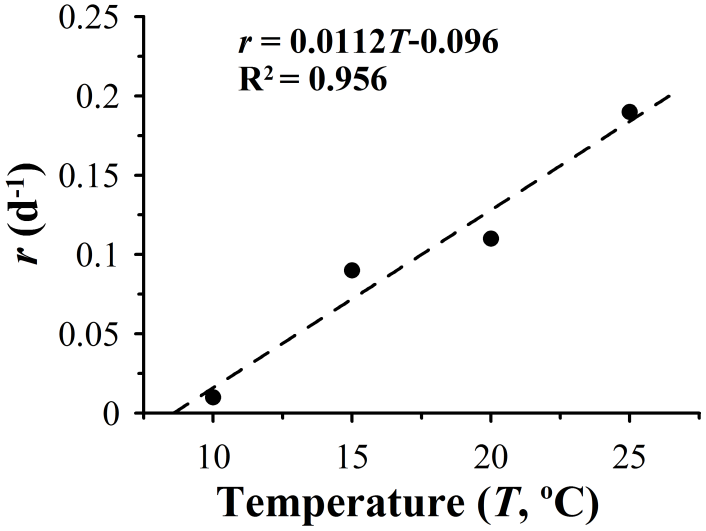

Fig. 1-7 Population growth rate (r, day^{-1}) of *Eodiaptomus japonicus* reared at four temperature conditions. The linear fit and its coefficient of determination are provided.

4. Discussion

In the present study, embryonic and post-EDT of *Eodiaptomus japonicus* strongly

depended on temperature, a common feature evident in other copepods (e.g., Landry, 1983, Ban 1994, Devreker et al. 2007). Additionally, post-EDT was isochronous, except for the shortest N1 and the longest female C5 stages, under sufficient food supply at all temperatures tested. This pattern of development was also identical to that observed in the same species by Kawabata (1989a) in the field and that observed in other calanoid copepod species, e.g., *Acartia clause* (Landry 1983), *Eurytemora affinis* (Ban 1994, Devreker et al. 2007), *Calanus helgolandicus* (Bonnet et al. 2009), and *Pseudocalanus newmani* (Lee et al. 2003), reared in the laboratory. The N1 stage of calanoid copepods is a non-feeding stage living on yolk (Mauchline 1998) and thus a rapid moult to the next feeding-stage, N2, is needed. The pre-adult C5 stage is usually longer than other developmental stages because individuals need time to mature their reproductive organs (Ban 1994, Devreker et al. 2007).

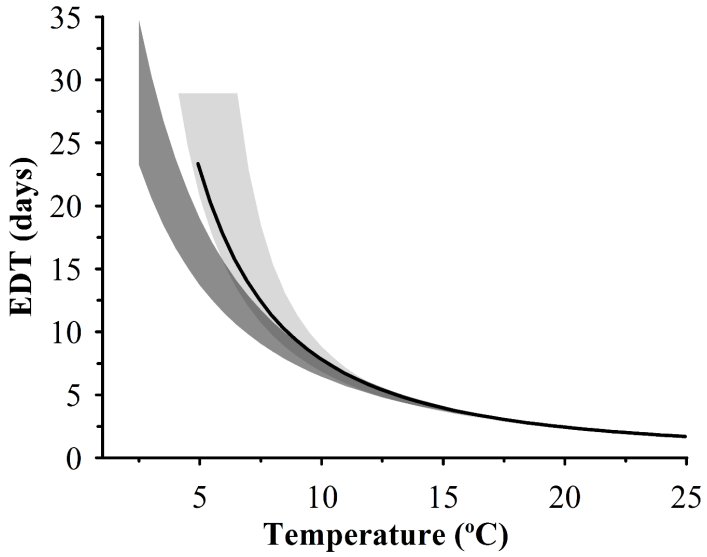

Fig. 1-8 Relationship between embryonic development time (EDT, days) and temperature (ºC) for freshwater copepods. The *black line* represents the fitted function on the median EDT of *Eodiaptomus japonicus* obtained in the present study, $EDT = 1360(T + 2.424)^{-2.05}$, $R^2>0.9$. *Dark* and *light* regions represent ranges of EDT against temperature for cold- and warm-water adapted species, respectively (Herzig 1983).

It has been shown that physiological responses of copepods to temperature vary among species or local populations of the same species living along different latitudes (McLaren et al. 1969, Landry 1975b, Herzig 1983). In marine copepods, EDT of cold-adapted species is shorter than that of warm-adapted ones, especially at low temperatures (McLaren et al. 1969,

Landry 1975b). From data obtained on several marine copepods, McLaren et al. (1969) found that biological zero indicated as *a* of Bělehrádek's temperature function for EDT was strongly related to the average temperature of their habitat. For *E. japonicus,* biological zero for EDT was −2.4 ºC. Based on Fig. 3 of McLaren et al. (1969), an average habitat temperature of ca. 17 ºC can be predicted, which is similar to the average temperature above 20 m in Lake Biwa during the growing season for *E. japonicus* from May to October (i.e., 18 ºC, SPFES unpublished data). For freshwater copepods, Herzig (1983) showed that temperature−EDT curves for warm-water species could be separated from that for cold-water species. The temperature−EDT curve obtained in the present study of *E. japonicus* lies at the lower edge for warm-water copepods (Fig. 1-8).

In many previous studies, EPR increased with temperature up to a maximum level, but decreased or even ceased beyond the maximum (Uye 1981, Rodríguez et al. 1995, Ianora 1998, Holste and Peck 2005, Dur et al. 2009, Jiménez-Melero et al. 2012). Such maximum temperature levels differ between species (Holste and Peck 2005) and depend on factors other than temperature, including female age (e.g., Parrish and Wilson 1978), food concentration and quality (e.g., Kiørboe et al. 1985, Broglio et al. 2003), and the difference between *in situ* and experimental temperatures (Kim 1995). The difference in egg production temperature optima is not only species-specific but also population-specific (Holste and Peck 2005), and is likely due to adaptation to local conditions. In *E. japonicus*, EPR increased with temperature to the highest temperature tested, i.e., 25 ºC. This may suggest that the threshold temperature for inhibiting EPR is over 25 ºC and supports the idea that this copepod is warm-water adapted.

On the other hand, the fact that the smallest CS observed in this study was produced by larger females at 10 ºC may be explained as a low-temperature stress. In general, CS is positively correlated with female body size in many temperate copepod species (cf., Uye et

al. 1983, Hirche 1992, Ban 1994). However, no significant relationship between CS and body size was found in the present study. The largest individual variability of the post-EDT at 10 ºC also supported this idea. Such individual development variability has been found to increase when the environment is non-optimal (Carlotti and Nival 1991).

Although high hatching success was obtained at all the temperatures tested, i.e., > 98 %, survival to adulthood varied among the temperatures and showed the lowest value at 10 ºC. Reproductive activity was also very low at 10 ºC; only 40 % of pairs tested could reproduce successfully, while 70 % could at higher temperatures, and 63 % of them produced only a single clutch during their life. Finally, the temperature function of population growth rate (*r*) predicted that population growth of this copepod may cease below 8.6 ºC. Somatic growth was also predicted from the equation obtained in this study to be quite low at 10 ºC. These results confirmed that 10 ºC is not optimal for *E. japonicus* and suggests delayed growth and development during winter at ca. 8 ºC.

The limited spatial distribution of *E. japonicus* in the epilimnion of Lake Biwa has been associated with poor food conditions (Okamoto 1984a, Kawabata 1987a). Nevertheless copepods, as ectotherms, exhibit a strong relation between water temperature and biogeographic range (Beaugrand et al. 2002, Beaugrand et al. 2009). Even in more restricted areas such as a lake, adaptation to a specific spatial niche can be associated with temperature preferences (Lampert 1989). For instance, besides the food-limitation hypothesis, the residence of *E. japonicus* populations in the epilimnion can be satisfactorily explained by the temperature responses obtained in this study. *E. japonicus* is restricted to above 20 m throughout the growing season in the lake, where the average temperatures exceed 10 ºC (Kawabata 1987a). In this study, we found that *E. japonicus* populations could no longer increase at temperatures below 10 ºC even with sufficient food supply. There are also strong interactions between these two environmental factors, as shown in *Daphnia* (Winder et al.

2004) that need thorough examination. Finally, organisms with short life cycles, such as copepods, may adapt through rapid evolution to fluctuating environments (Hairston and Dillon 1990). To clarify the spatial distribution of *E. japonicus* in Lake Biwa, further investigation of these aspects is required.

The cessation of growth below 10 ºC, suggests that temperature is an important force limiting *E. japonicus* populations in winter. Over a 3-year investigation, (Kawabata 1987a) found that the abundance of *E. japonicus* was low in winter and increased in late spring or early summer when water temperature started to increase. According to the Bělehrádek's function calibrated on our data, it takes about 3 months for individuals to complete their development at winter temperatures in Lake Biwa. This corresponds to the overwintering period observed in the field (Kawabata 1987a).

The long overwintering period may incur high risks for *E. japonicus* from fish and invertebrate predators. Few ovigerous females and mainly naupliar stages have been shown to occur in winter (Kawabata 1987a). This can be associated with the fact that ovigerous females suffer high predation from visually oriented predators during winter because they are more easily perceived (Hairston 1987, Mahjoub et al. 2011). Nevertheless, there is no proof of predation risk during winter in Lake Biwa, the predatory stages of *Mesocyclops dissimilis* being infrequent in winter (Kawabata 1989b, 2006). The fact that *E. japonicus* overwintered in naupliar stages (Kawabata 1987a) can be associated with growth cessation at low temperature suggested by our results.

The best estimate of different climate scenarios predicts that global average surface temperature will increase between 1.1 and 6.4 ºC, with an average rise of 2.8 ºC at the end of the 21st century (IPCC 2007). Our study showed that the population growth rate of *E. japonicus* was a positive linear function of temperature. In 2010, monthly average water temperature varied from 7.8 to 22.3 ºC (SPFES, unpublished data), giving a population

growth rate ranging between −0.009 and 0.15 day^{-1} from the function. The predicted increase in temperature of 2.8 ºC will give water temperatures varying between 10.6 and 25.1 ºC at the end of this century in Lake Biwa, resulting in a population growth rate ranging between 0.02 and 0.19 day^{-1}. Global warming may improve the environment for *E. japonicus*, which will be able to grow during winter. Additionally, we observed that the sex ratio is in favour of females at temperatures over 20 ºC. IPCC scenarios result in a prediction for an extended period of water temperatures over 20 ºC. In line with the present results, we predict that in the lake, under sufficient food supply, the population density of *E. japonicus* will be positively affected by the expected rise of temperature through a decrease of development times and an increase in both the survival rates and the reproduction frequency within a female-biased population. On the other hand, global warming may, in addition to raising water temperature, also change lake environments by altering thermocline depths and nutrient inputs, and by accelerating a decrease in dissolved oxygen (Magnuson et al. 1997). These physical changes in turn affect the primary production of a lake. For instance, an 80-year survey showed that climate change has contributed to diminishing Lake Tanganyika's primary productivity (Verburg et al. , O'Reilly et al. 2003). A decrease in primary production associated with a decrease in food availability would negatively affect the total zooplankton abundance in Lake Biwa, considering its strong correlation with total phytoplankton biomass (Hsieh et al. 2011). Such complex interactions may occur in Lake Biwa and negatively affect the population of *E. japonicus*. Further studies on the effect of food on the life cycle traits, thus, would be a continuation of the present work. The crucial information obtained from such a study would provide the necessary foundation for calibrating a mathematical model to enable testing of several scenarios on the consequences of climate changes on this copepod: for example, the match/mismatch hypothesis (Durant et al. 2007).

In conclusion, the responses of life history traits in *E. japonicus* to temperature

observed in this study help us understand the population dynamics observed in the field (Kawabata 1987a). According to field observations (Kawabata 1987a) and responses to temperature predicted from the experiments in the present study under sufficient food supply, this species may behave like a warm-water adapted species despite inhabiting a temperate lake, such as Lake Biwa. Present data on life history traits will be used to calibrate the individual-based model developed for egg-carrying copepods by Dur et al. (2013), and consequently provide further insight into the population dynamics of this species in the lake. Besides temperature, food supply is another important environmental factor that affects life history traits and population dynamics of the copepod. Sometimes food availability can have even more impact than temperature (Ban 1994). Thus, it is necessary in the future to determine the food supply effect on *E. japonicus* and evaluate the interaction between temperature and food conditions to predict changes in population dynamics due to global warming and modern human impacts.

CHAPTER 2:

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

This section is mainly based on the manuscript:

- "Combined effects of temperature and food concentration on growth and reproduction of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan)" by Xin Liu, Delphine Beyrend, Gaël Dur and Syuhei Ban published in ***Freshwater Biology***.

1. 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 and Lopez 1992, Ban 1994, Jiménez-Melero et al. 2012). Temperature 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 and 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 et al. 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 and 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 stabilization 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 et al.

1999, Ishikawa et al. 1999, Lee and Petersen 2002, Beyrend-Dur et al. 2009), or even on the combined effect of temperature and salinity (Roddie et al. 1984, Chinnery and Williams 2004, Devreker et al. 2004, Holste and Peck 2005, Devreker et al. 2007, Devreker et al. 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 and Kuosa 1999), few studies have dealt with the combined effect of these environmental factors on copepod development, growth and reproduction (Klein Breteler and Gonzalez 1986, Koski and 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 of water temperature due to global warming (Tsugeki et al. 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 1987a, Yoshida et al. 2001b). 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 ºC during winter, even with sufficient food supply. Kawabata (1987a) 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.

2. Methods

2.1 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 ºC 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 ca. 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 ºC 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).

2.2 Experimental conditions

Prior to the experiments, animals were acclimatized for at least one generation at 15 and 25 ºC 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 ºC, respectively. Frequency of water renewal was adapted to limit bacterial

growth and keep water clean from waste matter. All experiments were conducted under the same light conditions as those of the stock cultures.

2.3 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 acclimatized 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 ca. 200× magnification twice per day at 15 ºC and four times per day at 25 ºC 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.

2.4 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 (ca. 50 females per treatment). Then, N1s hatched within 12 h in each treatment were reared in 1-L jars (ca. 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 acclimatized 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 unfertilized 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.

2.5 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 and Burns 1996, Lee et al. 2003). The PL of adults was measured after death on individuals preserved in neutral 5% formalin.

2.6 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 and 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 (The 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 and Urabe 1998):

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

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

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

where A is the asymptotic value for body weight at time $t \rightarrow \infty$, 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 (The 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 duration 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 ºC), 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 (The MathWorks Inc. 2009).

3. Results

3.1 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. 2-1, Table 2-1). At 15 ºC, the MDTs of adult males (AdMs) reared at ≥5 × 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 2-2). At 25 ºC, 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 2-2). GLMs showed that temperature and FC significantly influenced post-EDT for both males and females (Table 2-3). 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 ºC (Kruskal−Wallis test, d.f. = 3, $H = 19.42$, $P < 0.001$), but not at 15 ºC (Kruskal−Wallis test, d.f. = 3, $H = 3.48$, $P = 0.323$).

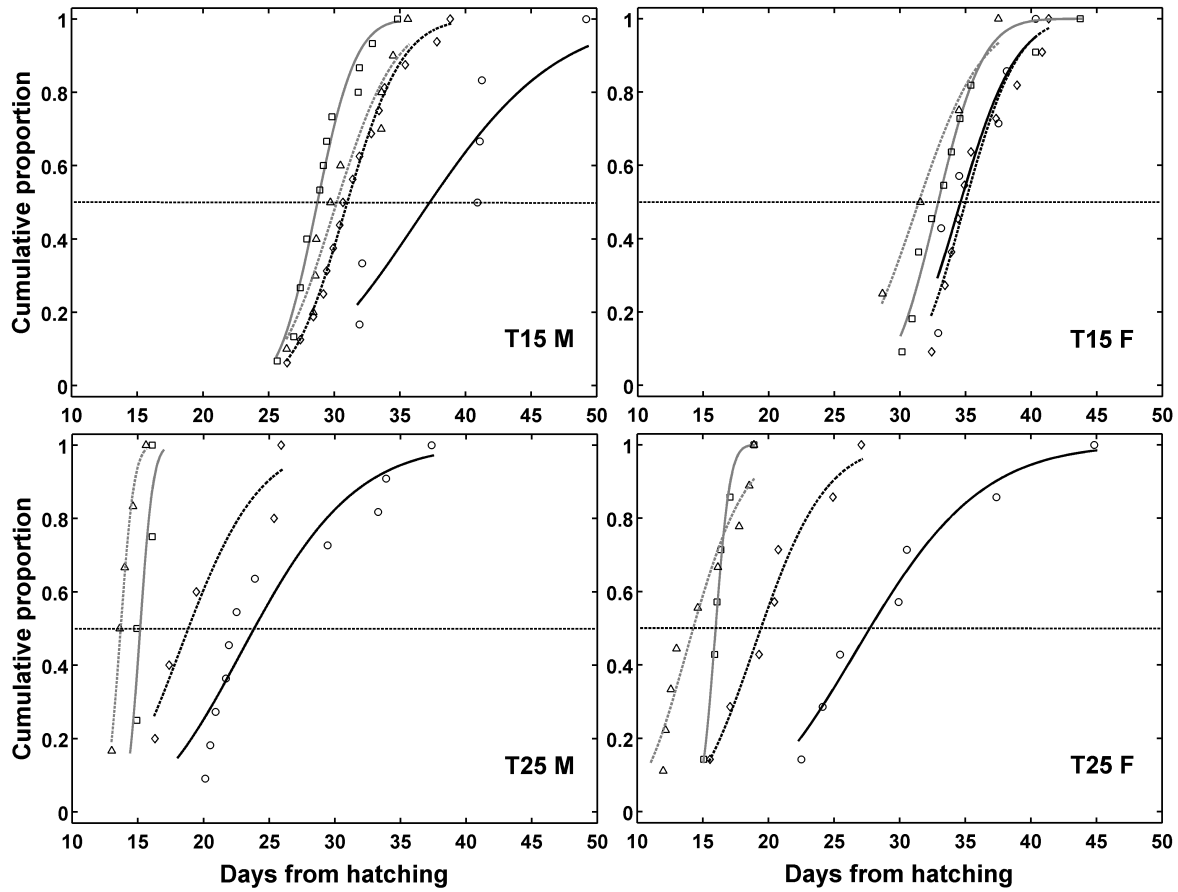

Fig. 2-1 Cumulative proportions of *Eodiaptomus japonicus* individuals moulting to adult stage against days from hatching. *Dots* represent observed data at food concentrations (FCs) of 10^3 (*circles*), 5 × 10^3 (*diamonds*), 10^4 (*squares*) and 5 × 10^4 (*triangles*) cells mL^{-1} at 15 ºC (T15) and 25 ºC (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 2-1.

The CV tended to decrease towards older stages (Table 2-2). The highest variation of post-EDT was always observed in the second naupliar stage (N2) (Table 2-2). CV of adult post-EDT strongly depended on FC at both experimental temperatures, especially at 25 ºC, 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 ºC at the medium FC, that is 10^4 cells mL^{-1} (Table 2-2).

Table 2-1 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

The shortest stage duration (SD) was observed in the N1 stage among developmental stages of all treatments (Table 2-2). 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 2-3). 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 2-3). 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 C1−C3, 12.82 and 15.85 in C4−C5 female, at 15 and 25 ºC, 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.

Table 2-2 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.

Sex ratios (female/male) at each treatment are shown in Table 2-2. The proportion of males was higher than the proportion of females except at the lowest FC at 15 ºC (Table 2-2). At 25 ºC, the opposite occurred; the proportion of females was higher than that of males except at the lowest FC (Table 2-2). 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 ºC where a survival rate of

>70% was recorded (Table 2-2). 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 2-4, $P < 0.001$). The most severe mortalities were found at the lowest FC, especially at 25 ºC (Table 2-2).

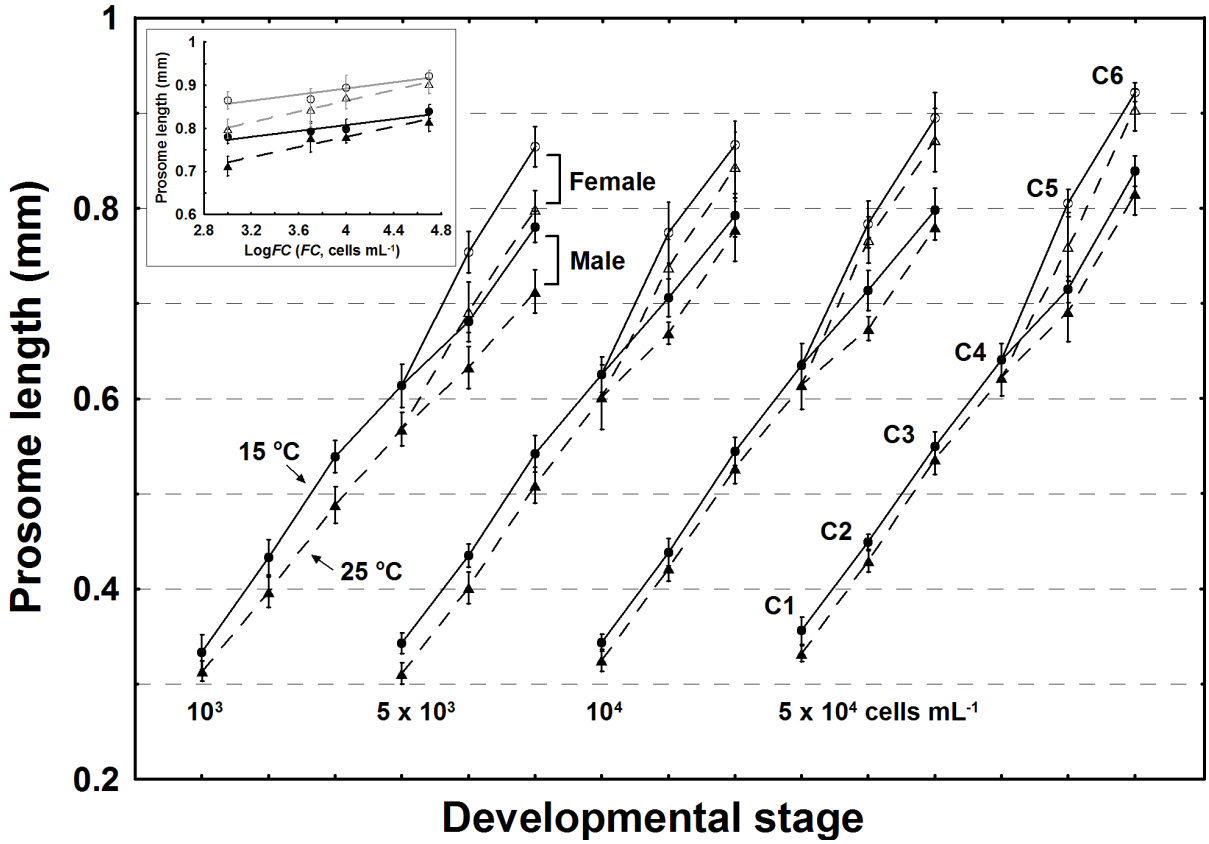

Fig. 2-2 Prosome length (PL, mm) of developmental stages from C1 to adult in *Eodiaptomus japonicus* males (*solid symbols after C5*) and females (*open symbols after C5*) at food concentrations of 10^3, 5×10^3, 10^4 and 5×10^4 cells mL^{-1} at 15 (*circles* and *solid lines*) and 25 ºC (*triangles*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

Table 2-3 Generalized linear models (GLM) show the effect of temperature (Temp) and food concentration (Food) on stage durations (*N1−N6*, *C1−C3*, *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.

PL of C1 increased with increasing FC similarly at both temperatures (Fig. 2-2), although the differences were quite small: just 6% larger in the highest FC compared to the lowest one (Fig. 2-2, Table 2-3). 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. 2-2, Figure 2-3). GLM showed an interaction between food and temperature in adults, but not in C1 (Table 2-3). 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

ºC, respectively, $P < 0.01$). The relationship between FC and PL was not the same at all stages (Figure 2-3, Table 2-5). ANCOVA showed that the slopes at every developmental stage were not homogeneous at 15 and 25 ºC (d.f. = 6 and 20, $F = 4.45$, $P < 0.01$ for 15 ºC; and d.f. = 6 and 20, $F = 4.67$, $P < 0.01$ for 25 ºC). Post hoc tests showed that at 15 ºC the slopes for AdM and AdF were significantly different from those of C3 (Figure 2-4a). At 25 ºC, the slope for C1 was significantly different from those of AdM and AdF (Figure 2-4b).

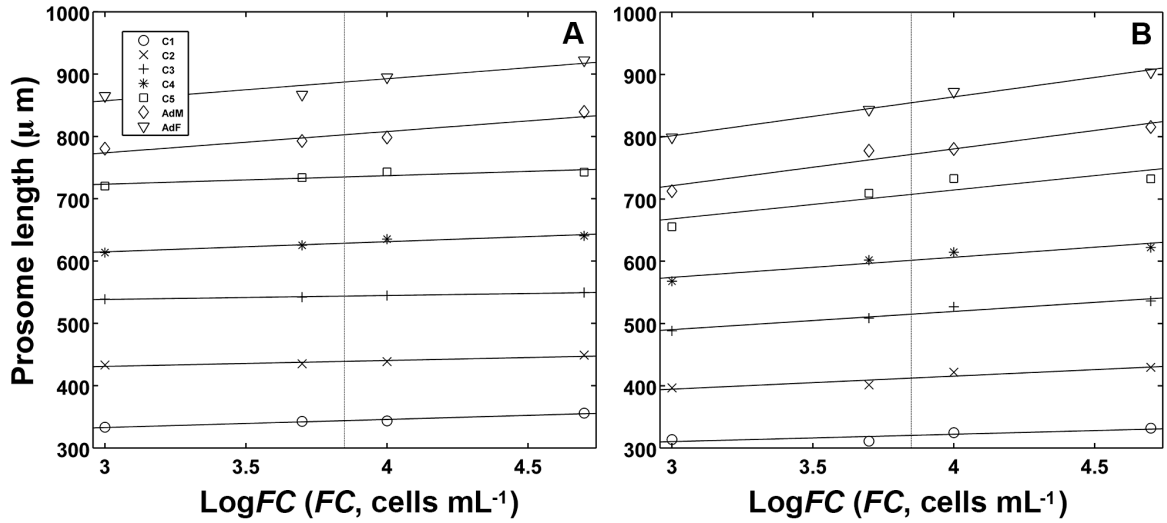

Fig. 2-3 Prosome length (μm) of juvenile (C1−C5) and adult stages (AdM, male; AdF, female) of *Eodiaptomus japonicus* over a food concentration gradient at 15 (A) and 25 ºC (B). Lengths are mean values and lines represent statistically significant regressions (see Table 2-5).

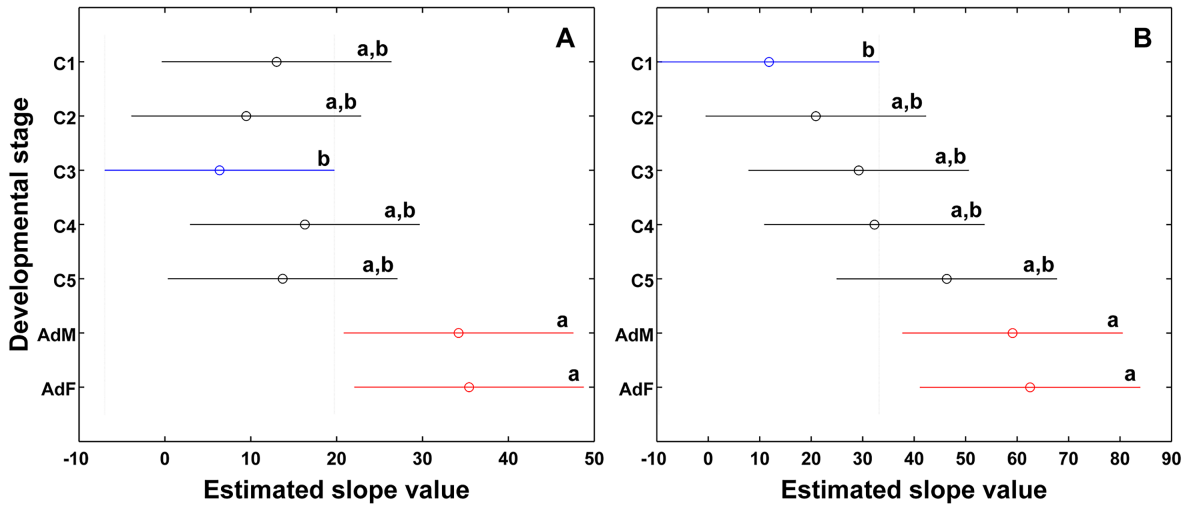

Fig. 2-4 Results of the multiple comparison of slopes following the ANCOVA testing the potential differences between stages in the response of prosome length to increasing food concentration at 15 (A) and 25 ºC (B). Estimated slope values and standard deviations (*horizontal bars*) are indicated. The same letters indicate statistically non-significant differences between the developmental stages with post-hoc test at $p < 0.05$.

Table 2-4 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	

Table 2-5 Parameter estimates (Estim.) for regression of prosome length of *Eodiaptomus japonicus* on food concentration for each post-larval stage (see Figure 2-3) 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**

3.2 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. 2-5, Table 2-6). 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 ºC (n = 4, t for the slope = 3.472 in males and 4.302 in females, $P < 0.05$), but not at 15 ºC (n = 4, t for the slope = 0.343 in males and 1.278 in females, $P > 0.05$) (Fig. 2-6). At 25 ºC, ANCOVA showed that the differences of k between the sexes were not statistically significant (d.f. = 1 and 13, $F = 0.13$, $P = 0.727$).

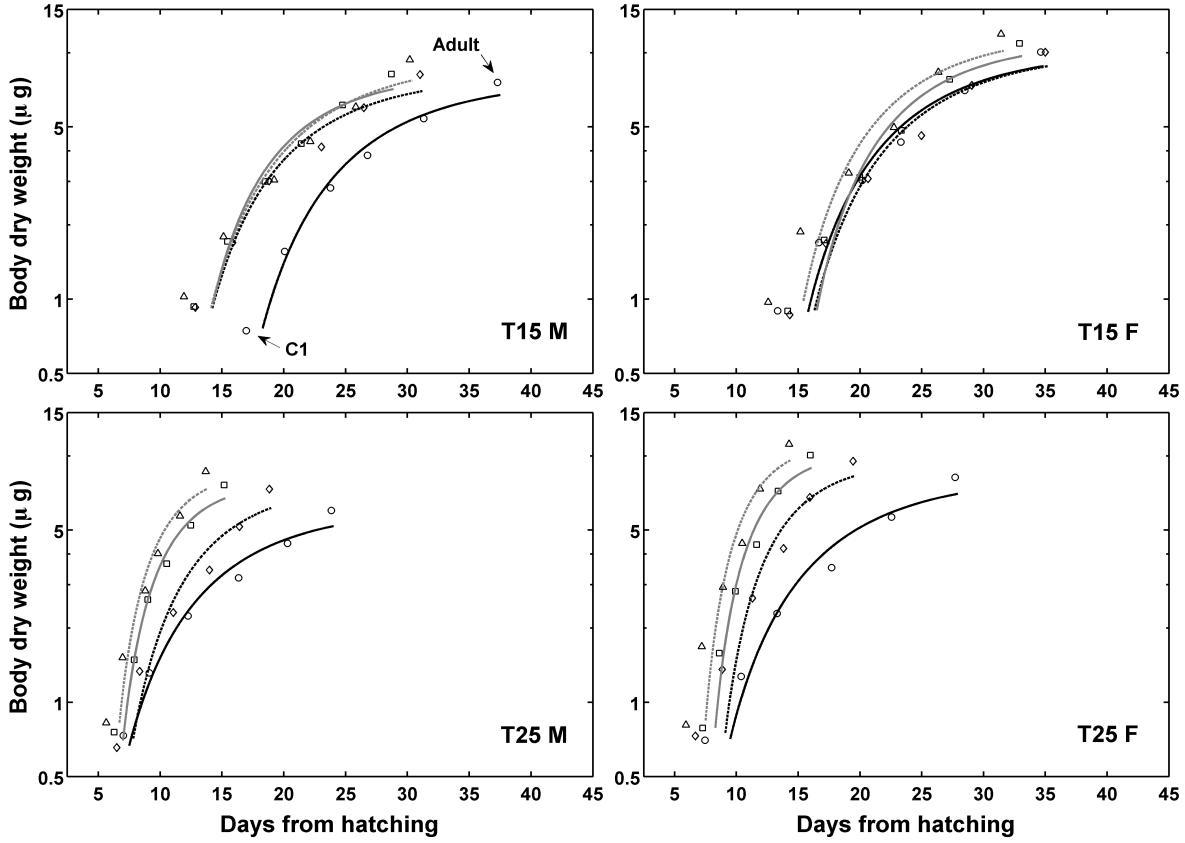

Fig. 2-5 Body dry weights of *Eodiaptomus japonicus* in copepodid stages as a function against the cumulative development time of each stage. *Dots* represent observed data at food concentrations of 10^3 (*circles*), 5×10^3 (*diamonds*), 10^4 (*squares*) and 5×10^4 (*triangles*) cells mL^{-1} at 15 (T15) and 25 ºC (T25). Males (M) and females (F) are shown separately. The lines represent the expected data from the Bertalanffy's functions at food concentrations 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 2-6.

Table 2-6 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

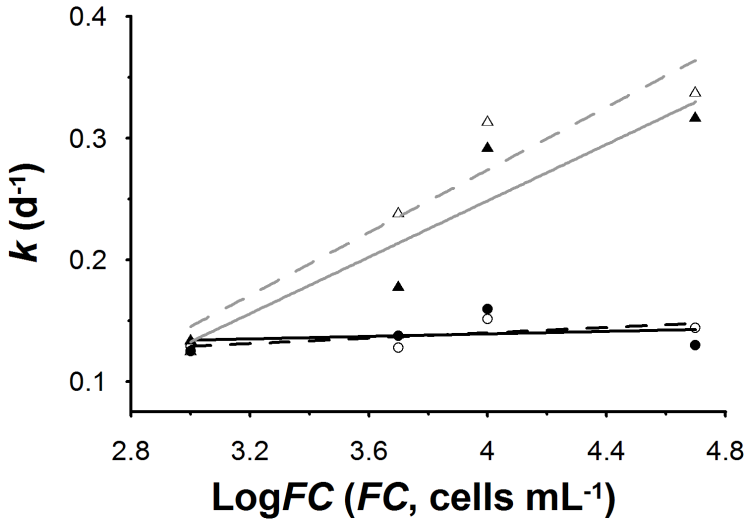

Fig. 2-6 Growth coefficient (k, day^{-1}) of *Eodiaptomus japonicus* of males (*filled circles* and *black solid line*) and females (*open circles* and *black dashed line*) at 15 ºC and males (*filled triangles* and *grey solid line*) and females (*open triangles* and *grey dashed line*) at 25 ºC reared under different food treatments.

3.3 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 ºC (Table 2-7). EDTs were significantly different between the two temperatures, but not among FCs, averaging 3.9 and 1.7 days at 15 and 25 ºC, respectively (Fig. 2-7, Tables 2-7 and 2-8). CS increased with FC at both temperatures (Fig. 2-7), 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. 2-7, Table 2-8). 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 ºC, respectively, $P < 0.05$).

Table 2-7 Mean and standard deviations (*s.d.*) 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).

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 ºC, respectively (Fig. 2-7, Table 2-7). Both temperature and FC significantly affected ICD with interactions between the two factors (Table 2-8). The Kruskal–Wallis test showed that ICDs were significantly different among the FCs at both 15 and 25 ºC (d.f. = 3, H = 36.31 and 13.02 at 15 and 25 ºC, $P < 0.01$), although the variations were larger at 15 ºC than at 25 ºC.

Latency times showed a similar trend as ICD, varying between 2.52 and 6.12 days at 15 ºC, and between 1.22 and 1.96 days at 25 ºC (Fig. 2-7, Table 2-7). 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 ºC, respectively, $P < 0.05$).

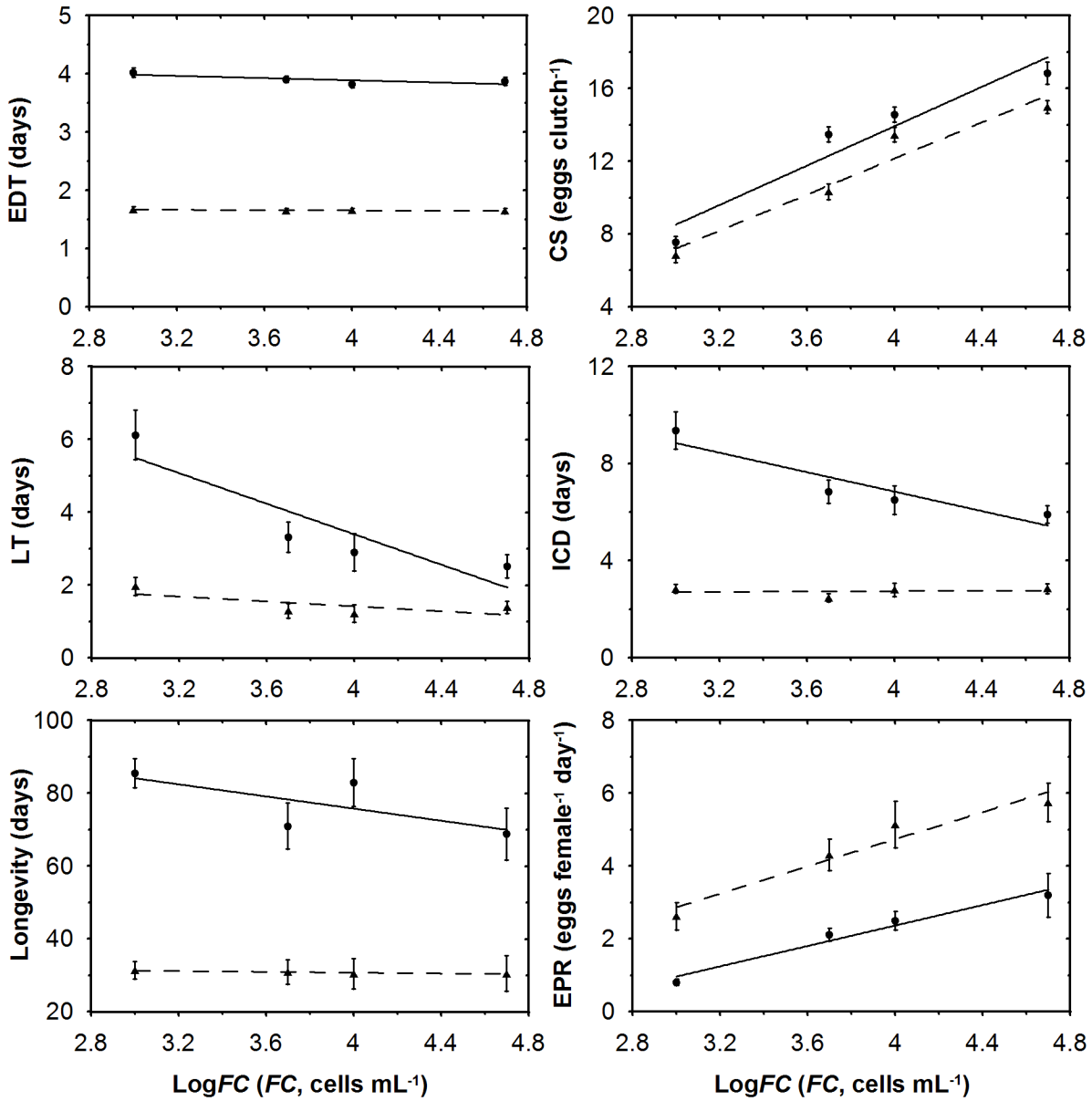

Fig. 2-7 Reproductive parameters of *Eodiaptomus japonicus* reared under four food concentrations (FC, cells mL^{-1}) at 15 (*circles*) and 25 ºC (*triangles*). The regression lines are for the experiments at 15 (*solid lines*) and 25 ºC (*dashed lines*), respectively. Acronyms are as follows: EDT (embryonic 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.

Table 2-8 Generalized 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.

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.

Finally, EPR calculated from CS and ICD, increased with increasing FC at both temperatures (Fig. 2-7), increasing by 4.0-fold and 2.2-fold at 15 and 25 ºC, respectively, from the lowest FC to the highest one. No interaction between the two factors on EPR was found (Table 2-8). Average longevity of *E. japonicus* females always exceeded 2 months at 15 ºC and was about a month at 25 ºC regardless of FC (Fig. 2-7, Table 2-7). Longevity was significantly different between the temperatures, but not among the FCs (Table 2-8).

3.4 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. 2-8). Regression analysis showed that r significantly increased with log-transformed FCs at 25 ºC ($n = 4$, $R^2 = 0.877$, t for the slope = 3.783, $P < 0.05$), but not at 15 ºC ($n = 4$, $R^2 = 0.435$, t for the slope = 1.241, $P > 0.05$).

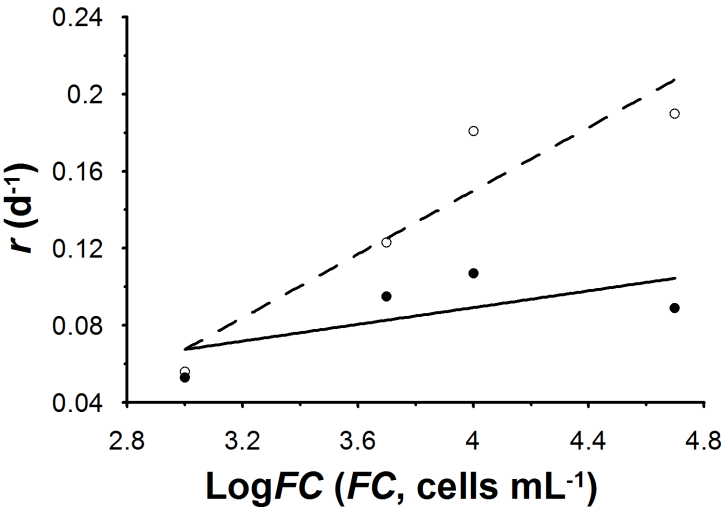

Fig. 2-8 Estimated population growth rate (r, day^{-1}) of *Eodiaptomus japonicus* reared under different food treatments at 15 (*filled symbols* and *solid line*) and 25 ºC (*open symbols* and *dashed line*).

4. Discussion

In this study, we demonstrated that growth and reproduction of *E. japonicus* were significantly influenced by FC and temperature. Kawabata (1989a) 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 (1989a) 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 and 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 1989a, Liu et al. 2014), as confirmed by complementary observation of empty guts in the N1 stage in the course of the present study. Therefore, 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 and McKinnon 1987, Lee et al. 2003). Williamson et al. (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 et al. 1988, Merrell and 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 and 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 and Krause 1987, Hagen 1988, Lee et al. 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 ºC, equivalent to ca. 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 and McKinnon 1990, Hart 1996). Zeller et al. (2004) suggested that two freshwater copepods, *Eudiaptomus gracilis* and *Eudiaptomus graciloides*,

suffered from fungal infection and exhibited low survival rates at high temperature (i.e. 24 ºC). 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 and Burns 1988, Amarasinghe et al. 1997, Devreker et al. 2004, Zeller et al. 2004, Devreker et al. 2005). Williams and 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 ºC. 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 and 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 ºC, 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 and 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 et al. 1997). Ban (1994) showed that female body size in *Eurytemora affinis* decreased by up to 32% under food-limited conditions at 15 ºC, but only declined by 10% when temperature increased from 10 to 20 ºC 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 ºC, 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 ºC, 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 ºC.

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 size (Kawabata 1987a, Kawabata and Urabe 1998). Therefore, natural populations may experience a limited food supply at times as suggested by Kawabata (1989a). 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 and 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 ºC but not at 15 ºC, 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 ºC) (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 ºC and 25 ºC, respectively (Castellani et al. 2005), while in *Centropages chierchiae*, it was ca. 0.03 and 0.15 μL O_2 μg C^{-1} day^{-1} at 8 and 24 ºC, 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 et al. 2003, Isla and 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 (Lampert 1977a, Jamieson 1986, Klein Breteler and Gonzalez 1986, Jamieson and 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 ºC, 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 ºC than at 15 ºC. Probably this difference may be attributed to prolonged and more variable ICDs at 15 ºC 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 and Haney 1980, Williamson and 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 ºC, but not at 15 ºC. This implies that *r* is more influenced by food shortage at higher temperatures than at 15 ºC, 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.

CHAPTER 3:

Effects of long-term acclimatization on metabolic plasticity of *Eodiaptomus japonicus* (Copepoda: Calanoida) using optical oxygen meter

This section is mainly based on the manuscript:

- "Effects of long-term acclimatization on metabolic plasticity of *Eodiaptomus japonicus* (Copepoda: Calanoida) using optical oxygen meter" by Xin Liu and Syuhei Ban in preparing to submitted to ***Journal of Plankton Research***.

1. Introduction

Metabolism provides a basic information for understanding the first principle to link the biology of aquatic organisms to the ecology of populations and ecosystems (Brown et al. 2004), and plays an important role in the cycling of organic matter in aquatic ecosystems through assimilation (Lampert 1977b, Teuber et al. 2013). It also used to describe zooplankton activities in biogeochemical models (Pahlow et al. 2008).

Copepods are the dominant zooplankton in aquatic food webs and the major link between primary production and higher trophic levels (Mauchline 1998). Previous studies on physiology of copepods can help ecologists tracing nutrient pathways and energy balance contributed to growth and reproduction, and consequently the population dynamics responses to environmental change (Kiørboe et al. 1985). It is necessary to understand the mechanisms of physiological plasticity in copepods for managing both natural populations and aquaculture stocks, and therefore huge studies have been published (Bradley 1978b, Hart and McLaren 1978, Penry and Frost 1991, Roche-Mayzaud et al. 1991, Mayzaud and Razouls 1992, Mayzaud et al. 1992, Lee and Petersen 2003).

It is well know that temperature is one of the most important factors determining somatic growth and reproduction of copepods (Landry 1975a, Jiménez-Melero et al. 2007, Beyrend-Dur et al. 2011, Liu et al. 2014), because temperature mostly influences metabolic rates in any kinds of aquatic organisms (Brown et al. 2004). Local adaptation for the metabolic rates involves beneficial interactions between genotypes and surrounding abiotic factors in copepods, being especially shown in extremely environmental factors, e.g. lower and higher temperatures than those in the habitats where the copepods colonize (Bradley 1978b, a, Edmands and Deimler 2004). The fact that boreal copepods cannot live at extremely high temperatures would imply that their physiology and biochemistry differ markedly from those of temperate forms (Hirche 1984).

Metabolic rates in various copepod species have been determined in the laboratory as respiration rates (Kiørboe et al. 1985, Lee et al. 2001, Castellani et al. 2005, Cruz et al. 2013). It has been shown that respiration rates in copepods are generally related to surrounding temperature (Lee et al. 2001, Castellani et al. 2005, Alcaraz et al. 2013, Cruz et al. 2013) and body mass (Ikeda 1970, 1985, Ikeda et al. 2001, Brown et al. 2004). Despite of huge number of studies on metabolic plasticity of aquatic organisms, there are few studies focused on the metabolic responses after an acclimatization, and the results were controversial even in an acclimation (i.e. short-term adaptation) (Sastry 1979, Hop and Graham 1995, Gaudy and Thibault-Botha 2007). For instance, respiration rates in Arctic cod *Boreogadus saida* was shifted downward after long-term acclimatization (five months) compared to those after short-term acclimation (two weeks) (Hop and Graham 1995). In marine copepods, metabolic responses on temperature would be different between summer and winter populations that would experience under different temperature regimes during their developments (Halcrow 1963, Mayzaud 1973, Kawall et al. 2001, Gaudy and Thibault-Botha 2007). Whereas, Robinson and Williams (Robinson and Williams 1993) found that short-term acclimation did not bias temperature responses of the respiration rates in Antarctic nano- and microplankton communities. Clarke (1993) emphasized that seasonal acclimatization of metabolic rates was a meaningless concept in his short review.

It is quite difficult to measure the oxygen consumption of mesozooplankton due to its small body mass and low oxygen demand especially at low temperatures (Lee et al. 2001, Alcaraz et al. 2013, Cruz et al. 2013). The methods mostly used for directly measuring oxygen consumption in zooplankton were Winkler titration method (Marshall et al. 1935, Williams and Jenkinson 1982, Nakamura and Turner 1997, Lee et al. 2001, Castellani et al. 2005) and oxygen electrode method (Roff 1973, Møhlenberg and Kiørboe 1981, Kiørboe et al. 1985, Ploug et al. 2008, Rosa et al. 2009, Geslin et al. 2011, Rosa et al. 2012), and the

method for indirectly measuring the amount of enzymes related to respiration, e.g. lactate dehydrogenase or pyruvate kinase in electron transfer system (ETS) (Devol 1979, Drits et al. 1993, Gómez and Hernández-León 1996). All of them involve sensitive and tedious procedures and require huge number of animals tested (ca. 200 ind. in each experiment) during entire measurements (Nakamura and Turner 1997, Castellani et al. 2005).

Among those of the published methods, the Winkler technique is the most-spread and precise method using a closed-bottle by measuring dissolved oxygen based on chemical determination at the start and end of the incubation for calculating respiration rates by an animal in an experimental vessel (Edmondson and Winberg 1971). Many modifications have been subjected for this method (Urabe and Watanabe 1990, Nakamura and Turner 1997, Lee et al. 2001, Castellani et al. 2005), which aim to improve the accuracy, for example, in a modified-Winkler method using a small chamber of 2.5-mL, detection limit for measuring dissolved oxygen concentration can be reached 20 $\mu LO_2\ L^{-1}$ with coefficient of variation of 0.1% (Urabe and Watanabe 1990), and then accuracy of the respiration rate was 10^{-3} μLO_2 $ind^{-1}\ h^{-1}$ when 15 individuals were incubated for 6 hours in an experiment.

Apart from the modified-Winkler technique, Cartesian diver method in a variable pressure system using the sensitive tool on enclosed micro-respirometry for measuring oxygen consumption in very small aquatic animals such as rotifer is the most accurate method; the detection accuracy drops down to 10^{-5} μLO_2 $ind^{-1}\ h^{-1}$ (Klekowski 1971), but requires the time for learning some measuring skills and tedious works for reading the diver's position (Epp and Lewis 1979, 1980, Downing and Rigler 1984).

It has been shown that the measurements for the first few hours need to be rejected because oxygen consumption was high due to increasing activity of experimental animals (Teuber et al. 2013). It is possible in an open-flow system (Franke 1977) but impossible in a closed-bottle method (Downing and Rigler 1984), i.e. Winkler titration and Cartesian diver

methods described above. Although dissolved oxygen concentration can be continuously monitored using an oxygen electrode even in a closed-bottle system, since the electrode consumes oxygen by itself, thus it needs further corrections for using it as a measuring instrument (Harris et al. 2000).

Recently, a contactless optical spot-fiber oxygen sensor was developed to measure dissolved oxygen concentration in water. It is relatively precise (1 $\mu LO_2\ L^{-1}$) and easier to measure, and less number of experimental animals required than those for any of the traditional methods. It is being popular to measuring oxygen consumption of copepod using this new device but only in large species (Bode et al. 2013, Teuber et al. 2013, Kiko et al. 2015). Considering all of these merits to measure oxygen concentrations, we designed an optical oxygen sensor system to determine the oxygen consumption rates in our target median size copepod (<17 μg dry weight) in more precise and less procedures than those in the previous methods.

The calanoid copepod *Eodiaptomus japonicus* was focused on in this study. It is an endemic species in Japan, and has been known that dominated in zooplankton community all year round in Lake Biwa (Kawabata 1987a). It has been shown to play a crucial role in transport of energy through the food chain, and important as food source for fish with high economic value in this lake (Kawabata et al. 2002). Previous studies showed that this copepod expressed extremely high population growth rate at high temperature under sufficient food supply (Liu et al. 2014), indicating temperature-mediated metabolic responses. Severely food effect was also shown at higher temperatures of more than 15 ºC (Liu et al. 2015), and might be attributed to temperature-mediated metabolic cost in the copepods (Lampert 1977b, Gillooly et al. 2001, Alcaraz et al. 2013).

The aim of this study is to determine temperature function of respiration rate in *E. japonicus* acclimatized at two different temperature conditions using an optical spot-fiber

oxygen sensor system. Net growth efficiency (K_2) was usually calculated from proportion of carbon budget, i.e. ratio of carbon weight of net production (somatic growth and/or reproduction) to assimilated carbon weight (net production and metabolic loss by respiration) in zooplankton (Kiørboe et al. 1985, Ikeda et al. 2001, Lee et al. 2001). Both of global metabolic and growth models were judged by K_2 and provide a basis for assessing energy flux and associated material cycling by zooplankton assemblages in aquatic ecosystem (Hirst and Lampitt 1998, Ikeda et al. 2001). We therefore calculated K_2 in our target copepod for evaluating metabolic costs under different environmental conditions.

2. Methods

2.1 Field collection and stock cultures

Females with egg sac in *Eodiaptomus japonicus* were sorted from zooplankton samples collected with vertical plankton net hauls (mouth diameter, 45 cm; mesh size, 200 μm) from 30 m to the surface at a sampling site situated in north basin of Lake Biwa (35º18'8.6"N, 136º09'8.8"E) from 25 July 2014 to 16 September 2015. The copepods were then cultivated in 1-L jars filled with tap water filtered with a glass-fiber filter (Whatman, GF/F), autoclaved and well-oxygenated (FTW) as stock cultures.

In order to determine difference of metabolic plasticity in *E. japonicus* between two different acclimatizations, two stock cultures maintained at 15 (T15) and 25 ºC (T25) until at least two generations prior to the experiments, under same photoperiod of 12L:12D with light intensity of 15.4 μmol m^{-2} s^{-1}, and food concentration at ca. 10^5 cells mL^{-1} of 1:1 (cell:cell) fresh algal mixture of *Chlamydomonas reinhardtii* (IAM C-9) and *Cryptomonas tetrapyrenoidosa* (NIES 282). FTW was changed weekly, and fresh food suspensions were provided every 2 days. Algal cultures were grown in 1-L flasks at 20 ºC under a photoperiod of 12L:12D with a light intensity of 125 μmol m^{-2} s^{-1} in each algal medium of C and VT for

C. reinhardtii and *C. tetrapyrenoidosa*, respectively (Provasoli and Pintner 1959, Ichimura 1971, Starr 1973).

2.2 Experimental procedure

Metabolic rate was expressed as respiration rate in oxygen consumption over time. Oxygen consumption rates of adult males and females sorted from T15 and T25 was measured at 8, 10, 15, 20, 25, 28 and 30 ºC to determine the difference of metabolic responses on temperature between the different acclimatized individuals. The temperature range experienced by wild *E. japonicus* in Lake Biwa is 8−25 ºC (Liu et al. 2014). The extreme temperatures of 28 and 30 ºC were also carried out to evaluate a tolerance to extremely high temperatures. Adult male and female were separately measured to avoid energy lose due to moving and mating activities (Dur et al. 2011). Additionally, oxygen consumption rates in copepodid stages of C3, C4 and C5 from T15 were measured at 15 and 25 ºC to determine effect of body mass on its respiration rates. All of the experiments lasted at least 12 hours with 2−4 replicates under dark condition. More than 80% of initial concentrations of dissolved oxygen (DO) remained at the end of all experiments. Animals were not fed during entire period of the experiments.

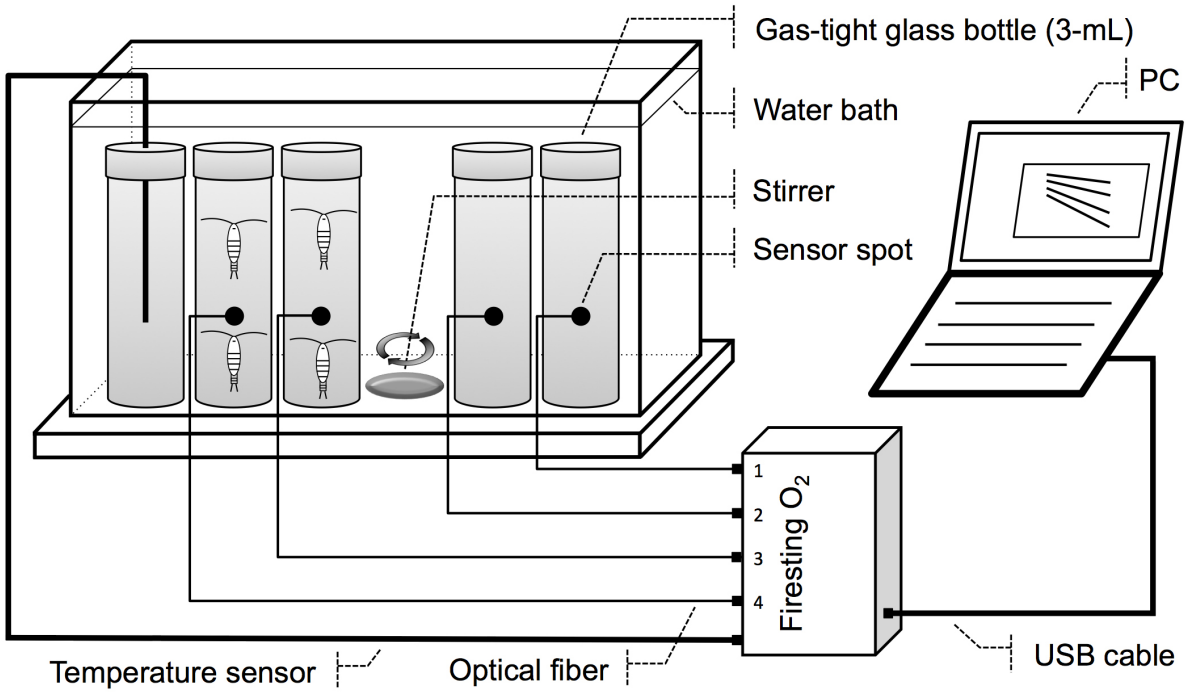

Fig. 3-1 Schematic diagram on a water-bath and closed-bottle unit for measuring oxygen consumption of copepods using a fiber-optic oxygen meter in an incubator.

Experimental setup is shown in Fig. 3-1. Five gas-tight glass bottles were situated in a water bath in an incubator (CN-25C, Mitsubishi, Tokyo, Japan) to keep constant and same temperature among these five bottles. All experimental bottles were cleaned with an ultra sonic cleaner (HZ-630, AS ONE, Osaka, Japan) without using any antibiotics. The DO concentration in each bottle was measured using a fiber-optic oxygen meter (Firesting O_2, PyroScience, Aachen, Germany), fitted with a spot-fiber oxygen sensor (SPFIB, PyroScience, Aachen, Germany), that allowed semi-continuous (every 1 minutes) measurements using four oxygen sensors (two for experimental bottles with animals and remaining two for control bottles without animals) with a submersible temperature sensor (TSUB21, PyroScience, Aachen, Germany) in the last one bottle. An oxygen sensor spot (OXSP5, PyroScience, Aachen, Germany) was glued to the inner wall of each experimental bottle to non-invasively and non-destructively measure the dissolved oxygen concentration by the oxygen sensors from the outside of the bottles. This new technology for measuring dissolved oxygen is based on red light irradiances detected with a spot-fiber oxygen sensor up to the detection limit of

dissolved oxygen, 1 μLO_2 L^{-1}, and the coefficients of variations are 0.02−0.2% (http://www.pyro-science.com).

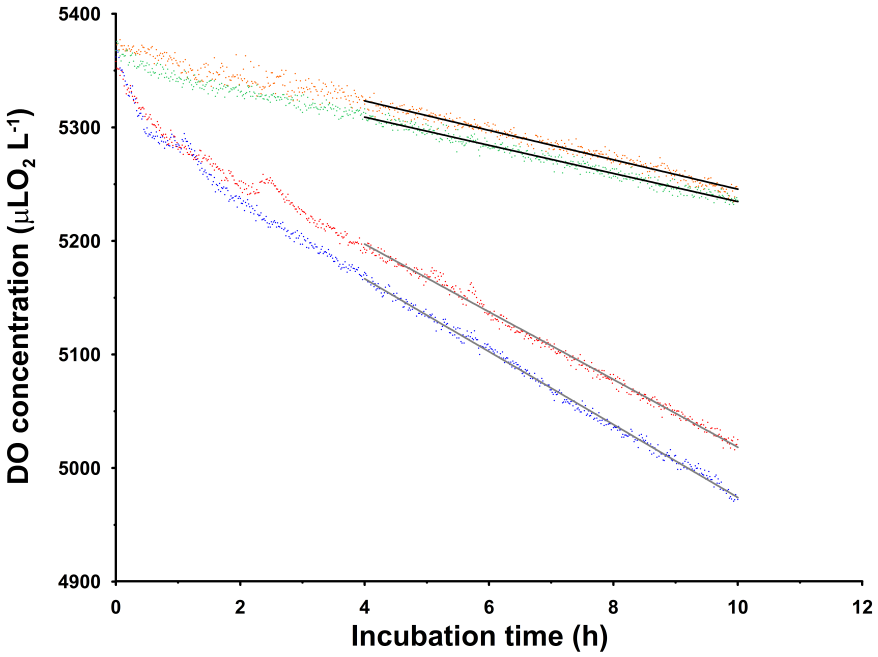

Fig. 3-2 Dissolved oxygen (DO) concentration (μLO_2 L^{-1}) decreased with incubation time (h) in each experimental and control bottle. Each dot (*blue* and *red* in two experimental bottles, *green* and *orange* in two controlled bottles) indicated the DO concentration detected by a fiber-optic oxygen meter every one minute. Fitted lines (*grey* in experimental bottles and *black* in controlled bottles) represented the linear regression of DO concentration against incubation time for six hours after four hours from the start of the incubation.

Experimental animals were sorted from each stock culture, transferred to a 20-mL vial filled with FTW and exposed with gradually changing temperatures from those in the stock cultures (15 or 25 ºC) to those in the experiment (8−30 ºC) during at least 12 hours in an incubator, in order to avoid temperature shock. Then, they were gently washed at three times with FTW at each experimental temperature, to remove remaining algae and bacteria from the stock culture. Finally, 2−4 individuals were placed into a gas-tight experimental glass bottle (3-mL) filled with FTW, and monitoring the DO concentrations was started. Despite the excellent precision of DO detection in the oxygen meter, unstable DO concentrations were monitored during a couple of hours from the start of the incubation, because of low near infrared-emission under high DO concentrations (>4.5 mLO_2 L^{-1}) due to its principle, so that the high variation of DO (±0.2%) was detected (Fig. 3-2). DO concentrations in the

experimental bottles also fluctuated during the first few hours of the incubation probably due to increasing activity of animals as pointed out by Teuber et al. (2013). Therefore, the data for the following analyses were taken after four hours from the start of the incubation. After that, DO concentrations linearly decreased with incubation time, always more rapidly in experimental bottles compared to those in controls (Fig. 3-2). The periods for estimating oxygen consumption rates with regression analysis were 3 to 12 hours depending on temperature and size of the animals tested. In each experiment, differences between slopes of the regression lines in experimental bottles and a slope in control ones were tested with analysis of covariance (ANCOVA). When the differences were not statistically significant, the results were discarded, not used for the following calculations. At the end of the experiments, the animals were preserved with 5% neutral sugar formalin, and then the prosome length was measured with an eyepiece micrometer under a dissecting microscope (SZX12, Olympus, Tokyo, Japan) at magnification of 900×.

2.3 Data transformation and statistical analysis

The weight-specific respiration rate (R, μLO_2 mg-dry-weight^{-1} h^{-1}) was estimated from the slope of linear regression line of oxygen concentration in both experimental and control bottles against incubation time using following equation:

$$R = (\Delta O_{exp} - \Delta O_c) \times V \times 1000 / N / W',$$

where ΔO_{exp} and ΔO_c are coefficient of oxygen consumption (μLO_2 L^{-1} h^{-1}) as each slope of the regression lines in the two experimental bottles and average slope in the two control bottles, respectively, with least square method, and V is volume of experimental bottle (L). The detection accuracy of a slope is 0.1 μLO_2 L^{-1} h^{-1}, therefore the accuracy of DO consumption was at least 10^{-4} μLO_2 ind^{-1} h^{-1} when four individuals were incubated in a 3-ml experimental bottle. N and W' are number of animals in an experimental bottle and average

body dry weight (μg), respectively. Body dry weight of each experimental animal (W, μg) was calculated from its prosome length (PL, mm) using a following exponential equation;

$W = \mathrm{e}^{(2.59 \ln PL + 2.6995)}$ (Kawabata and Urabe 1998).

Therefore, the accuracy of R can be expressed with at least 10^{-2} μLO_2 mg-dry-weight^{-1} h^{-1}.

Rs with temperature range between 8 and 28 ºC were fitted to an exponential temperature function with least square method after natural log-transformation of R. The magnitude of the acceleration of the metabolic rate is generally characterized by Q_{10} value, the ratio of rates resulting from a temperature increase of 10 ºC (Downing and Rigler 1984), calculated from the equation:

$$Q_{10} = (R_{T2} / R_{T1})^{10 / (T2 - T1)},$$

where R_{T1} and R_{T2} are the rates of the studied process at temperature $T1$ and $T2$ (in ºC), respectively.

In order to evaluate the physiological efficiency of *E. japonicus* at different environmental conditions, the net growth efficiency (K_2, %) for well-fed and food-limited individuals at 15 and 25 ºC was calculated using following equation;

$$K_2 = G \times 100 / (G + M),$$

where G is the somatic growth of copepodites, and calculated with

$$G = (W_{C6} - W_{C1}) \times 0.447,$$

where W_{C1} and W_{C6} is the body dry weight (μg) of first and sixth (adult) copepodid stages (C1 and C6), respectively. Body weights of the individuals were used at algal density of 10^3 (food-limited) and 5×10^4 (well-fed) cells mL^{-1} at 15 and 25 ºC in the previous study (Liu et al. 2015). M is the total respiration loss during copepodid development and calculated from the equation;

$$M = \sum_{n=i}^{\omega} R \times W_{ci} \times D_{ci} \times RQ \times 0.536 .$$

W_{ci} and D_{ci} are the body dry weight (μg) and the stage duration (day), respectively, in each

copepodid stage i (ω = 6) at each different condition as *G*. *RQ* is respiratory quotient of 0.97 for protein metabolism (Lee et al. 2001). The two factors of 0.447 and 0.536 were used for converting μg body dry weights and μL oxygen consumptions into carbon weights, respectively.

Differences of *R* among acclimatizing temperatures, experimental temperatures and genders in adult were tested with generalized linear model (GLM). Kruskal−Wallis test was employed to test the difference of *R*s in adults at 30 ºC between different acclimatizing treatments, between the two experimental temperature treatments of 28 and 30 ºC, and between the two experimental treatments of 15 and 25 ºC in copepodid stages. Regression analysis of *R* against *W* was made to test the difference between the slope and zero at 15 and 25 ºC. All statistical analyses were performed with SPSS (IBM Inc. 2011) and MATLAB (The MathWorks Inc. 2009) software.

3. Results

Body weights of copepodid and adult stages in *Eodiaptomus japonicus* (C3−C6) used for measuring oxygen consumption ranged from 3.079 to 16.186 μg (Table 3-1). On average, coefficients of oxygen consumption ranged from 25.6 to 204.8 $\mu LO_2\ L^{-1}\ h^{-1}$ in experiments, while from 5.1 to 101.2 $\mu LO_2\ L^{-1}\ h^{-1}$ in controls (Table 3-1). In all cases, the oxygen consumption rates were 1.5–12.8 fold greater in experimental bottles than those in control ones. Oxygen consumptions in control bottles ($\Delta O_c \times V$) increased with temperature probably due to contaminated bacteria (Fig. 3-3). It had 10-fold difference at each temperature, varied from 0.004−0.05 $\mu LO_2\ h^{-1}$ to 0.03−0.4 $\mu LO_2\ h^{-1}$ at 8−30 ºC.

Table 3-1 Average coefficients of oxygen consumption in experimental bottles including *Eodiaptomus japonicus* (Stage C3−C6) at seven experimental temperatures (Exp temp, ºC) for two acclimatization temperatures (Acclim. temp, ºC). Sex, M in male, F in Female; Rep *no.*, number of replicates, Ind *no.*, numbers of animal used in an experimental chamber; I_t, incubation time performed for linear regression analysis (h); W, body dry weight (μg ind^{-1}), ΔO_{exp} and ΔO_c, coefficient of oxygen consumption in experimental and control bottle, respectively (μLO$_2$ L^{-1} h^{-1}). Ranges represent in a parenthesis.

Acclim. temp (ºC)	Exp temp (ºC)	Stage	Sex	Rep *no.*	Ind *no.*	I_t (h)	W (μg ind^{-1}) mean (range)	ΔO_{exp} (μLO$_2$ L^{-1} h^{-1}) mean (range)	ΔO_c (μLO$_2$ L^{-1} h^{-1}) mean (range)
T15	8	C6	M	6	3−4	12	8.650 (7.194−11.318)	25.6 (9.8−38.5)	6.8 (3.7−10.8)
	8	C6	F	6	3	12	12.927 (10.264−15.739)	27.2 (15.6−33.5)	5.1 (1.8−9.4)
	10	C6	M	4	4	7	8.108 (7.194−8.955)	42.6 (36.1−60)	17 (10.1−26.3)
	10	C6	F	6	3	7	10.992 (9.596−12.056)	37.7 (33.6−43.6)	17.4 (15.2−20.5)
	15	C3	-	4	4	9	3.299 (3.079−3.415)	33.2 (29.7−35.2)	14 (11.2−19.3)
	15	C4	-	4	4	9	5.158 (3.960−6.401)	36.3 (29.9−46.4)	13.8 (11.8−19)
	15	C5	-	4	4	9	6.859 (6.149−9.272)	45.4 (37−54)	16.8 (9.1−26.8)
	15	C6	M	6	3−4	9	8.458 (7.194−10.608)	44 (26.9−63.4)	9.1 (2.5−13.9)
	15	C6	F	4	3	9	11.011 (10.094−12.056)	54.8 (48.6−63.3)	11.8 (9.5−13.9)
	20	C6	M	4	3	5	7.806 (7.471−8.342)	63.3 (50.6−71)	18.9 (16.7−20.5)
	20	C6	F	6	3	5	11.595 (10.264−12.822)	91.2 (46.9−155.3)	31.9 (14.8−38.6)
	25	C3	-	4	4	7	3.640 (3.244−4.765)	80 (64.8−90.3)	35.7 (29.9−40.4)
	25	C4	-	4	4	7	5.324 (3.773−6.923)	64.9 (50.3−74.3)	20.5 (11.1−27.5)
	25	C5	-	4	4	7	6.818 (3.244−9.926)	90.5 (85.2−96.8)	30.7 (22.9−43.5)
	25	C6	M	4	3	7	8.007 (7.471−8.955)	85.8 (69.5−110.4)	21.9 (17.1−27.7)
	25	C6	F	4	3	7	11.032 (8.645−12.822)	108.8 (74−148.6)	26.4 (21−30.7)
	28	C6	M	6	3	4	7.729 (7.194−8.342)	130.2 (79.4−196.7)	39.2 (16.1−84.8)
	28	C6	F	6	3	4	11.742 (10.608−14.028)	173.6 (139.1−211.7)	49 (38.9−58.5)
	30	C6	M	4	4	6	10.486 (9.596−11.683)	138.4 (125−154.5)	53.1 (47.9−59.6)
	30	C6	F	4	3	6	14.843 (13.619−16.186)	131.6 (128.7−135.6)	49 (34.3−58.7)
T25	8	C6	M	6	4	8	6.906 (5.903−7.755)	29.0 (22.1−32.7)	10.6 (8.6−13.0)
	8	C6	F	6	3	8	10.437 (8.800−11.683)	34.8 (22.5−46.2)	10.5 (8.9−11.9)
	10	C6	M	8	3−4	10	7.798 (6.149−10.608)	26.7 (17.7−33.2)	8.6 (3.1−16.8)
	10	C6	F	4	3	10	9.777 (8.800−10.959)	40.6 (31.9−57.5)	14.9 (13.2−17.9)
	15	C6	M	4	4	6	7.224 (6.149−8.342)	43.4 (38.9−53.4)	16.3 (13.4−21.9)
	15	C6	F	4	2−3	6	11.167 (9.272−12.435)	65.1 (39.7−86.1)	18.6 (16.3−22.1)
	20	C6	M	4	3−4	7	7.176 (5.903−8.955)	52.9 (35.0−65.9)	20.6 (11.9−30.6)
	20	C6	F	6	3	7	11.187 (9.272−14.444)	87.6 (67.3−116.9)	32.4 (14.5−50.6)
	25	C6	M	4	4	3	7.177 (6.149−7.755)	173.6 (102.4−240.0)	74.7 (38.2−127.4)
	25	C6	F	4	3	3	11.222 (9.272−14.444)	179.7 (121.3−248.2)	67.5 (52.2−86.9)
	28	C6	M	4	2−4	3	7.998 (6.149−9.926)	170.2 (129.3−207.3)	70.2 (55.2−87.8)
	28	C6	F	4	2−3	3	11.560 (10.264−12.822)	204.8 (182.2−225.0)	101.2 (72.3−132.3)
	30	C6	M	6	3−4	7	8.694 (8.045−9.596)	184.4 (99.1−249.6)	94.4 (33.5−134.3)
	30	C6	F	6	2−3	7	12.537 (11.683−13.619)	133.6 (89.7−179.7)	56.6 (33.4−104.2)

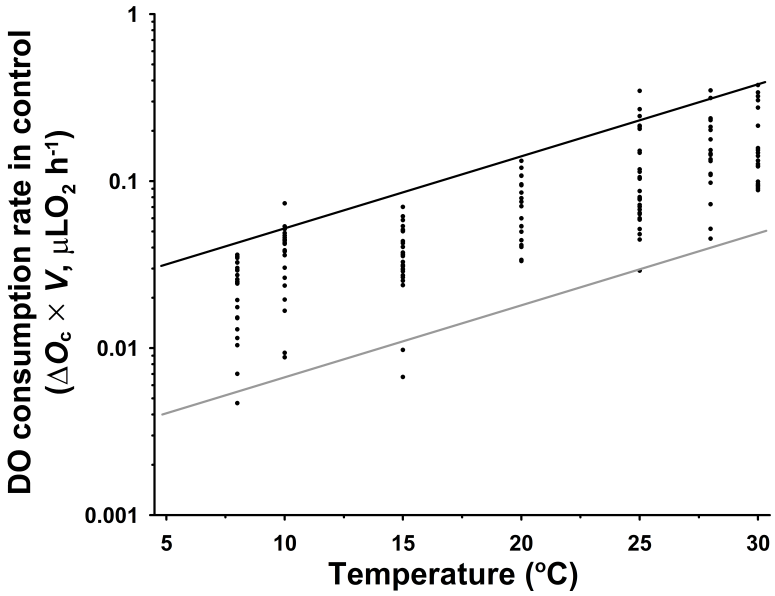

Fig. 3-3 Oxygen consumption rates in control ($\Delta O_c \times V$, *solid symbols*, μLO_2 h^{-1}) at each experimental temperature, (see details in Methods). Two regression lines were drawn using maximum (*black line*) and minimum values (*grey line*) at each temperature.

Weight-specific respiration rates (R, μLO_2 mg-dry-weight^{-1} h^{-1}) of adult *E. japonicus* exponentially increased with increasing temperature from 8 to 28 ºC for both males and females acclimatized at both 15 and 25 ºC (Fig. 3-4). Average R varied from 1.64 to 10.78 and 1.55 to 9.77 μLO_2 mg-dry-weight^{-1} h^{-1} for males and females, respectively, acclimatized at 15 ºC, while 1.71 to 11.13 and 1.98 to 10.10 μLO_2 mg-dry-weight^{-1} h^{-1} for those acclimatized at 25 ºC. Rs at 30 ºC deviated from the exponential phase, being significantly lower than those at 28 ºC for the animals acclimatized at both 15 and 25 ºC (Kruskal−Wallis test, df = 1, H = 8 and 7.35 at 15 and 25 ºC, respectively, $P < 0.05$). Rs at 30 ºC for the animals acclimatized at 15 ºC were slightly lower than those for the animals acclimatized at 25 ºC, though the difference was not statistically significant (Kruskal−Wallis test, df = 1, H = 2.91, P = 0.088). Generalized linear model (GLM) showed that Rs were significantly influenced by experimental temperature, but not by acclimatization and gender without any interactions (Table 3-2).

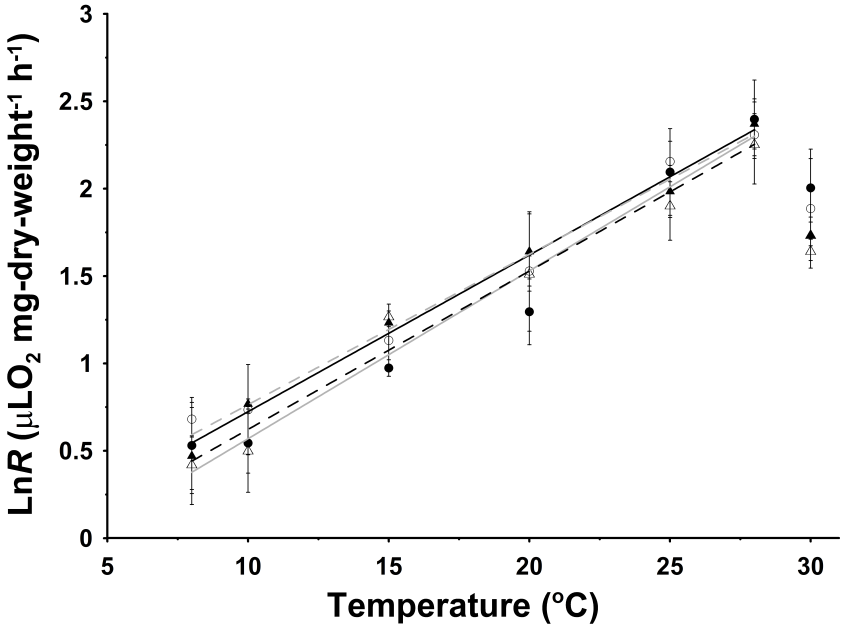

Fig. 3-4 Ln-transformed weight-specific respiration rates (R, μLO$_2$ mg-dry-weight^{-1} h^{-1}) of *Eodiaptomus japonicus* acclimatized at 15 ºC (T15, *triangles*) and 25 ºC (T25, *circles*) at seven experimental temperatures (*solid symbols* in male and *open symbols* in female). The regression lines of R against temperature except for 30 ºC (*black* in T15 and *grey* in T25, *solid* in male and *dashed* in female). Error bars indicate standard deviation.

Table 3-2 Results of generalized linear models (GLM) for a three-way analysis: effects of acclimatization (Acclim.), temperature (Temp) and gender (Sex) on weight-specific respiration rates of *Eodiaptomus japonicus*.

Factor	*df*	x^2	*P* value
Acclim.	1	0.059	0.808
Temp	5	542.401	<0.001
Sex	1	0.194	0.660
Acclim. × Temp	5	3.447	0.631
Acclim. × Sex	1	3.146	0.076
Temp × Sex	5	3.265	0.659
Acclim. × Temp × Sex	5	2.015	0.847

df, degrees of freedom

*R*s in copepodid and adult stages from C3 to C6 were always higher at 25 ºC than those at 15 ºC, while exhibited low correlation with W ($r^2 < 0.03$) (Fig. 3-5). Statistical analyses showed that the slopes were not significantly deviated from zero at both temperatures ($n = 30$ and 28, $t = 0.875$ and 0.446 at 15 and 25 ºC, respectively, $P > 0.05$), indicating that R was independent from W. *R*s were significantly different between these two temperatures (Kruskal−Wallis test, $df = 1$, $H = 21.77$, $P < 0.001$).

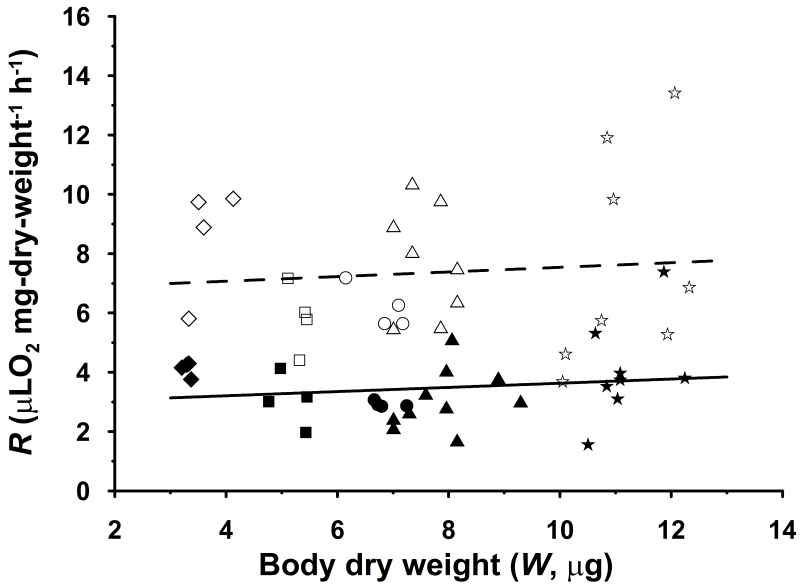

Fig. 3-5 Weight-specific respiration rates (R, μLO_2 mg-dry-weight^{-1} h^{-1}) of *Eodiaptomus japonicus* in copepodid stages C3 (*diamonds*), C4 (*squares*), C5 (*circles*), C6 male (*triangles*) and C6 female (*stars*) at 15 (*solid symbols*) and 25 ºC (*open symbols*) against the body dry weight (W, μg). Fitted lines represent linear regression of R against W at 15 (*solid line*, $r^2 = 0.027$) and 25 ºC (*dashed line*, $r^2 = 0.005$).

Since there were no significant differences of R between the acclimatizations and independence from body weight, all data were pooled, and the relationship between R and temperature (T, ºC) could be expressed as an exponential function at the temperature range of 8−28 ºC;

$$R = 0.8072\ e^{0.0897T}\ (n = 144,\ r^2 = 0.995).$$

The value of Q_{10} calculated was 2.3 when temperature increasing from 15 to 25 ºC.

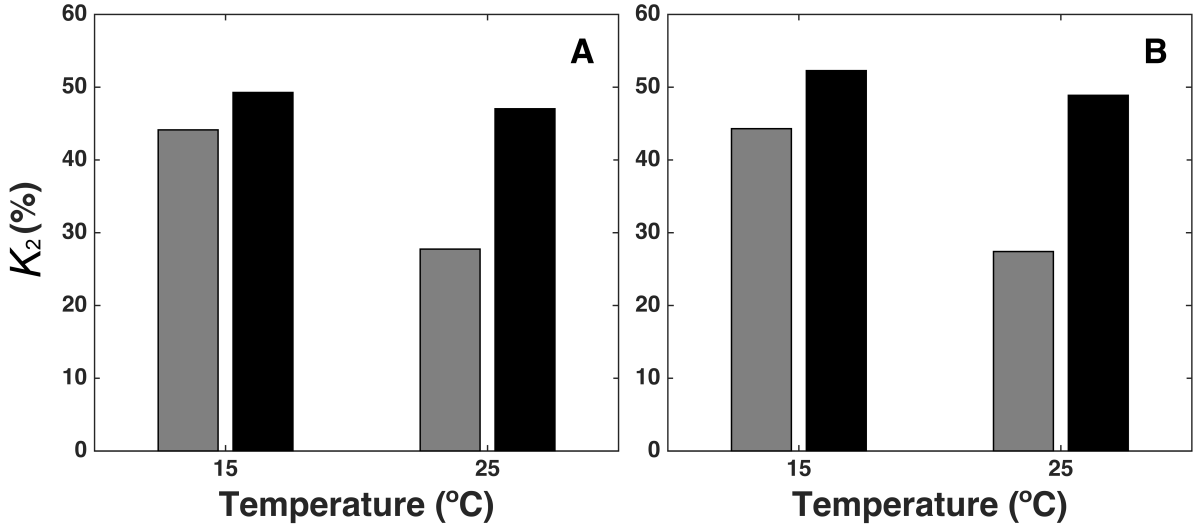

Fig. 3-6 Estimated net growth efficiencies (K_2, %) for food-limited (*grey bars*) and well-fed (*black bars*) *Eodiaptomus japonicus* males (A) and females (B) at 15 and 25 ºC.

Finally, we estimated net growth efficiency (K_2) from carbon accumulation and metabolic loss in *E. japonicus*. K_2 in well-fed individuals were 47−49% in males and 49−52% in females at both 15 and 25 ºC, while K_2 in food-limited ones for both males and females were 44% at 15 ºC but decreased to 27−28% at 25 ºC (Fig. 3-6).

4. Discussion

Small-volume of experimental chamber have to need for measuring respiration rates in micrometer-sized animals such as micro- to mesozooplankton so that the oxygen reduction can be determined with good precision (Downing and Rigler 1984). In the traditional methods, however, relatively large chambers (60−250 mL) were often used because large number of animals needed in an experiment (Ikeda 1971, Nakamura and Turner 1997, Castellani et al. 2005). Even in a new method using an optical contactless oxygen sensor, 10−60 mL chambers were used due to larger size of the copepods tested (>33 μg dry-weight) (Bode et al. 2013, Teuber et al. 2013, Kiko et al. 2015). In this study, we used a 3-mL gas-tight glass bottle for the measurements and successfully detected the oxygen consumption of our small copepod *Eodiaptomus japonicus*, and the precision was ten-fold higher (10^{-4} μLO_2 ind^{-1} h^{-1}) than those obtained with the modified-Winkler technique using similar size experimental bottles, as described in the introduction section (Urabe and Watanabe 1990). The volume of 3-mL was suitable and convenient size for handling and checking small to median sized animals under a dissecting microscope, and provided enough space for the animals to swim freely in a bottle.

On the other hand, a large number of individuals are required in order that the respiration rates of zooplankton are as precisely measured as possible with traditional procedures. For example, since respiration rate in a small marine copepod *Oithona similis* was 0.01 μLO_2 ind^{-1} h^{-1} at 19 ºC (Nakamura and Turner 1997), more than hundred individuals

needed for measurement. However, the density of experimental animals tends to bias the results. In marine zooplankton, the respiration rates have been shown to increase with increasing the densities of experimental animals in a container (Satomi and Pomeroy 1965). In *Daphnia magna*, crowding seemed to enhance respiration, though such kind of crowding effect was not found in marine calanoid *Calanus finmarchicus* (Zeiss 1963). Therefore, it is better to conduct the respiration experiments with as small number of animals tested as possible in an experiment. A single individual measurement has been conducted just in large marine copepods whose body dry weights were >360 μg ind^{-1}, e.g. *Gaetanus pileatus*, *Eucalanus hyalinus*, *Rhincalanus nasutus* (Teuber et al. 2013). Few studies for single individual measurements have been made in median or small sized copepods (<5 μg dry weight) with the methods other than the micro-Winkler and Cartesian diver methods.

In our method, the respiration rates were calculated from the differences between the coefficients of oxygen consumption in the experimental bottles and the control ones (ΔO_{exp} and ΔO_c) (see Methods), so that the detection limits for respiration rates in an experimental animal might be related to those in the control. The oxygen consumption rates in control (i.e. $\Delta O_c \times V$) due to microbes (i.e. bacteria) increased with increasing temperature (see Fig. 3-3). Altough the large variations of ΔO_c might indicate contamination of microbes into the incubation bottles at different levels in each of the experiment in spite of careful procedure, the values of ΔO_c were enough small to detect the difference from those in the experimental bottles with animals in this study. Since ΔO_{exp} needs to enough exceed ΔO_c for calculating respiration rates of the animals, conservative detection limits for calculating the respiration rates should be more than the largest values of oxygen consumption rates in control ($\Delta O_c \times V$), >0.03−0.4 μLO_2 h^{-1}. According to the previous studies, oxygen consumption rates in small to median sized copepods (>3 μg dry weight) were mostly ranged 0.01−1.6 μLO_2 ind^{-1} h^{-1} depending on temperature (5−30 ºC) (Raymont and Gauld 1951, Gauld and Raymont

1953, Conover 1960, Ikeda 1971, Newrkla 1978, Isla and Perissinotto 2004, Bode et al. 2013, Teuber et al. 2013). This implies that a couple of individuals in an experimental chamber should be needed in our method proposed in this study. To measure it from a single individual, careful procedure, e.g. preperaing FTW, handling the animals during the whole procedure, and using antibiotics if necessary. In our preliminary experiments, respiration rates of adult female in *Arctodiaptomus dorsalis* (ca. 10 μg dry weight) could be successfully measured in a single individual with more careful procedure (author's unpublished data). Further studies for measuring oxygen consumption rates from a single individual might be possible in clarifying the individual variability of metabolic rates in micro- and mesozooplankton.

It has been shown that the respiration rates varied considerably with incubation period (Kamler 1969). Starvation due to long-term incubation could induce variability of respiration rates in copepods (Comita 1968, Conover and Corner 1968, Ikeda 1971, Thor 2003). For example, some species reduced their respiration rates under starvation, e.g. *Diaptomus siciloides*, *Diaptomus leptopus*, *Mixdiaptomus laciniatus*, *Temora stylifera* (Comita 1968, Abou Debs 1984), whereas those in *Calanus cristatus* and *Acartia tonsa* increased 2−3 days after the prolonged starvation (Ikeda 1971, Thor 2003). Short incubation period could be therefore beneficial for precise measurements in active zooplankton that are less tolerant to prolonged starvation during the incubation (Harris et al. 2000). A large number of individuals tested and long-term incubation for measuring respiration rates with the traditional methods induces mortality in experimental animals during the incubation, e.g. 2 % of individuals dead during measurements using 600 individuals in some marine copepods (Raymont and Gauld 1951). Bacteria associated with copepod carcass have been shown to increase within just 15 minutes after the copepod death (Elliott et al. 2010). If experimental animals would die during the incubation, the respiration rates might be over estimated due to enhancement of

bacterial production (Del Giorgio et al. 1997).

Apparent seasonal variation of temperature responses in respiration rates has been shown in marine copepods *Centropages* spp., suggesting effects of acclimatization to the seasonal thermal conditions (Gaudy and Thibault-Botha, 2007). The *E. japonicus* population was mostly distributed in the epilimnion of Lake Biwa (Kawabata 1987a), where the temperatures seasonally varied between 8 and 25 ºC (Liu et al. 2014). We found that *E. japonicus* acclimatized at two constant temperatures, i.e. spring (15 ºC) and summer temperatures (25 ºC) experienced by the copepod in the lake, during two generations in the laboratory showed almost the same temperature responses on respiration rates within *in situ* temperature range. This suggests that both spring and summer populations in the lake show the same responses to temperature changes. Bradley (1978b) has shown that copepods could not need to change genetically throughout the year if the range of individual tolerance would be sufficiently wider than the seasonal variation. Although two generations might be short for acclimatizing the copepods in a certain temperature, it is enough long for the wild population to be exposed to wider than 10 ºC of temperature changes because of just four generations during the growing season in Lake Biwa (Kawabata 1989a). Additionally, this implies that temperature function of respiration rate obtained in this study can be applicable to the wild population in any seasons.

Metabolic rates have been shown to be depressed when surrounding temperatures exceeded over the tolerance ranges, e.g. that is >20 ºC in *Daphnia pulex* (Lampert 1977a), >19 ºC in *Boeckella dilatata* (Green and Chapman 1977), ca. 10 ºC in *Calanus glacialis*, >11 ºC in *Metridia longa* (Hirche 1987), and 8 ºC in *Cyclops bicuspidatus* (Laybourn-Parry and Strachan 1980). Bradley (Bradley 1978b) showed that acclimatization at higher temperatures than usual in *Eurytemora affinis* could lead to greater tolerance at high temperatures. Harpacticoid copepod *Tigriopus japonicus* could also tolerate higher

temperatures than usual once it would be acclimated to the high temperatures (Damgaard and Davenport 1994). In *Daphnia magna*, one-generation acclimatization at higher limit temperature of tolerance prolonged the period until immobilization due to heat shock (Yampolsky et al. 2013). In the present study, the weight-specific respiration rates (R) in the both temperature acclimatized animals declined over 28 ºC, but the depression of R at 30 ºC seemed to be more relaxed for the copepods acclimatized at 25 ºC though not significantly different from those of the copepods acclimatized at 15 ºC.

Q_{10} in a given species has been shown to vary with its habitat temperature to which it is adapted (Rao and Bullock 1954), being 2−3 for some marine copepods (Lee et al. 2001, Isla and Perissinotto 2004, Castellani et al. 2005). Q_{10} of *E. japonicus* from Lake Biwa was 2.3, being similar values as those from other temperate species. For example, it is ca. 2.5 from copepods inhabited northwest coast in Morocco (33ºN, 10ºW) (Nival et al. 1974), 2.2−3.4 from estuarine species of *Pseudodiaptomus hessei* in Mpenjati Estuary, South Africa (38º58'E, 30º17'S) (Isla and Perissinotto 2004), and 2−3.8 from freshwater species of *Diaptomus* spp. in Fargo, USA (47ºN, 97ºW) (Comita 1968). On the other hand, Q_{10} of 1.4−2 for three tropical species collected from eastern tropical Atlantic (10ºN, 20ºW) (Kiko et al. 2015), and the median value of Q_{10} from 11 tropical species in Mindelo Bay (17ºN, 25ºW) was 1.8, being smaller than those for temperate species (Teuber et al. 2013). These imply that Q_{10} values of >2.0 was obtained from temperate species but not from tropical one. This may be related to large seasonal fluctuation of water temperatures in temperate regions. High Q_{10} values in copepods might indicate high sensitivity on temperature (Mauchline 1998). Therefore, copepods having higher Q_{10} might adapt wide range of temperature fluctuation in higher latitudes, while copepods having lower Q_{10} might adapt narrow range in lower latitudes.

In this study, no relationship between R and body weight among copepodid and adult

stages (C3−C6) in *E. japonicus* was found. Generally, in copepods, a negative correlation between *R* and body dry weights having two to three orders differences between naupliar and adult stages (Comita 1968, Champalbert and Gaudy 1972, Fernández 1978, Vidal 1980). The differences of body dry weight between C3 and adult in *E. japonicus* was just 3.4-fold (Liu et al. 2014). This narrow range of the difference in body weights of the animals used in this study may lead independency from the body weight on *R*.

Temperature is the most influential factor on determining net growth efficiency (K_2) in copepods due to higher metabolic costs or carbon losses for respiration at higher temperatures (Ikeda et al. 2001, Lee et al. 2001). Besides temperature, food quantity is second influential factor for K_2 in zooplankton (Lampert 1977b), because metabolic costs, i.e. respiration rates, are also associated to food quantity (Kiørboe et al. 1985). Abou Debs (1984) showed that metabolic costs of *Temora stylifera* significantly increased with food concentration. On the contrary, those in *Oithona nana* (Lampitt and Gamble 1982), *Calanus pacificus* (Vidal, 1980) and *Diaptomus oregonensis* (Richman 1964, Comita 1968) did not appear to be directly depended on the food concentration. In two cladocerans *Bosmina longirostris* and *Daphnia galeata*, the respiration losses increased with increasing food concentrations (Urabe and Watanabe 1990). These response curves in the two cladocerans were slightly different, and those in the smaller *B. longirostris* were less conspicuous. Although the food effects on metabolic costs seem to give some controversial results among species, the increases of respiration rates with increasing food concentration are considered to be associated with energetic cost required for biochemical processes with feeding known as specific dynamic action (SDA) (Kiørboe et al. 1985, Urabe and Watanabe 1990). Kiørboe et al. (1985) also showed that respiration rates in *A. tonsa* were relatively constant during first 8 hours of starvation but rapidly declined after that, and suggested that SDA is largely associated with biosynthesis rather than feeding cost. In this study, experimental animals were exposed no

food condition for 7−16 hours, but this short-term starvation condition had never influenced respiration in the experimental animals throughout the monitoring the DO concentrations in the experimental bottles (see Fig. 3-2). K_2 in the food-limited individuals calculated in this study therefore would be conservative, if it could be lowered at the food-limited condition.

CHAPTER 4:

Long-term trends in biomass and production of *Eodiaptomus japonicus* (Copepoda: Calanoida) in Lake Biwa, related to eutrophication and global warming

This section is mainly based on the manuscript:

- "Long-term trends in biomass and production of *Eodiaptomus japonicus* (Copepoda: Calanoida) in Lake Biwa, related to eutrophication and global warming" by Xin Liu, Gaël Dur, Shinsuke Oomae, Takashi Morita, Yoichiro Sakai and Syuhei Ban *in preparing the publication*.

1. Introduction

Freshwater lakes have been considered as a sentinel for anthropogenic influences, such as eutrophication and global warming (Williamson et al. 2009). Eutrophication dramatically influenced phytoplankton biomass and community structure in freshwater lakes (Anneville and Pelletier 2000, Anneville et al. 2002, Dokulil and Teubner 2005), and often extended up to higher trophic levels, through the food web dynamics (Molinero et al. 2006, Anneville et al. 2007, Hsieh et al. 2011). On the other hand, IPCC (2014) reported that water temperatures in freshwater lakes increased due to global warming during the last 5 decades. It has been shown to influence phytoplankton biomass (Winder et al. 2009, Hsieh et al. 2010) and zooplankton communities in lake ecosystems (Hampton et al. 2008, Hsieh et al. 2011). For example, global warming resulted in the changing vertical distribution of phytoplankton in Lake Superior through the varying physical structure of water column (Fahnenstiel and Glime 1983), and the warmer winters and spring recorded since 1988 led to an earlier clear-water phase due to zooplankton grazing in Lake Geneva (Anneville et al. 2002). In Lake Baikal, cladocerans increased with increasing temperature regardless of algal biomass (Hampton et al. 2008). In a temperate Lake Neusiedler See, both phyto- and zooplankton biomasses showed significantly increasing trend during last 39 years due to shortened ice cover period (Dokulil and Herzig 2009).

Lake Biwa (35.1°N, 136.1°E) is the largest and oldest lake in Japan. The lake waters are supplied as water resources for 14 million peoples living in the Kansai area (Yoshida et al. 2001b). It has been subjected to many human activities, including eutrophication from early 1960's to mid 1980's and global warming from 1980's (Kawabata 1987a, Yoshida et al. 2001b, Hsieh et al. 2010). Eutrophication in freshwater lakes had induced large summer phytoplankton blooms and influenced a negative impact on the traditional use of the lakes (Anneville and Pelletier 2000). In a lot of lakes in Europe, US and Japan, including Lakes

Geneva, Mjøsa, Constance, Washington and Biwa, a restoration plan for reduction of phosphorus input from a point source effluent into the lakes has been done after 1980's through an act for environmental loading reduction, resulting in re-oligotrophication proceeded during the last 4 decades (Edmondson 1970, Einsle 1983, Løvik and Kjellberg 2003, Tsugeki et al. 2003, Anneville et al. 2007). Those of the changes in physical and chemical conditions due to eutrophication and global warming might induce the reorganization of plankton communities in lake ecosystems (Hsieh et al. 2010, Hsieh et al. 2011).

Copepods are the dominant planktonic crustaceans and play a major link between primary production and higher trophic levels in aquatic ecosystems (Mauchline 1998). They are usually used as an indicator for responses to the long-term environmental changes because they are sensitive to environmental fluctuation (Anneville et al. 2007, Peterson 2009). The calanoid copepod *Eodiaptomus japonicus* is dominant zooplankton for at least over 50 years in spite of environmental variability in Lake Biwa (Hsieh et al. 2011). Because of its high abundance and importance as a food resource for commercial fish (Kawabata et al. 2002), this copepod plays a key role in the food web of this lake. A lot of studies had been focused on this key species in order to understand its biology and ecology in the lake (Okamoto 1984a, Kawabata 1987a, Nagata and Okamoto 1988, Kawabata 1989a, 1993, Kawabata 1995, Yoshida et al. 2001a, Liu et al. 2014, Liu et al. 2015).

Previous studies have been shown that total zooplankton abundance exhibited a significantly positive correlation with phytoplankton biomass in several freshwater lakes (Dokulil and Herzig 2009, Hsieh et al. 2011), but some long-term studies suggested that phytoplankton abundance could not better explain variation of zooplankton community biomass in lake ecosystems (Hampton et al. 2008, Hsieh et al. 2011). No significant correlation between copepod density and phytoplankton biomass has been shown in North

Sea (Wiltshire et al. 2008). In general, copepods can not only feed on nano- to micro-sized particulate organic matters, including phytoplankton and detritus, but also attack micro-sized zooplankton, e.g. rotifers and ciliates, as potential foods (Poulet 1983). Abundances of the potential food resources with temperature fluctuation may induce complex effects on the dynamics and production in copepods.

It is necessary to, therefore, establish an efficient indicator to estimate *in situ* food conditions and population growth for the copepods. Seasonal variations in body size of a copepod in a water column depend on both temperature and food conditions, i.e. quantity and quality (Klein Breteler and Gonzalez 1988, Ban 1994, Lee et al. 2003, Jónasdóttir et al. 2005, Beyrend-Dur et al. 2011). Copepod body size decreases with increasing temperature under satiated food condition, and therefore the deviation from the body size predicted from the body size – temperature equation represents food effect at a given temperature. In other words, *in situ* food conditions can be evaluated from the differences between *in situ* and predicted body sizes.

In this study, we determined *in situ* food indices estimated from differences between body size of the copepods collected from the lake and those predicted from the equations obtained from laboratory experiments (see Chapter 1 and 2) and ambient temperatures. Then, we calculated *in situ* growth rates and production during the last 4 decades, and finally evaluated how the copepod responded to the eutrophication and global warming in the lake.

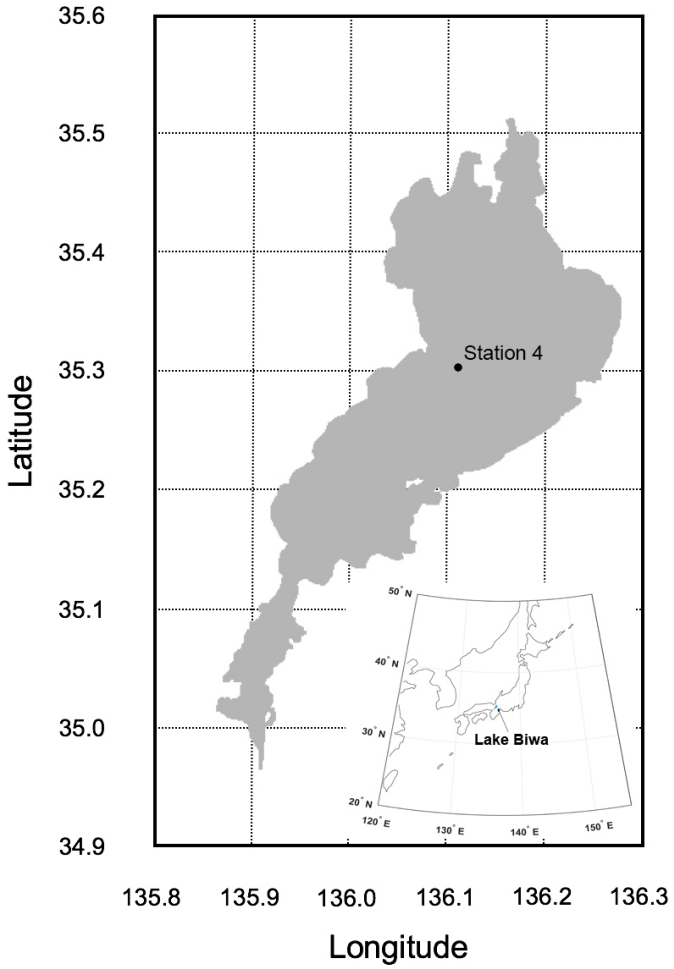

Fig. 4-1 The data analyses used in this study was the zooplankton sample collected from Station 4 of Shiga Prefecture Fisheries Experimental Station (SPFES) in Lake Biwa.

2. Methods

2.1 Field collection and long-term data sets in *in situ E. japonicas* population

Long-term data sets of body size distribution and biomass in *Eodiaptomus japonicus* collected from a station in north basin of Lake Biwa from January in 1971 to December in 2010 by the Shiga Prefectural Fisheries Experimental Station (SPFES) were used for the following analyses. Zooplankton was collected monthly with a discrete vertical haul using a Kitahara closing net (mouth diameter, 25 cm; mesh size, 95 μm) from four depth strata (0−10m, 10−20m, 20−40m and 40−75m) at St. 4 (35º18'34.2'' N, 136º07'19.1''E, 77m deep) located in north basin of Lake Biwa (Fig. 4-1). Adult males and females, copepodites and nauplii of *E. japonicus* were counted from 0−10 m and 10−20 m samples, because the most *E. japonicus* population was distributed in the epilimnion (above 20 m) of the lake (Kawabata 1987a). Body sizes of each category of the sexes and developmental stages were measured

with an eye-piece micrometer attached to a binocular microscope, and converted to dry weights with appropriate weight-length regression equations (see Chapter 1). Biomass (g dry weight m^{-2}) in a 20 m water column was calculated from the following equation;

Biomass = $\Sigma N_i W_i'$ (i = nauplii, copepodites, adult males and females),

where N and W' are number of individuals in a 20 m water column and average body dry weights of each development category i, respectively.

At each sampling occasion, a vertical temperature profile was recorded from the surface to the bottom using a thermistor thermometer or a Conductivity Temperature and Depth system (CTD). Those temperature data were provided from SPFES.

2.2 Parameter estimation and data analyses

Water temperatures recorded at each sampling occasion were different from those experienced by the copepods during their development in the field. According to previous field (Kawabata 1989a) and laboratory studies (see Chapter 1 and 2), the generation time of *E. japonicus* seasonally varied 1−3 months in a year. Then, we calculated the average water temperature experienced by the copepods collected at each sampling occasion during their development with an appropriate rule, i.e. when temperature in the date for collecting the copepods was <10 ºC, an average temperature in the last two and this months was used for following analysis, when it is 10 to 20 ºC, average in the last and this months was used, and when it is >20 ºC, just this month temperature was used (Table 4-1).

Table 4-1 An example for predicting rule of estimated water temperature (ºC) experience by *Eodiaptomus japonicus* during its development in Lake Biwa.

Year	Month	Average temperature at each month (ºC)	Months used for calculation	Estimated temperature experienced by copepods (ºC)
1970	Nov	14.6	-	-
1970	Dec	9.7	-	-
1971	Jan	7.6	Nov, Dec, Jan	10.6
1971	Feb	6.5	Dec, Jan, Feb	7.9
1971	Mar	6.2	Jan, Feb, Mar	6.7
1971	Apr	9.0	Feb, Mar, Apr	7.2
1971	May	13.4	Apr, May	11.2
1971	Jun	15.0	May, Jun	14.2
1971	Jul	18.4	Jun, Jul	16.7
1971	Aug	21.1	Aug	21.1
1971	Sep	19.6	Aug, Sep	19.6
1971	Oct	18.8	Sep, Oct	19.2
1971	Nov	14.6	Oct, Nov	16.7
1971	Dec	10.8	Nov, Dec	12.7

Most of the freshwater calanoid copepods have been known to be omnivores, i.e. they cannot only eat phytoplankton but also micro zooplankton, such as rotifers and ciliates (Poulet 1983). Therefore, chlorophyll *a* concentration or phytoplankton carbon stock, that is usually used as a food indicator for zooplankton (Lampert 1977a, Kiørboe et al. 1985, Koski and Kuosa 1999), is not necessarily good indicator for food conditions in *E. japonicus*. Potential growth in calanoids can be obtained from traditional growth experiments in the laboratory under satiated food condition (see Chapter 1). So, we can predict the food conditions from a ratio of a body size at a given food-limited condition to the maximum or potential body size at food satiated ones. A food index (*f*) was consequently calculated as PL_{obs} / PL_{max}, where PL_{obs} was the median value of prosome length (*PL*, mm) observed from field samples, and PL_{max} was the potential *PL* predicted from the following equation determined under food satiated condition (see Chapter 1) and ambient temperature (*T*, ºC) that the copepods exposed during the development,

$PL_{max} = 1.077T^{-0.0547}$.

Specific growth rate (k, day^{-1}) at those of different conditions (Liu et al. 2015) was also plotted against f at those of the same conditions, in order to clarify the relationship between k and f at different temperatures, and consequently the long-term fluctuation of k of this copepod in the lake. Then, the multiple linear regression analyses were employed for estimating parameter as the equation with multi-predictors as follows (Vadstein et al. 2004):

$$k = a_1 + a_2 f + a_3 T + a_4 f T,$$

where k is specific growth rate determined at 8 different temperature (T) and food (f) conditions in the previous study (Liu et al. 2015). The f values were calculated from the body size of adult females raised in each experimental condition to potential body size at a certain temperature under satiated food concentration. a_1, a_2, a_3 and a_4 were regression coefficients estimated using the *regress* function included in the statistics tool box of MATLAB software (The MathWorks Inc. 2009).

Then, monthly production (P_m, g m^{-2} d^{-1}) was calculated from

$$P_m = B \times k \times d_m,$$

where B was the *E. japonicus* biomass (g dry weight m^{-2}), k was the specific growth rate (day^{-1}), and d_m was the days of each month. Finally, annual production (P_a, g dry weight m^{-2} y^{-1}) was calculated from

$$P_a = \Sigma P_{mj} \quad (j = 1, 2, ..., 12),$$

where P_{mj} was the monthly production in month j.

2.3 Statistical analyses

Food index f and specific growth rate k against long-term year series were fitted with a non-linear smooth spline curve fitting analyses in the curve fitting tool box of MATLAB software (The MathWorks Inc. 2009). Regression analysis of annual average temperatures

against calendar year was carried out in order to test the difference between the slope and zero. The same analyses were also performed for k against f at different temperatures. All analyses were performed with IBM SPSS Statistics software (IBM Inc. 2011).

3. Results

Average water temperature throughout a 0−20 m water column in Lake Biwa showed seasonal fluctuation, varying between 5 and 25 ºC in each year during the last 4 decades (Fig. 4-2a). The slope of regression of annual average water temperature was significantly different from zero (n = 40, r^2 = 0.377, t for the slope = 4.855, P < 0.001), indicated a significant warming trend of this lake at 0.036 ºC year^{-1} during the last 4 decades in the lake (Fig. 4-2b).

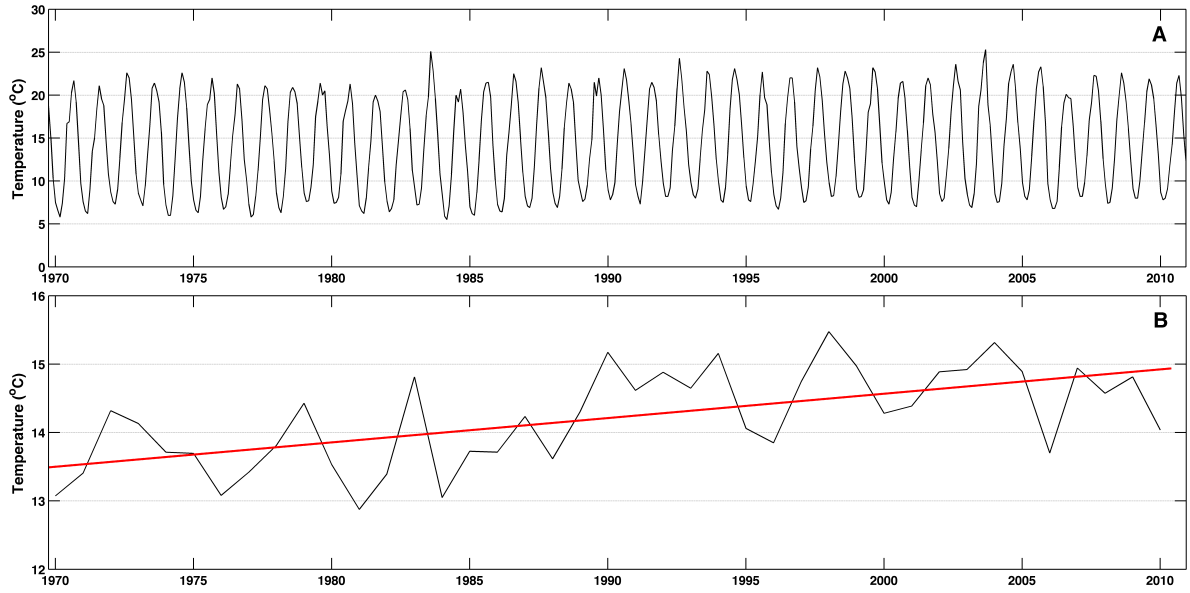

Fig. 4-2 Monthly variation of average water temperature (ºC) in epilimnion (0−20m) at St. 4 in Lake Biwa from 1971 to 2010 (data from SPFES) (A), and long-term trend in annual average temperature and a regression line indicated significant relationships (B) (*Temperature* = 0.0356*Year* − 56.61, r^2 = 0.377, n = 40, t = 4.855, P < 0.001).

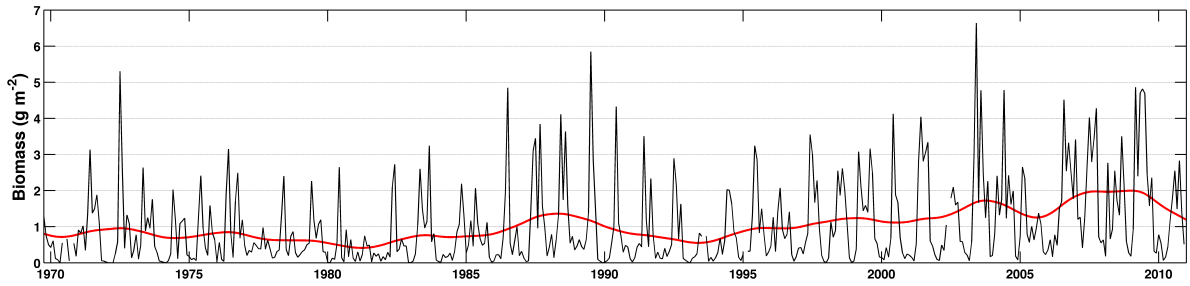

Fig. 4-3 Monthly variation of *Eodiaptomus japonicus* biomass (g m^{-2}) at St. 4 in Lake Biwa from 1971 to 2010. Regression curve showed the fluctuation trend in a ten years window.

Biomasses of *Eodiaptomus japonicus* varied between 0.01 and 6.97 g m^{-2} during the last 4 decades in Lake Biwa, being mostly less than 2 g m^{-2} before 1985. The biomasses increased from 1985 but declined during 1990's, and then showed increasing trend after 1995 (Fig. 4-3).

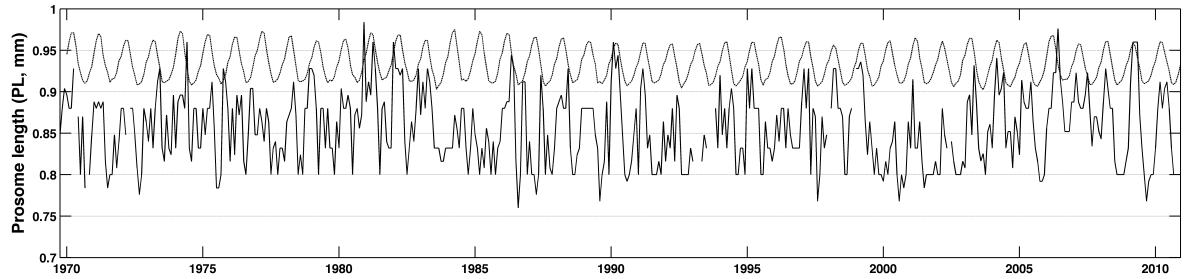

Fig. 4-4 Monthly variation in median prosome length (PL_{obs}, mm) of adult female in *Eodiaptomus japonicus* collected at St. 4 in Lake Biwa from 1971 to 2010 (*solid line*), and predicted maximum prosome length (PL_{max}, *broken line*) according to the equation, $PL_{max} = 1.077T^{-0.0547}$ (see Chapter 1) at ambient temperature (T, ºC).

The predicted potential body size (PL_{max}) of adult female showed clearly seasonal fluctuation due to temperature variation in a year (Fig. 4-4). PL_{max} showed a narrow variation range of 0.903−0.979 mm during the last 4 decades, whereas the *in situ* body size (PL_{obs}) varied in a wide range of 0.760−0.984 mm. PL_{obs} were mostly smaller than PL_{max} during the last 4 decades (Fig. 4-4).

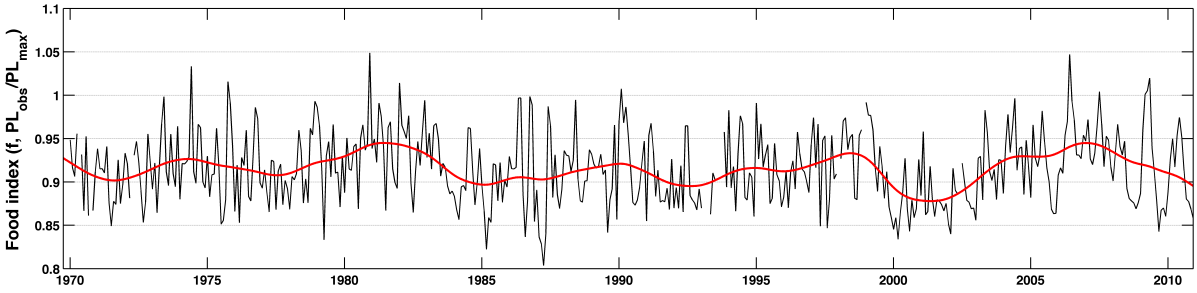

Fig. 4-5 Monthly variation of food index (*f*, PL_{obs}/PL_{max}) in *Eodiaptomus japonicus* at St. 4 in Lake Biwa from 1971 to 2010. Regression line showed the fluctuation trend in a ten years window.

Food index (*f*) of *E. japonicus* varied between 0.804 and 1.049 with large seasonal fluctuation during the last 4 decades (Fig. 4-5). Seasonal fluctuations, 0.825–1.009, were mostly larger than annual variations, 0.872–0.951. It seemed decadal fluctuations in *f* values were found, and the most prominent ones in early 1980's and the late 2000's with a suddenly depress in the early 2000's.

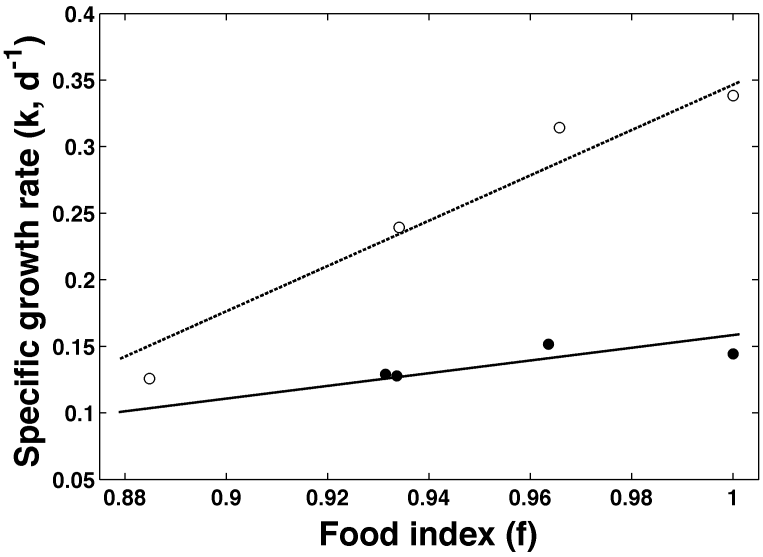

Fig. 4-6 Relationship between specific growth rate (*k*, day^{-1}) and food index (*f*) in *Eodiaptomus japonicus* at 15 (*solid circles*) and 25 ºC (*open circles*). Regression lines showed the significant linear relationship between *k* and *f* at the two temperatures (*solid line* in 15 ºC and *broken line* in 25 ºC).

The specific growth rates (*k*, day^{-1}) linearly increased with increasing *f* values at both 15 and 25 ºC (Fig. 4-6), and the regression equations were

$k = 0.4774\,f - 0.3189$ at 15 ºC ($n = 4$, $r^2 = 0.200$, $t = 9.320$, $P = 0.043$),

$k = 1.7028\,f - 1.3562$ at 25 ºC ($n = 4$, $r^2 = 0.949$, $t = 16.140$, $P = 0.020$).

Then, *k* can be expressed with the two parameters of *f* and *T*, assuming the slopes linearly increase with increasing temperature,

$$k = -1.3607\,f - 0.1037\,T + 0.1225\,f \times T + 1.237 \quad (n = 8,\ r^2 = 1,\ \mathrm{SSE} < 0.001) \qquad (4\text{-}1).$$

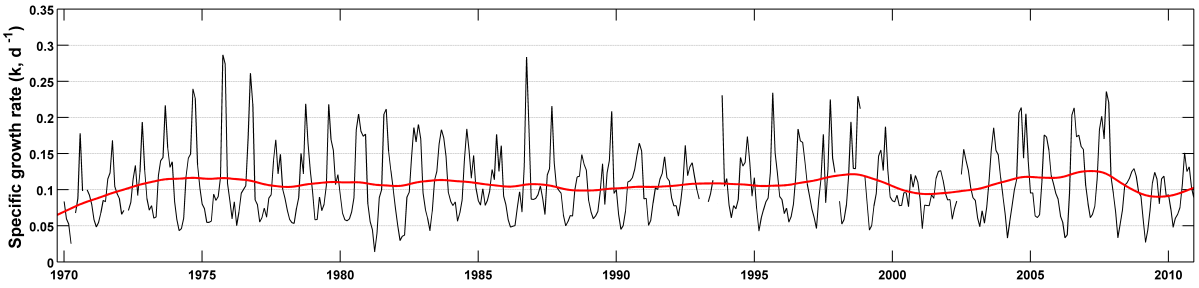

Fig. 4-7 Monthly variation of estimated specific growth rate (k, day^{-1}) in *Eodiaptomus japonicus* at St. 4 in Lake Biwa from 1971 to 2010. Regression line shows the fluctuation trend in a ten years window.

Then, the k values of *E. japonicus* population in the lake were estimated according to equation (4-1) (Fig. 4-7). The k values estimated varied between 0.011 and 0.273 day^{-1} with large seasonal fluctuations during the last 4 decades. The seasonal fluctuations, 0.039–0.204 day^{-1}, were larger than variation of annual mean values, 0.092–0.140 day^{-1}. The fitted curve showed that k increased during the early 1970's and being almost constant after the following years.

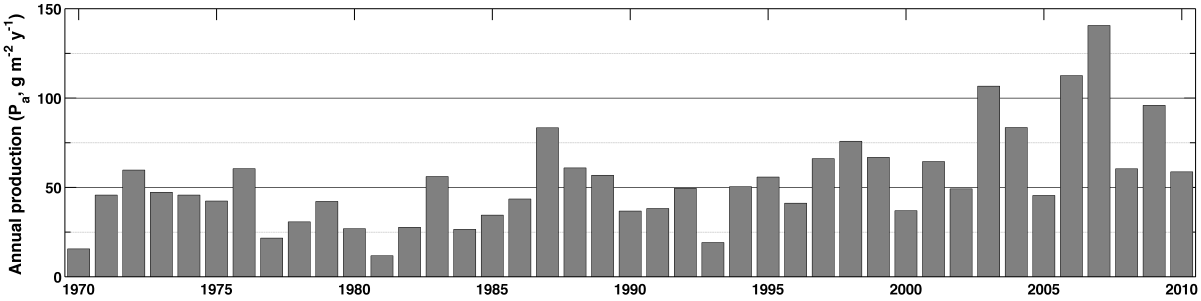

Fig. 4-8 Year-to-year variation of annual production (P_a, g m^{-2} y^{-1}) in *Eodiaptomus japonicus* at St. 4 in Lake Biwa from 1970 to 2010.

Finally, we calculated the annual production (P_a) of *E. japonicus* during the last 4 decades in Lake Biwa (Fig. 4-8). P_a varied from 11.7 to 140.6 g m^{-2} y^{-1}, and showed increasing trend after 2000. An average P_a during the last 4 decades was 54.4 g m^{-2} y^{-1}.

4. Discussion

During the last 4 decades, body sizes of *in situ Eodiaptomus japonicus* adult females

varied 29%, whereas the potential body sizes predicted from the ambient temperature assuming sufficient food supply just 8%. The difference of the body size variation between *in situ* and the potential ones supported the idea that food was the most important factor affected somatic growth of copepod (Ban 1994, Koski and Kuosa 1999, Liu et al. 2015). In Lake Biwa, *E. japonicus* could never reach to the potential growth in the most occasions during the last 4 decades, suggesting that the copepod population in the lake was always suffered with food shortage even in the eutrophication period in 1970's and 1980's (Hsieh et al. 2011). Anneville et al. (2007) showed that copepods were exposed to interspecies competition in Lake Geneva. In Lake Biwa, *E. japonicus* was less abundant in the zooplankton community even in the eutrophication period, whereas potential superior competitors, e.g. *Daphnia* spp. and predator, i.e. *Mesocyclops* sp., were relatively abundant at that time (Hsieh et al. 2011). Those of results suggested that the food shortage and low biomass in *E. japonicus* during the eutrophication period might be due to the complexity of competition and predator-prey interactions in zooplankton communities in the lake.

Zooplankton biomass was positively correlated with food supply and it also will reduce food resources due to their grazing (Holeck et al. 2008). Unstable *f* values in *E. japonicus* were found during the last 4 decades in Lake Biwa. The large depression of *f* in the early 2000's implied that the copepods might be suffered with extremely food shortage. On the contrary, relatively high *f* values were shown in the early 1980's and the late 2000's, indicating the high food supply for the copepods. The former period was identical to the end of the eutrophication period in this lake, when the chlorophyll *a* concentrations were the maximum level in the lake, ca. 8 μg L^{-1} (Hsieh et al. 2010). Whereas, the later period was in the reoligotrophication period, when the chlorophyll *a* concentrations were ca. 2 μg L^{-1} (Hsieh et al. 2010). These results coincided with the knowledge of omnivorous copepod feeding habit, suggesting that food supply for *E. japonicus* in the lake cannot necessarily

explained by just phytoplankton biomass.

Annual productions of *E. japonicus* were relatively stable until 2000, but gradually increased after that in Lake Biwa. Since year-to-year variation of specific growth rates was relatively stable, the trend in the production was related to that of the biomass. In North Sea, global warming seems to be related to high survival rates of copepods especially in Autumn (Wiltshire et al. 2008). More abundant copepodid larvae of *Arctodiaptomus spinosus* survived in winter led the higher winter biomass in a temperate lake (Dokulil and Herzig 2009). Hsieh et al. (2010) suggested that global climate change might enhance increasing lake water temperature in Lake Biwa through atmospheric forcing especially in winter. In the previous studies, the naupliar abundance of *E. japonicus* always increased in spring when the large well-fed and over-wintering females produced large number of offsprings in Lake Biwa (Kawabata 1987a, 1989a). Global warming might positively affect the population growth in spring due to the greater survivals of over-wintering population in the lake (Liu et al. 2014).

In Lake Biwa, *Mesocyclops* sp. often impacted upon its preys, i.e. *E. japonicus* nauplii, and can reduce their number (Kawabata 2006). Planktivorous fish is also potential predator for copepods, especially large females and ovigerous females (Jeppesen et al. 2003). Top-down control by such predators has been shown in a lot of water bodies from freshwater to marine ecosystems (McQueen et al. 1986, McQueen et al. 1989). *Plecoglossus altivelis* has been shown to be the most efficient predator on *E. japonicus* in Lake Biwa; occurrences of this copepod in its stomach contents represented 88% of total fishes tested, and 91% of them was adult stage (Kawabata et al. 2002). The predation pressure by *Mesocyclops* sp. and *P. altivelis* might depress the *E. japonicus* population size in Lake Biwa. The high predation pressure on the copepod may result in decreasing population size, and consequently lead high phytoplankton abundance (Jeppesen et al. 1997).

Table 4-2 Average annual productions (P_a, g m^{-2} y^{-1}) of *Eodiaptomus japonicus* in Lake Biwa during the last 4 decades, and P_a predicted from the temperature (3 ºC plus annual average during the last 4 decades) and food condition (6% less than the current value) at the end of 21st century (IPCC 2014).

Past & future remarks	Temperature (ºC)	Food index (f)	Biomass (B, g m^{-2})	Specific growth rate (k, day^{-1})	Annual production (P_a, g m^{-2} y^{-1})
Average of 1971–2010	14.2	0.915	1.15	0.111**	46.9
Average of 1971–2010 + 3 ºC	17.2	0.915	1.15	0.137**	57.5
Average of 1971–2010 + 3 ºC & less food	17.2	0.860*	1.15	0.095**	40.2

* Decreasing of 6 % in f induced by declining of global primary production at the end of 21st century

** Estimated from equation (4-1): $k = -1.3607\,f - 0.1037\,T + 0.1225\,f \times T + 1.237$

At the end of the 21st century, the best estimate of climate scenarios predicts that the average surface temperature in temperate lakes will increase 3 ºC in Asian region (IPCC 2014). In Lake Biwa, the annual mean temperature in epilimnion during the last 4 decades was 14.2 ºC (Table 4-2), the current annual mean food index was 0.915 for *E. japonicus* community, and consequently the specific growth rate was 0.111 day^{-1} in this lake calculated from equation (4-1). If considering the average biomass of 1.15 g m^{-2} during the last 4 decades in Lake Biwa, mean annual production (P_a) of *E. japonicus* predicted from the annual mean temperature and food index was 46.9 g m^{-2} y^{-1}. Additional 3 ºC plus the annual mean temperature predicted from a global warming scenario, i.e. 17.2 ºC induces larger P_a, 57.5 g m^{-2} y^{-1} compared to the present state (Table 4-2). The Nutrient-Phytoplankton-Zooplankton (NPZ) models can be coupled to atmospheric General Circulation Models of the Earth's climate change, allowing forecast the potential future states of plankton production of aquatic system (Richardson 2008). Results from the NPZ models predicted that global primary production would slightly decline, ca. 6 %, due to the warming (Bopp et al. 2001). Assuming that the food index will reduce to 0.860 at the end of this century, P_a predicted declines to 40.2 g m^{-2} y^{-1} (Table 4-2). These results suggest that the warming would be

beneficial for *E. japonicus* population in the lake under current nutritional status, while it would induce disadvantage assuming to depress the food conditions due to declining primary production induced by the warming.

SUMMARY

1. Chapter 1

Effects of temperature on life history traits of the dominant calanoid, *Eodiaptomus japonicus* were examined to evaluate its population dynamics in Lake Biwa (Japan). Embryonic and post-embryonic development times and reproduction were determined in the laboratory at four temperature conditions (10, 15, 20 and 25 ºC) and ad libitum food condition. Post-embryonic development time of *E. japonicus* from hatching to adult female decreased with increasing temperature from 67.9 to 15.1 days. Males reached the adult stage 1 to 6 days earlier than the females. Only 15% of the individuals survived until the adult stage at 10 ºC, while 40% did so at >15 ºC. Egg production also depended on temperature. A power function of temperature on instantaneous growth rate predicted a value of <0.06 d^{-1} when water temperature was below 10 ºC, suggesting that *E. japonicus* retards its growth during winter. The null value obtained at 8.6 ºC for the computed population growth rate supports the idea of an overwintering strategy. Responses of life history traits to temperature suggested that in conditions where there was no food limitation *E. japonicus* in Lake Biwa would be able to take advantage of the rise of temperature predicted in the context of global climate change.

2. Chapter 2

Life history traits of the freshwater calanoid copepod *Eodiaptomus japonicus* from Lake Biwa were examined in the laboratory. Four different food concentrations (10^3, 5×10^3, 10^4 and 5×10^4 cells mL^{-1}) and two temperature conditions (15 and 25 ºC) were used to clarify the combined effects of those two factors on life history traits. More than a 70% survival rate was observed at the two medium food concentrations at 15 ºC, 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 food concentration and temperature;

median development times ranged from 28.7–37.3 and 31.4–35.0 days at 15 ºC and 13.7–23.9 and 14.3–27.7 days at 25 ºC, respectively for males and females. An interaction between the two experimental factors was found only for females: i.e., food shortage was most acute at 25 ºC. Clutch sizes also increased with food concentration at both temperatures and interaction occurred between those two factors. Egg production rates increased with increasing food concentration similarly at both temperatures without an interaction effect. Adult body size increased with increasing food concentration at both temperatures: for example, average female prosome length increased from 0.865 mm to 0.922 mm at 15 ºC and from 0.799 mm to 0.904 mm at 25 ºC. Somatic and population growth rates calculated from the experimental data increased with food concentration but the increase was more important at 25 ºC. These responses to food concentration and temperature suggested that both growth and population dynamics of this copepod might be more influenced by food shortage at temperatures >15 ºC. Adult body sizes under food limited conditions in this study are in the lower 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.

3. Chapter 3

Oxygen consumption rates (R, μLO_2 mg-dry-weight^{-1} h^{-1}) of freshwater copepod *Eodiaptomus japonicus* collected from Lake Biwa were determined in a temperature range of 8−30 ºC (i.e. 8, 10, 15, 20, 25, 28 and 30 ºC) using an optical oxygen meter after two different temperature acclimatizations. Average R in adult stage varied from 1.64 to 10.78 and 1.55 to 9.77 μLO_2 mg-dry-weight^{-1} h^{-1} with experimental temperatures for males and females, respectively, acclimatized at 15 ºC, while 1.71 to 11.13 and 1.98 to 10.10 μLO_2 mg-dry-

weight^{-1} h^{-1} for those acclimatized at 25 ºC. *R* exponentially increased with increasing temperature from 8 to 28 ºC and deviated from the exponential phase at 30 ºC for the animals acclimatized at both 15 and 25 ºC. No significant differences of *R* from acclimatizations and genders were shown without any interactions. *R*s in various copepodid stages acclimatized at 15 ºC were always higher at 25 ºC than those at 15 ºC, but not correlated with body weight at both temperatures. According to these results, relationship between *R* and experimental temperature (*T*, ºC) ranged from 8 to 28 ºC could be expressed as an exponential function; $R = 0.8072\ e^{0.0897T}$ ($r^2 = 0.995$, $P < 0.05$) for C1 to C6. Q_{10} between 15 and 25 ºC could be calculated as 2.3. Net growth efficiencies (K_2) calculated in well-fed individuals were 47−52% at both 15 and 25 ºC, whereas those in food-limited ones were 44% at 15 ºC but decreased to 27−28% at 25 ºC. The low K_2 in the food-limited animals at 25 ºC may imply that the metabolic cost at higher temperatures induces lowering the growth rate under food-limited environment.

4. Chapter 4

In order to evaluated the eutrophication and global warming impacts on secondary production during the last 4 decades in Lake Biwa, we analysed long-term (1971−2010) data sets in the body size and biomass of the dominant calanoid copepod *Eodiaptomus japonicus* with the laboratory studies. An efficient food index *f* was calculated from the ratio between *in situ* and potential body size of this copepod to evaluate the food supply levels for *E. japonicus* in the lake, showing a large fluctuation during the last 4 decades. Then, the specific growth rate *k* was also calculated from an equation with multiple factors of *f* and ambient temperature (*T*, ºC): $k = -1.3607\ f - 0.1037\ T + 0.1225\ f \times T + 1.237$ ($n = 8$, $r^2 = 1$, SSE < 0.001), showing less oscillation even in the eutrophication period in 1970's and 1980's. Finally, we calculated the annual production (P_a) of *E. japonicus* from the biomass and *k*

during the last 4 decades. The productions calculated were relatively stable until the late 1990's, but tended to increase after 2000, related to biomass trend. According to the global warming scenarios, we predicted average P_a at the end of this century. Average P_a could increase from 46.9 to 57.5 g m^{-2} y^{-1} due to 3 ºC temperature raise at the end of this century, if *in situ* population would be exposed to current nutritional status, while P_a might be depressed by food shortage if the primary production would be reduced at 6% due to global warming.

REFERENCES

Abou Debs, C. 1984. Carbon and nitrogen budget of the calanoid copepod *Temora stylifera*: effect of concentration and composition of food. Marine Ecology Progress Series **15**:213-223.

Alcaraz, M., R. Almeda, E. Saiz, A. Calbet, C. M. Duarte, S. Agustí, R. Santiago, and A. Alonso. 2013. Effects of temperature on the metabolic stoichiometry of Arctic zooplankton. Biogeosciences **10**:689-697.

Amarasinghe, P. B., M. Boersma, and J. Vijverberg. 1997. The effect of temperature, and food quantity and quality on the growth and development rates in laboratory-cultured copepods and cladocerans from a Sri Lankan reservoir. Hydrobiologia **350**:131-144.

Anneville, O., J. C. Molinero, S. Souissi, G. Balvay, and D. Gerdeaux. 2007. Long-term changes in the copepod community of Lake Geneva. Journal of Plankton Research **29**:i49-i59.

Anneville, O. and J. P. Pelletier. 2000. Recovery of Lake Geneva from eutrophication: quantitative response of phytoplankton. Archiv fur Hydrobiologie **148**:607-624.

Anneville, O., S. Souissi, F. Ibanez, V. Ginot, J. C. Druart, and N. Angeli. 2002. Temporal mapping of phytoplankton assemblages in Lake Geneva: Annual and interannual changes in their patterns of succession. Limnology and Oceanography **47**:1355-1366.

Baloch, A. w., H. Maeta, and T. Saisho. 1998. Seasonal Abundance and Vertical Distribution of Zooplanton in Lake Ikeda. Journal of Plankton Research **13**:1-8.

Ban, S. 1994. Effect of temperature and food concentration on post-embryonic development, egg production and adult body size of calanoid copepod *Eurytemora affinis*. Journal of Plankton Research **16**:721-735.

Beaugrand, G., C. Luczak, and M. Edwards. 2009. Rapid biogeographical plankton shifts in the North Atlantic Ocean. Global Change Biology **15**:1790-1803.

Beaugrand, G., P. C. Reid, F. Ibañez, J. A. Lindley, and M. Edwards. 2002. Reorganization of North Atlantic marine copepod biodiversity and climate. Science **296**:1692-1694.

Berggreen, U., B. Hansen, and T. Kiørboe. 1988. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: Implications for determination of copepod production. Marine Biology **99**:341-352.

Beyrend-Dur, D. 2010. Life history traits of key brackish copepods from temperate to tropical environments. University of Lille, France / National Taiwan Ocean University, Taiwan, pp. 216.

Beyrend-Dur, D., R. Kumar, T. R. Rao, S. Souissi, S.-H. Cheng, and J.-S. Hwang. 2011. Demographic parameters of adults of *Pseudodiaptomus annandalei* (Copepoda: Calanoida): Temperature–salinity and generation effects. Journal of Experimental Marine Biology and Ecology **404**:1-14.

Beyrend-Dur, D., S. Souissi, D. Devreker, G. Winkler, and J. S. Hwang. 2009. Life cycle traits of two transatlantic populations of *Eurytemora affinis* (Copepoda: Calanoida): salinity effects. Journal of Plankton Research **31**:713-728.

Bode, M., A. Schukat, W. Hagen, and H. Auel. 2013. Predicting metabolic rates of calanoid copepods. Journal of Experimental Marine Biology and Ecology **444**:1-7.

Bonnet, D. and F. Carlotti. 2001. Development and egg production in *Centropages typicus* (Copepoda: Calanoida) fed different food types: a laboratory study. Marine Ecology Progress Series **224**:133-148.

Bonnet, D., R. P. Harris, L. Yebra, F. Guilhaumon, D. V. P. Conway, and A. G. Hirst. 2009. Temperature effects on *Calanus helgolandicus* (Copepoda: Calanoida) development time and egg production. Journal of Plankton Research **31**:31-44.

Bopp, L., P. Monfray, O. Aumont, J. L. Dufresne, H. L. Treut, G. Madec, L. Terray, and J. C. Orr. 2001. Potential impact of climate change on marine export production. Global Biogeochemical Cycles **15**:81-99.

Boxshall, G. A. and D. Defaye. 2008. Global diversity of copepods (Crustacea: Copepoda) in freshwater. Hydrobiologia **595**:195-207.

Bradley, B. P. 1978a. Genetic and physiological adaptation of the copepod *Eurytemora affinis* to seasonal temperatures. Genetics **90**:193-205.

Bradley, B. P. 1978b. Increase in range of temperature tolerance by acclimation in the copepod *Eurytemora affinis*. Biological Bulletin **154**:177-187.

Broglio, E., S. H. Jónasdóttir, A. Calbet, H. H. Jakobsen, and E. Saiz. 2003. Effect of heterotrophic

versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition. Aquatic Microbial Ecology **31**:267-278.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771-1789.

Burns, C. W. 1984. Fungal parasitism in a copepod population: the effects of *Aphanomyces* on the population dynamics of *Boeckella dilatata* Sars. Journal of Plankton Research **7**:201-205.

Carlotti, F. and S. Nival. 1991. Individual variability of development in laboratory-reared *Temora stylifera* copepodites: consequences for the population dynamics and interpretation in the scope of growth and development rules. Journal of Plankton Research **13**:801-813.

Castellani, C., C. Robinson, T. Smith, and R. S. Lampitt. 2005. Temperature affects respiration rate of *Oithona similis*. Marine Ecology Progress Series **285**:129-135.

Cervetto, G., R. Gaudy, and M. Pagano. 1999. Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). Journal of Experimental Marine Biology and Ecology **239**:33-45.

Champalbert, G. and R. Gaudy. 1972. Etude de la respiration chez des copépodes de niveaux bathymétriques variés dans la région sud marocaine et canarienne. Marine Biology **12**:159-169.

Checkley, D. M. 1980. Food limitation of egg production by a marine, planktonic copepod in the sea off southern California. Limnology and Oceanography **25**:991-998.

Chinnery, F. E. and J. A. Williams. 2004. The influence of temperature and salinity on *Acartia* (Copepoda: Calanoida) nauplii survival. Marine Biology **145**:733-738.

Clarke, A. 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? Functional Ecology **7**:139-149.

Comita, G. W. 1968. Oxygen consumption in *Diaptomus*. Limnology and Oceanography **13**:51-57.

Conover, R. J. 1960. The feeding behavior and respiration of some marine planktonic crustacea. The Biological Bulletin **119**:339-415.

Conover, R. J. and E. D. S. Corner. 1968. Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. Journal of the Marine Biological Association of the United Kingdom **48**:49-75.

Cook, K. B., A. Bunker, S. Hay, A. G. Hirst, and D. C. Speirs. 2007. Naupliar development times and survival of the copepods *Calanus helgolandicus* and *Calanus finmarchicus* in relation to food and temperature. Journal of Plankton Research **29**:757-767.

Cruz, J., S. Garrido, M. S. Pimentel, R. Rosa, A. M. P. Santos, and P. Ré. 2013. Reproduction and respiration of a climate change indicator species: effect of temperature and variable food in the copepod *Centropages chierchiae*. Journal of Plankton Research **35**:1046-1058.

Dam, H. G. 2013. Evolutionary adaptation of marine zooplankton to global change. Annual Review of Marine Science **5**:349-370.

Damgaard, R. M. and J. Davenport. 1994. Salinity tolerance, salinity preference and temperature tolerance in the high-shore harpacticoid copepod *Tigriopus brevicornis*. Marine Biology **118**:443-449.

Deevey, G. B. 1960. Relative effects of temperature and food on seasonal variations in length of marine copepods in some eastern American and western European waters. Bulletin of the Bingham Oceanographic Collection **17**:54-84.

Del Giorgio, P. A., J. J. Cole, and A. Cimbleris. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature **385**:148-151.

Devol, A. H. 1979. Zooplankton respiration and its relation to plankton dynamics in two lakes of contrasting trophic state. Limnology and Oceanography **24**:893-905.

Devreker, D., S. Souissi, J. Forget-Leray, and F. Leboulenger. 2007. Effects of salinity and temperature on the post-embryonic development of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: a laboratory study. Journal of Plankton Research **29**:i117-i133.

Devreker, D., S. Souissi, and L. Seuront. 2004. Development and mortality of the first naupliar stages of *Eurytemora affinis* (Copepoda, Calanoida) under different conditions of salinity and temperature. Journal of Experimental Marine Biology and Ecology **303**:31-46.

Devreker, D., S. Souissi, and L. Seuront. 2005. Effects of chlorophyll concentration and temperature

variation on the reproduction and survival of *Temora longicornis* (Copepoda, Calanoida) in the Eastern English Channel. Journal of Experimental Marine Biology and Ecology **318**:145-162.

Devreker, D., S. Souissi, G. Winkler, J. Forget-Leray, and F. Leboulenger. 2009. Effects of salinity, temperature and individual variability on the reproduction of *Eurytemora affinis* (Copepoda; Calanoida) from the Seine estuary: A laboratory study. Journal of Experimental Marine Biology and Ecology **368**:113-123.

Dokulil, M. T. and A. Herzig. 2009. An analysis of long-term winter data on phytoplankton and zooplankton in Neusiedler See, a shallow temperate lake, Austria. Aquatic Ecology **43**:715-725.

Dokulil, M. T. and K. Teubner. 2005. Do phytoplankton communities correctly track trophic changes? An assessment using directly measured and palaeolimnological data. Freshwater Biology **50**:1594-1604.

Downing, J. A. and F. H. Rigler. 1984. A manual on methods for the assessment of secondary productivity in fresh waters, Second Edition, IBP HAND BOOK 17, Black Scientific Publications, Oxford Londen Edinburgh, Boston Melbourne, 501 p.

Drits, A. V., A. F. Pasternak, and K. N. Kosobokova. 1993. Feeding, metabolism and body composition of the Antarctic copepod *Calanus propinquus* Brady with special reference to its life cycle. Polar Biology **13**:13-21.

Dur, G., R. Jiménez-Melero, D. Beyrend-Dur, J. S. Hwang, and S. Souissi. 2013. Individual-based model for the phenology of egg-bearing copepods : application to *Eurytemora affinis* from the Seine estuary, France. Ecological Modelling **269**:21-36.

Dur, G., S. Souissi, D. Devreker, V. Ginot, F. G. Schmitt, and J.-S. Hwang. 2009. An individual-based model to study the reproduction of egg bearing copepods: Application to *Eurytemora affinis* (Copepoda Calanoida) from the Seine estuary, France. Ecological Modelling **220**:1073-1089.

Dur, G., S. Souissi, F. G. Schmitt, D. Beyrend-Dur, and J. S. Hwang. 2011. Mating and mate choice in *Pseudodiaptomus annandalei* (Copepoda: Calanoida). Journal of Experimental Marine Biology and Ecology **402**:1-11.

Durant, J. M., D. Ø. Hjermann, G. Ottersen, and N. C. Stenseth. 2007. Climate and the match or mismatch between predator requirements and resource availability. Climate research **33**:271-283.

Durbin, E. G., A. G. Durbin, T. J. Smayda, and P. G. Verity. 1983. Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnology and Oceanography **28**:1199-1213.

Edmands, S. and J. K. Deimler. 2004. Local adaptation, intrinsic coadaptation and the effects of environmental stress on interpopulation hybrids in the copepod *Tigriopus californicus*. Journal of Experimental Marine Biology and Ecology **303**:183-196.

Edmondson, W. T. 1970. Phosphorus, nitrogen, and algae in Lake Washington after diversion of sewage. Science **169**:690-691.

Edmondson, W. T. and G. G. Winberg. 1971. A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook No. 17, Oxford and Edinburgh: Blackwell Scientific Publications, 358 p.

Einsle, U. 1983. Long-term changes in planktonic associations of crustaceans in Lake Constance and adjacent waters and their effects on competitive situations. Hydrobiologia **106**:127-134.

Elliott, D. T., C. K. Harris, and K. W. Tang. 2010. Dead in the water: The fate of copepod carcasses in the York River estuary, Virginia. Limnology and Oceanography **55**:1821-1834.

Epp, R. W. and W. M. J. Lewis. 1979. Metabolic responses to temperature change in a tropical freshwater copepod (*Mesocyclops brasilianus*) and their adaptive significance. Oecologia **42**:123-138.

Epp, R. W. and W. M. J. Lewis. 1980. The nature and ecological significance of metabolic changes during the life history of copepods. Ecology **61**:259-264.

Escribano, R., C. Irribarren, and L. Rodriguez. 1997. Influence of food quantity and temperature on development and growth of the marine copepod *Calanus chilensis* from northern Chile. Marine Biology **128**.

Fahnenstiel, G. L. and J. M. Glime. 1983. Subsurface chlorophyll maximum and associated *Cyclotella*

pulse in Lake Superior. Internationale Revue der gesamten Hydrobiologie und Hydrographie **68**:605-616.

Fernández, F. 1978. Metabolismo y alimentacion en copepodos planctonicos del Mediterraneo: respuesta a la temperatura. Investigacion Pesquera **42**:97-139.

Fitzhugh, H. A. 1976. Analysis of growth curves and strategies for altering their shape. Journal of animal Science **42**:1036-1051.

Franke, U. 1977. Experimentelle untersuchungen zur respiration von *Gammarus fossarum* Koch 1835 (Crustacea-Amphipoda) in abhängigkeit von temperatur, sauerstoff-konzentration und wasserbewegung. Archiv für Hydrobiologie (Supplement) **48**:369-411.

Gaudy, R. and D. Thibault-Botha. 2007. Metabolism of *Centropages* species in the Mediterranean Sea and the North Atlantic Ocean. Progress in Oceanography **72**:151-163.

Gauld, D. T. and J. E. G. Raymont. 1953. The respiration of some planktonic copepods II. The effect of temperature. Journal of the Marine Biological Association of the United Kingdom **31**:447-460.

Geslin, E., N. Risgaard-Petersen, F. Lombard, E. Metzger, D. Langlet, and F. Jorissen. 2011. Oxygen respiration rates of benthic foraminifera as measured with oxygen microsensors. Journal of Experimental Marine Biology and Ecology **396**:108-114.

Gillooly, J. F. 2000. Effect of body size and temperature on generation time in zooplankton. Journal of Plankton Research **22**:241-251.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science **293**:2248-2251.

Gómez, M. and S. Hernández-León. 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science **17**:15-20.

Green, J. D. and M. A. Chapman. 1977. Temperature effects on oxygen consumption by the copepod *Boeckella dilatata*. New Zealand Journal of Marine and Freshwater Research **11**:375-382.

Guisande, C. and R. P. Harris. 1995. Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. Limnology and Oceanography **40**:476-482.

Haga, H., T. Nagata, and M. Sakamoto. 1995. Size-fractionated NH4+ regeneration in the pelagic environments of two mesotrophic lakes. Limnology and Oceanography **40**:1091-1099.

Hagen, W. 1988. Zur Bedeutung der Lipide im antarktischen Zooplankton. Berichte zur Polarforschung **49**:1-129.

Hairston, N. G. 1987. Diapause as a predator-avoidance adaptation. In predation. Direct and indirect impacts in aquatic communities, (eds. W. C. Kerfoot and A. Sih), pp. 281-290. University Press of New England, Hanover, NH.

Hairston, N. G. and T. A. Dillon. 1990. Fluctuating selection and response in a population of freshwater copepods. Evolution **44**:1796-1805.

Håkanson, J. L. 1984. The long and short term feeding condition in field-caught *Calanus pacificus*, as determined from the lipid content. Limnology and Oceanography **29**:794–804.

Halcrow, K. 1963. Acclimation to temperature in the marine copepod, *Calanus finmarchicus* (Gunner.). Limnology and Oceanography **8**:1-8.

Halsband-Lenk, C., F. Carlotti, and W. Greve. 2004. Life-history strategies of calanoid congeners under two different climate regimes: a comparison. Journal of Marine Science **61**:709-720.

Halsband-Lenk, C., H. J. Hirche, and F. Carlotti. 2002. Temperature impact on reproduction and development of congener copepod populations. Journal of Experimental Marine Biology and Ecology **271**:121-153.

Hampton, S. E., L. R. Izmest'eva, M. V. Moore, S. L. Katz, B. Dennis, and E. A. Silow. 2008. Sixty years of environmental change in the world's largest freshwater lake – Lake Baikal, Siberia. Global Change Biology **14**:1947-1958.

Harris, R. P., P. H. Wiebe, J. Lenz, H. R. Skjoldal, and M. E. Huntley. 2000. ICES Zooplankton Methodology Manual. Academic Press, London, 684 p.

Hart, R. C. 1996. Naupliar and copepodite growth and survival of two freshwater calanoids at various food levels: Demographic contrasts, similarities, and food needs. Limnology and Oceanography **41**:648-658.

Hart, R. C. and I. A. McLaren. 1978. Temperature acclimation and other influences on embryonic duration in the copepod *Pseudocalanus* sp. Marine Biology **45**:23-30.

Herzig, A. 1983. The ecological significance of the relationship between temperature and duration of embryonic development in planktonic freshwater copepods. Hydrobiologia **100**:65-91.

Hirche, H. J. 1984. Temperature and metabolism of plankton−I. Respiration of antarctic zooplankton at different temperatures with a comparison of antarctic and nordic krill. Comparative Biochemistry and Physiology **77**:361-368.

Hirche, H. J. 1987. Temperature and plankton. II. Effect on respiration and swimming activity in copepods from the Greenland Sea. Marine Biology **94**:347-356.

Hirche, H. J. 1992. Egg production of *Eurytemora affinis*-Effect of k-strategy. Estuarine Coastal and Shelf Science **35**:395-407.

Hirst, A. G. and R. S. Lampitt. 1998. Towards a global model of in situ weight-specific growth in marine planktonic copepods. Marine Biology **132**:247-257.

Hirst, A. G., M. Sheader, and J. A. Williams. 1999. Annual pattern of calanoid copepod abundance, prosome length and minor role in pelagic carbon flux in the Solent, UK. Marine Ecology Progress Series **177**:133-146.

Holeck, K. T., J. M. Watkins, E. L. Mills, O. Johannsson, S. Millard, V. Richardson, and K. Bowen. 2008. Spatial and long-term temporal assessment of Lake Ontario water clarity, nutrients, chlorophyll a, and zooplankton. Aquatic Ecosystem Health and Management Society **11**:377-391.

Holste, L. and M. A. Peck. 2005. The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. Marine Biology **148**:1061-1070.

Hop, H. and M. Graham. 1995. Respiration of juvenile Arctic cod (*Boreogadus saida*): effects of acclimation, temperature, and food intake. Polar Biology **15**:359-367.

Hsieh, C. H., K. Ishikawa, Y. Sakai, T. Ishikawa, S. Ichise, Y. Yamamoto, T. C. Kuo, H. D. Park, N. Yamamura, and M. Kumagai. 2010. Phytoplankton community reorganization driven by eutrophication and warming in Lake Biwa. Aquatic Sciences **72**:467-483.

Hsieh, C. H., Y. Sakai, S. Ban, K. Ishikawa, T. Ishikawa, S. Ichise, N. Yamamura, and M. Kumagai. 2011. Eutrophication and warming effects on long-term variation of zooplankton in Lake Biwa. Biogeosciences Discussions **8**:593-629.

Huntley, M. E. and M. G. Lopez. 1992. Temperature-Dependent Production of Marine Copepods: A Global Synthesis. The American Naturalist **140**:201-242.

Ianora, A. 1998. Copepod life history traits in subtemperate regions. Journal of Marine Systems **15**:337-349.

IBM Inc. 2011. IBM SPSS Statistics (Predictive analytics software and solutions), version 20.0.0, Armonk, New York, USA.

Ichimura, T. 1971. Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum*. In Proceedings of the Seventh International Seaweed Symposium, University of Tokyo Press, Tokyo, p. 208-214.

Ikeda, T. 1970. Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. Bulletin of the faculty of fisheries Hokkaido University **21**:91-112.

Ikeda, T. 1971. Change in respiration rate and in composition of organic matter in *Calanus cristatus* (Crustacea Copepoda) under starvation. Bulletin of the faculty of fisheries Hokkaido University **21**:280-298.

Ikeda, T. 1985. Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Marine Biology **85**:1-11.

Ikeda, T., Y. Kanno, K. Ozaki, and A. Shinada. 2001. Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. Marine Biology **139**:587-596.

IPCC. 2007. Summary for Policymakers. In: Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M., & Miller H.L. (Eds), *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, USA. pp. 1-18.

IPCC. 2014. Hijioka, Y., E. Lin, J.J. Pereira, R.T. Corlett, X. Cui, G.E. Insarov, R.D. Lasco, E. Lindgren, and A. Surjan, 2014: Asia. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros, V.R., C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1327-1370.

Ishikawa, A., S. Ban, and N. Shiga. 1999. Effects of salinity on survival, and embryonic and postembryonic development of *Eurytemora affinis*. Plankton Biology and Ecology **46**:113-119.

Isla, J. A. and R. Perissinotto. 2004. Effects of temperature, salinity and sex on the basal metabolic rate of the estuarine copepod *Pseudodiaptomus hessei*. Journal of Plankton Research **26**:579-583.

Jamieson, C. and C. Burns. 1988. The effects of temperature and food on copepodite development, growth and reproduction in three species of *Boeckella* (Copepoda: Calanoida). Hydrobiologia **164**:235-257.

Jamieson, C. D. 1986. The effects of temperature and food on naupliar development, growth and metamorphosis in three species of *Boeckella* (Copepoda:Calanoida). Hydrobiologia **139**:277-286.

Jeppesen, E., J. P. Jensen, C. Jensen, B. Faafeng, D. O. Hessen, M. Søndergaard, T. L. Lauridsen, P. Brettum, and K. Christoffersen. 2003. The impact of nutrient state and lake depth on top-down control in the pelagic zone of lakes: A study of 466 lakes from the temperate zone to the Arctic. Ecosystems **6**:313-325.

Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen, L. J. Pedersen, and L. Jensen. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. Hydrobiologia **342/343**:151-164.

Jiménez-Melero, R., G. Parra, and F. Guerrero. 2012. Effect of temperature, food and individual variability on the embryonic development time and fecundity of *Arctodiaptomus salinus* (Copepoda: Calanoida) from a shallow saline pond. Hydrobiologia **686**:241-256.

Jiménez-Melero, R., G. Parra, S. Souissi, and F. Guerrero. 2007. Post-embryonic developmental plasticity of *Arctodiaptomus salinus* (Copepoda: Calanoida) at different temperatures. Journal of Plankton Research **29**:553-567.

Jiménez-Melero, R., B. Santer, and F. Guerrero. 2005. Embryonic and Naupliar Development of *Eudiaptomus gracilis* and *Eudiaptomus graciloides* at Different Temperatures: Comments on Individual Variability. Journal of Plankton Research **27**:1175-1187.

Jónasdóttir, S. H., N. H. Trung, F. Hansen, and S. Gärtner. 2005. Egg production and hatching success in the calanoid copepods *Calanus helgolandicus* and *Calanus finmarchicus* in the North Sea from March to September 2001. Journal of Plankton Research **27**:1239-1259.

Kamler, E. 1969. A comparison of the closed-bottle and flowing-water methods for measurement of respiration in aquatic invertebrates. Polskie Archiwum hydrobiologii **16**:31-49.

Kattner, G. and M. Krause. 1987. Changes in lipids during the development of *Calanus finmarchicus* s.l. from copepodid I to adult. Marine Biology **96**:511-518.

Kawabata, K. 1987a. Abundance and distribution of *Eodiaptomus japonicus* (Copepoda: Calanoida) in Lake Biwa. Bulletin of the Plankton Society of Japan **34**:173-183.

Kawabata, K. 1987b. Ecology of large phytoplankton in Lake Biwa: population dynamics and food relations with zooplankters. Bulletin of the Plankton Society of Japan **34**:165-172.

Kawabata, K. 1989a. Natural development time of *Eodiaptomus japonicus* (Copepoda: Calanoida) in Lake Biwa. Journal of Plankton Research **11**:1261-1272.

Kawabata, K. 1989b. Seasonal changes in abundance and vertical distribution of *Mesocyclops thermocyclopoides*, *Cyclops vicinus* and *Daphnia longispina* in Lake Biwa. Japanese Journal of Limnology **50**:9-13.

Kawabata, K. 1993. Mortality rate of *Eodiaptomus japonicus* (Copepoda: Calanoida) in Lake Biwa. Japanese Journal of Limnology **54**:131-136.

Kawabata, K. 1995. Survival of *Eodiaptomus japonicus* in the laboratory. Kanazawa University

Repository for Academic resources **44**:61-64.
Kawabata, K. 2006. Clearance rate of the cyclopoid copepod Mesocyclops dissimilis on the calanoid copepod *Eodiaptomus japonicus*. Plankton Benthos Research **1**:68-70.
Kawabata, K., T. Narita, M. Nagoshi, and M. Nishino. 2002. Stomach contents of the landlocked dwarf ayu in Lake Biwa, Japan. Japanese Society of Limnology **3**:135-142.
Kawabata, K. and J. Urabe. 1998. Length-weight relationships of eight freshwater planktonic crustacean species in Japan. Freshwater Biology **39**:199-205.
Kawall, H. G., J. J. Torres, and S. P. Geiger. 2001. Effects of the ice-edge bloom and season on the metabolism of copepods in the Weddell Sea, Antarctica. Hydrobiologia **453/454**:67-77.
Kiko, R., H. Hauss, F. Buchholz, and F. Melzner. 2015. Ammonium excretion and oxygen respiration of tropical copepods and euphausiids exposed to oxygen minimum zone conditions. Biogeosciences Discussions **12**:17329-17366.
Kim, W. S. 1995. The effect of food on the egg production rates of *Acartia tonsa* (Calanoid Copepod) in Long Island Sound. Ocean and Polar Research **17**:1-7.
Kimmerer, W. J. and A. D. McKinnon. 1987. Growth, mortality, and secondary production of the copepod *Acartia tranteri* in Westernport Bay, Australia. Limnology and Oceanography **32**:14-28.
Kimmerer, W. J. and A. D. McKinnon. 1990. High mortality in a copepod population caused by a parasitic dinoflagellate. Marine Biology **107**:449-452.
Kiørboe, T., F. Møhlenberg, and K. Hamburger. 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Marine Ecology Progress Series **26**:85-97.
Klein Breteler, W. C. M. and S. R. Gonzalez. 1986. Culture and development of *Temora longicornis* (Copepoda, Calanoida) at different conditions of temperature and food. Syllogeus **58**:71-84.
Klein Breteler, W. C. M. and S. R. Gonzalez. 1988. Influence of temperature and food concentration on body size, weight and lipid content of two calanoid copepod species. Hydrobiologia **167**:201-210.
Klein Breteler, W. C. M., S. R. Gonzalez, and N. Schogt. 1995. Development of *Pseudocalanus elongatus* (Copepoda, Calanoida) cultured at different temperature and food conditions. Marine Ecology Progress Series **119**:99-110.
Klekowski, R. Z. 1971. Cartesian diver microrespirometry for aquatic animals. Polskie Archiwum hydrobiologii **18**:93-114.
Koski, M. and H. Kuosa. 1999. The effect of temperature, food concentration and female size on the egg production of the planktonic copepod *Acartia bifilosa*. Journal of Plankton Research **21**:1779-1790.
Laabir, M., S. A. Poulet, A. Ianora, A. Miralto, and A. Cueff. 1995. Reproductive response of *Calanus helgolandicus*. II. *In situ* inhibition of embryonic development. Marine Ecology Progress Series **129**:97-105.
Lampert, W. 1977a. Studies on the carbon balance of *Daphnia pulex* DE GEER as related to environmental conditions. II. The dependence of carbon assimilation on animal size, temperature, food concentration and diet species. Hydrobiologia **48**:310-335.
Lampert, W. 1977b. Studies on the carbon balance of *Daphnia pulex* DE GEER as related to environmental conditions. III. Production and production efficiency. Hydrobiologia **48**:336-360.
Lampert, W. 1989. The adaptive significance of diel vertical migration of zooplankton. Functional Ecology **3**:21-27.
Lampitt, R. S. and J. C. Gamble. 1982. Diet and respiration of the small planktonic marine copepod *Oithona nana*. Marine Biology **66**:185-190.
Landry, M. R. 1975a. The relationship between temperature and the development of life stages of the marine copepod *Acartia clausi* Giesbr. Limnology and Oceanography **20**:854-857.
Landry, M. R. 1975b. Seasonal temperature effects and predicting development rate of marine copepod eggs. Limnology and Oceanography **20**:434-440.
Landry, M. R. 1983. The development of marine calanoid copepods with comment on the isochronal rule. Limnology and Oceanography **28**:614-624.
Laybourn-Parry, J. and I. M. Strachan. 1980. Respiration metabolism of *Cyclops bicuspidatus* (*sensu*

stricta) (Claus) (Copepoda: Cyclopoida) From Esthwaite Water, Cumbria. Oecologia **46**:386-390.

Lee, C. E. and C. H. Petersen. 2002. Genotype - by - Environment Interaction for Salinity Tolerance in the Freshwater - Invading Copepod *Eurytemora affinis*. Physiological and Biochemical Zoology **75**:335-344.

Lee, C. E. and C. H. Petersen. 2003. Effects of developmental acclimation on adult salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. Physiological and Biochemical Zoology **76**:296-301.

Lee, H. W., S. Ban, T. Ikeda, and T. Matsuishi. 2003. Effect of temperature on development, growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food condition. Journal of Plankton Research **25**:261-271.

Lee, H. W., T. Ikeda, and S. Ban. 2001. Metabolism, body composition (C and N) and estimated net growth efficiency of a calanoid copepod *Pseudocalanus newmani* raised at different temperatures in the laboratory. Plnakton Biology and Ecology **48**:114-120.

Lee, R. F., W. Hagen, and G. Kattner. 2006. Lipid storage in marine zooplankton. Marine Ecology Progress Series **307**:273-306.

Liu, X., D. Beyrend, G. Dur, and S. Ban. 2015. Combined effects of temperature and food concentration on growth and reproduction of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan). Freshwater Biology **60**:2003-2018.

Liu, X., D. Beyrend-Dur, G. Dur, and S. Ban. 2014. Effects of temperature on life history traits of *Eodiaptomus japonicus* (Copepoda: Calanoida) from Lake Biwa (Japan). Limnology **15**:85-97.

Løvik, J. E. and G. Kjellberg. 2003. Long-term changes of the crustacean zooplankton community in Lake Mjøsa, the largest lake in Norway. Journal of Limnology **62**:143-150.

Magnuson, J. J., K. E. Webster, R. A. Assel, C. J. Bowser, P. J. Dillon, J. G. Eaton, H. E. Evans, E. J. Fee, R. I. Hall, L. R. Mortsch, D. W. Schindler, and F. H. Quinn. 1997. Potential effects of climate changes on aquatic systems: Laurentian Great Lakes and Precambrian Shield Region. Hydrological processes **11**:825-871.

Mahjoub, M.-S., S. Souissi, F.-G. Michalec, F. G. Schmitt, and J.-S. Hwang. 2011. Swimming kinematics of *Eurytemora affinis* (Copepoda, Calanoida) reproductive stages and differential vulnerability to predation of larval *Dicentrarchus labrax* (Teleostei, Perciformes). Journal of Plankton Research **33**:1095-1103.

Marshall, S. M., A. G. Nicholls, and A. P. Orr. 1935. On the biology of *Calanus finmarchicus*. Part VI. Oxygen consumption in relation to environmental conditions. Journal of the Marine Biological Association of the United Kingdom **20**:1-27.

Mauchline, J. 1998. The Biology of Calanoid Copepods. In: Blaxter, J.H.S., Southward, A.J., Tyler, P.A. (Eds.), Academic Press, London, 710 p.

Mayzaud, P. 1973. Respiration and nitrogen excretion of zooplankton. II. Studies of the metabolic characteristics of starved animals. Marine Biology **21**:19-28.

Mayzaud, P. and S. Razouls. 1992. Degradation of gut pigment during feeding by a subantarctic copepod: Importance of feeding history and digestive acclimation. Limnology and Oceanography **37**:393-404.

Mayzaud, P., O. Roche-Mayzaud, and S. Razouls. 1992. Medium term time acclimation of feeding and digestive enzyme activity in marine copepods: influence of food concentration and copepod species. Marine Ecology Progress Series **89**:197-212.

McKinnon, A. D. 1996. Growth and development in the subtropical copepod *Acrocalanus gibber*. Limnology and Oceanography **41**:1438-1447.

McLaren, I. A., C. I. Corkett, and E. J. Zillioux. 1969. Temperature adaptations of copepod eggs from the arctic to the tropics. The Biological Bulletin **137**:486-493.

McQueen, D. J., M. R. S. Johannes, J. R. Post, T. J. Stewart, and D. R. Lean. 1989. Bottom-up and top-down impacts on freshwater pelagic community structure. Ecological Monographs **59**:289-309.

McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Journal of Fisheries and Aquatic Science **43**:1571-1581.

Merrell, J. R. and D. K. Stoecker. 1998. Differental grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. Journal of Plankton Research **20**:289-304.

Mizuno, T. 1984. Nihon no rikusui san Calanoida (Freshwater Calanoida in Japan), p. 475-499. In *Freshwater Copepoda* (ed. Mizuno, T.). Tatara Shobo, Yonago. (In Japanese.).

Møhlenberg, F. and T. Kiørboe. 1981. Growth and energetics in *Spisula subtruncata* (da Costa) and the effect of suspended bottom material. Ophelia **20**:79-90.

Molinero, J. C., O. Anneville, S. Souissi, G. Balvay, and D. Gerdeaux. 2006. Anthropogenic and climate forcing on the long-term changes of planktonic rotifers in Lake Geneva, Europe. Journal of Plankton Research **28**:287-296.

Munch, S. B. and S. Salinas. 2009. Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. Proceedings of the National Academy of Sciences of the United States of America **106**:13860-13864.

Munro, I. G. 1974. Effect of temperature on the development of egg naupliar copepodite stage of two species of copepods *Cyclops vicinus* Uljanin and *Eudiaptomus gracilis* sars. Oecologia **16**:355-367.

Nagata, T. and K. Okamoto. 1988. Filtering rates on natural bacteria by *Daphnia longispina* and *Eodiaptomus japonicus* in Lake Biwa. Journal of Plankton Research **10**:835-850.

Nakamura, Y. and J. T. Turner. 1997. Predation and respiration by the small cyclopoid copepod *Oithona similis*: How important is feeding on ciliates and heterotrophic flagellates? Journal of Plankton Research **19**:1275-1288.

Newrkla, P. 1978. The influence of ionic concentration on population parameters, development time, activity, and respiration rate of *Arctodiaptomus spinosus* (Daday) (Calanoida, Copepoda). Oecologia **33**:87-99.

Nival, P., G. Malara, R. Charra, I. Palazzoli, and S. Nival. 1974. Étude de la respiration et de l'excrétion de quelques copépodes planctoniques (crustacea) dans la zone de remontée d'eau profonde des côtes marocaines. Journal of Experimental Marine Biology and Ecology **15**:231-260.

O'Reilly, C. M., S. R. Alin, P. D. Plisnier, A. S. Cohen, and B. A. McKee. 2003. Climate change decreases aquatic ecosystem productivity of Lake Tanganyika, Africa. Nature **424**:766-768.

Okamoto, K. 1984a. Diurnal changing patterns of the in situ size-selective feeding activities of *Daphnia longispina hyalina* and *Eodiaptomus japonicus* in a pelagic area of Lake Biwa. Memoirs of the Faculty of Science Kyoto University Series of Biology **9**:107-132.

Okamoto, K. 1984b. Size-selective feeding of *Daphnia longispina hyalina* and *Eodiaptomus japonicus* on a natural phytoplankton assemblage with the fractionizing method. Memoirs of the Faculty of Science Kyoto University Series of Biology **9**:23-40.

Paffenhöfer, G.-A. and K. D. Lewis. 1989. Feeding behavior of nauplii of the genus *Eucalanus* (Copepoda, Calanoida). Marine Ecology Progress Series **57**:129-136.

Pahlow, M., A. F. Vézina, B. Casault, H. Maass, L. Malloch, D. G. Wright, and Y. Lu. 2008. Adaptive model of plankton dynamics for the North Atlantic. Progress in Oceanography **76**:151-191.

Parrish, K. K. and D. F. Wilson. 1978. Fecundiy studies on *Acartia tonsa* (Copepoda: Calanoida) in standardized culture. Marine Biology **46**:65-81.

Penry, D. L. and B. W. Frost. 1991. Chlorophyll *a* degradation by *Calanus pacificus*: Dependence on ingestion rate and digestive acclimation to food resources. Limnology and Oceanography **36**:147-159.

Peterson, W. T. 2009. Copepod species richness as an indicator of long term changes in the coastal ecosystem of the northern California Current. CalCOFI Reports **50**:73-81.

Ploug, H., M. H. Iversen, M. Koski, and E. T. Buitenhuis. 2008. Production, oxygen respiration rates, and sinking velocity of copepod fecal pellets: Direct measurements of ballasting by opal and calcite. Limnology and Oceanography. **53**:469-476.

Plounevez, S. and G. Champalbert. 1999. Feeding behaviour and trophic environment of *Engraulis encrasicolus* (L.) in the Bay of Biscay. Estuarine, Coastal and Shelf Science **49**:177-191.

Poulet, S. A. 1983. Factors controlling utilization of non-algal diets by particle-grazing copepods. A review. Oceanologica Acta **6**:221-234.

Provasoli, L. and I. J. Pintner. 1959. Artificial media for fresh-water algae: problems and suggestions.

In The Ecology of Algae. Spec. Pub. No. 2, Eds. by Tryon, C. A., Jr. & Hartmann, R. T., Pymatuning Laboratory of Field Biology, University of Pittsburgh, Pittsburgh, pp. 84-96.

Rao, K. P. and T. H. Bullock. 1954. Q10 as a function of size and habitat temperature in poikilotherms. The American Society of Naturalists **88**:33-44.

Raymont, J. E. G. and D. T. Gauld. 1951. The respiration of some planktonic copepods. Journal of the Marine Biological Association of the United Kingdom **29**:681-693.

Richardson, A. J. 2008. In hot water: zooplankton and climate change. ICES Journal of Marine Science **65**:279-295.

Richman, S. 1964. Energy transformation studies on *Diaptomus oregonensis*. Verhandlungen des Internationalen Verein Limnologie **15**:654-659.

Robinson, C. and P. J. L. Williams. 1993. Temperature and Antarctic plankton community respiration. Journal of Plankton Research **15**:1035-1051.

Roche-Mayzaud, O., P. Mayzaud, and D. C. Biggs. 1991. Medium-term acclimation of feeding and of digestive and metabolic enzyme activity in the neritic copepod *Acartia clausi*. I. Evidence from laboratory experiments. Marine Ecology Progress Series **69**:25-40.

Roddie, B. D., R. J. G. Leakey, and A. J. Berry. 1984. Salinity-temperature tolerance and osmoregulation in *Eurytemora affinis* (Poppe) (Copepoda: Calanoida) in relation to its distribution in the zooplankton of the upper reaches of the Forth Estuary. Journal of Experimental Marine Biology and Ecology **79**:191-211.

Rodríguez, V., F. Guerrero, and B. Bautista. 1995. Egg production of individual copepods of *Acartia grani* Sars from coastal waters: seasonal and diel variability. Journal of Plankton Research **17**:2233-2250.

Roff, J. C. 1973. Oxygen consumption of *Limnocalanus macrurus* Sars (Calanoida, Copepoda) in relation to environmental conditions. Canadian Journal of Zoology **51**:877-885.

Rosa, R., M. S. Pimentel, J. Boavida-Portugal, T. Teixeira, K. Trübenbach, and M. Diniz. 2012. Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone squid. PLoS ONE **7**:e38282.

Rosa, R., L. Trueblood, and B. A. Seibel. 2009. Ecophysiological influence on scaling of aerobic and anaerobic metabolism of pelagic gonatid squids. Physiological and Biochemical Zoology **82**:419-429.

Sastry, A. N. 1979. Metabolic adaptation of *Cancer irroratus* developmental stages to cyclic temperatures. Marine Biology **51**:243-250.

Satomi, M. and L. R. Pomeroy. 1965. Respiration and phosphorus excretion in some marine populations. Ecology **46**:877-881.

Sekiguchi, H. 1974. Relation between the ontogenetic vertical migration and the mandibular gnathobase in pelagic copepods. . Bulletin of the Faculty of Fisheries, Mie University **1**:1-10.

Souissi, S. and S. Ban. 2001. The consequence of individual variability in moulting probability and the aggregation of stages for modelling copepod population dynamics models. Journal of Plankton Research **23**:1279-1296.

Starr, R. C. 1973. Special methods-dry soil samples. In Handbook of Phycological Methods. Culture Methods and Growth Measurements, Ed. by Stein, J. R., Cambridge University Press, Cambridge, p. 159-167.

Svensson, J. E. 1997. Fish predation on *Eudiaptomus gracilis* in relation to clutch size, body size, and sex: a field experiment. Hydrobiologia **344**:155-161.

Teuber, L., R. Kiko, F. Séguin, and H. Auel. 2013. Respiration rates of tropical Atlantic copepods in relation to the oxygen minimum zone. Journal of Experimental Marine Biology and Ecology **448**:28-36.

The MathWorks Inc. 2009. MATLAB (The Language of Technical Computing):Version 7.9.0 (R2009b), Natick, Massachusetts, USA.

Thor, P. 2003. Elevated respiration rates of the neritic copepod *Acartia tonsa* during recovery from starvation. Journal of Experimental Marine Biology and Ecology **283**:133-143.

Tsuda, A. 1994. Starvation tolerance of a planktonic marine copepod *Pseudocalanus newmani* Frost. Journal of Experimental Marine Biology and Ecology **181**:81-89.

Tsugeki, N., H. Oda, and J. Urabe. 2003. Fluctuation of the zooplankton community in Lake Biwa during the 20th century: a paleolimnological analysis. Limnology **4**:101-107.

Turner, J. T. 2004. The Importance of Small Planktonic Copepods and Their Roles in Pelagic Marine Food Webs. Zoological Studies **43**:255-266.

Twombly, S. and C. W. Burns. 1996. Exuvium analysis: a nondestructive method of analyzing copepod growth and development. Limnology and Oceanography **41**:1324-1329.

Urabe, J., K. Kawabata, M. Nakanishi, and K. Shimizu. 1996. Grazing and food size selection of zooplankton community in Lake Biwa during BITEX '93. Japanese Journal of Limnology **57**.

Urabe, J., M. Nakanishi, and K. Kawabata. 1995. Contribution of metazoan plankton to the cycling of nitrogen and phosphorus in Lake Biwa. Limnology and Oceanography **40**:232-241.

Urabe, J. and Y. Watanabe. 1990. Influence of food density on respiration rate of two crustacean plankters, *Daphnia galeata* and *Bosmina longirostris*. Oecologia **82**:362-368.

Uye, S. 1981. Fecundity studies of neritic calanoid copepods *Acartia clausi* Giesbrecht and *A. steueri* Smirnov: a simple empirical model of daily egg production. Journal of Experimental Marine Biology and Ecology **50**:255-271.

Uye, S. 2000. Why does *Calanus sinicus* prosper in the shelf ecosystem of the Northwest Pacific Ocean? ICES Journal of Marine Science **57**:1850-1855.

Uye, S., Y. Iwai, and S. Kasahara. 1983. Growth and production of the inshore marine copepod *Pseudodiaptomus marinus* in the central part of the Inland Sea of Japan. Marine Biology **73**:91-98.

Uye, S., N. Nagano, and T. Shimazu. 2000. Abundance, Biomass, Production and Trophic Roles of Micro- and Net-Zooplankton in Ise Bay, Central Japan, in Winter. Journal of Oceanography **56**:389-398.

Vadstein, O., H. Stibor, B. Lippert, K. Løseth, W. Roederer, L. Sundt-Hansen, and Y. Olsen. 2004. Moderate increase in the biomass of omnivorous copepods may ease grazing control of planktonic algae. Marine Ecology Progress Series **270**:199-207.

Verburg, P., R. E. Hecky, and H. Kling. 2003. Ecological consequences of a century of warming in Lake Tanganyika. Science **301**:505-507.

Vidal, J. 1980. Physioecology of zooplankton. III. Effects of phytoplankton concentration, temperature, and body size on the metabolic rate of *Calanus pacificus*. Marine Biology **56**:195-202.

Vuorinen, I., J. Hänninen, M. Viitasalo, U. Helminen, and H. Kuosa. 1998. Proportion of copepod biomass declines with decreasing salinity in the Baltic Sea. ICES Journal of Marine Science **55**:767-774.

Watras, C. J. and J. F. Haney. 1980. Oscillations in the reproductive condition of *Diaptomus leptopus* (Copepoda: Calanoida) and their relation to rates of egg-clutch production. Oecologia **45**:94-103.

Williams, P. J. l. and N. W. Jenkinson. 1982. A transportable microprocessor-controlled precise Winkler titration suitable for field station and shipboard use. Limnology and Oceanography **27**:567-584.

Williams, T. D. and M. B. Jones. 1994. Effects of temperature and food quantity on postembryonic development of *Tisbe battagliai* (Copepoda: Harpacticoida). Journal of Experimental Marine Biology and Ecology **183**:283–298.

Williamson, C. E. and J. W. Reid 2001. Copepoda, pp. 915-954. In: J. H. Thorp and A. P. Covich (Second Edition) Ecology and classification of North American freshwater invertebrates. Academic Press, Inc., San Diego.

Williamson, C. E. and N. M. Butler. 1986. Temperature, food and mate limitation of copepod reproductive rates: separating the effects of multiple hypotheses. Journal of Plankton Research **9**:821-836.

Williamson, C. E., N. M. Butler, and L. Forcina. 1985. Food limitation in naupliar and adult *Diaptomus pallidus*. Limnology and Oceanography **30**:1283-1290.

Williamson, C. E., J. E. Saros, and D. W. Schindler. 2009. Calimate change: Sentinels of change. Science **323**:887-888.

Wiltshire, K. H., A. M. Malzahn, K. Wirtz, W. Greve, S. Janisch, P. Mangelsdorf, B. F. J. Manly, and M. Boersma. 2008. Resilience of North Sea phytoplankton spring bloom dynamics: An analysis of long-term data at Helgoland Roads. Limnology and Oceanography **53**:1294-1302.

Winder, M., J. E. Reuter, and S. G. Schladow. 2009. Lake warming favours small-sized planktonic

diatom species. Proceedings of the Royal Society of London B: Biological Sciences **276**:427-435.

Winder, M., P. Spaak, and W. M. Mooij. 2004. Trade-offs in *Daphnia* habitat selection. Ecology **85**:2027-2036.

Yampolsky, L. Y., T. M. M. Schaer, and D. Ebert. 2013. Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. Proceedings of the Royal Society B, Biological Sciences **281**:20132744, doi:10.1098/rspb.2013.2744.

Yoshida, T., T. B. Gurung, M. Kagami, and J. Urabe. 2001a. Contrasting effects of a cladoceran (*Daphnia galeata*) and a calanoid copepod (*Eodiaptomus japonicus*) on algal and microbial plankton in a Japanese lake, Lake Biwa. Oecologia **129**:602-610.

Yoshida, T., M. Kagami, G. Bahadur, and J. Urabe. 2001b. Seasonal succession of zooplankton in the north basin of Lake Biwa. Aquatic Ecology **35**:19-29.

Zeiss, F. R. 1963. Effects of population densities on zooplankton respiration rates. Limnology and Oceanography **8**:110-115.

Zeller, M., R. Jiménez-Melero, and B. Santer. 2004. Diapause in the calanoid freshwater copepod *Eudiaptomus graciloides*. Journal of Plankton Research **26**:1379-1388.

APPENDIX

Appendix 1

C media

To 950 ml of distilled water, add the elements. Bring the final volume to 1L with distilled water. Use 1 mol HCl to regulate pH to 7.5. Autoclave (120°C 20 min).

Elements	Stock solution	Quantity
$Ca(NO_3)_2 \cdot 4H_2O$	15 g/100 ml	1 ml
KNO_3	10 g/100 ml	1 ml
β-Na_2glycerophosphate·$5H_2O$ (Disodium β-Glycerophosphate)	5 g/100 ml	1 ml
$MgSO_4 \cdot 7H_2O$	4 g/100 ml	1 ml
Vitamin B_{12}[b]	0.01 mg/100 ml	1 ml
Biotin[b]	0.01 mg/100 ml	1 ml
Thiamine HCl	1 mg/100 ml	1 ml
P IV metals[a]	-	3 ml
Tris (hydroxymethyl) aminomathane	50 g/1000 ml	10 ml

[a] See P IV metals
[b] Store in refrigerator or freezer

Add 1.5 g agar to 100 ml of medium to give a solid medium.

Reference

Ichimura, T. (1971) Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum*. In *Proceedings of the Seventh International Seaweed Symposium*, University of Tokyo Press, Tokyo, pp. 208-214.

Appendix 2

VT media

To 950 ml of distilled water, add the elements. Bring the final volume to 1L with distilled water. Use 1 mol NaOH to regulate pH to 7.5. Autoclave (120°C 20 min).

Elements	Stock solution	Quantity
$Ca(NO_3)_2 \cdot 4H_2O$	11.78 g/100 ml	1 ml
β-Na_2glycerophosphate$\cdot 5H_2O$ (Disodium β-Glycerophosphate)	5 g/100 ml	1 ml
$MgSO_4 \cdot 7H_2O$	4 g/100 ml	1 ml
KCl	5 g/100 ml	1 ml
Vitamin$\cdot B_{12}$[b]	0.01 mg/100ml	1 ml
Biotin[b]	0.01 mg/100 ml	1 ml
Thiamine HCl	1 mg/100 ml	1 ml
P IV metals[a]	-	3 ml
Glycylglycine	50 g/1000 ml	10 ml

[a] See P IV metals
[b] Store in refrigerator or freezer

Reference

Provasoli, L. and I. J. Pintner. (1959) Artificial media for fresh-water algae: problems and suggestions. In *The Ecology of Algae. Spec. Pub. No. 2,,* Eds. by Tryon, C. A., Jr. & Hartmann, R. T., Pymatuning Laboratory of Field Biology, University of Pittsburgh, Pittsburgh, pp. 84-96.

Starr, R. C. (1973) Special methods-dry soil samples. In *Handbook of Phycological Methods. Culture Methods and Growth Measurements*, Ed. by Stein, J. R., Cambridge University Press, Cambridge, pp. 159-167.

Appendix 3

P IV metals

To 450 ml distilled water, add the elements. Bring the final volume to 500 ml with distilled water.

Elements	Stock solution	Quantity
Na_2 EDTA·$2H_2O$	-	0.5 g
$FeCl_3 \cdot 6H_2O$	1.96 g/100 ml	5 ml
$MnCl_2 \cdot 4H_2O$	0.36 g/100 ml	5 ml
$ZnSO_4 \cdot 7H_2O$	0.22 g/100 ml	5 ml
$CoCl_2 \cdot 6H_2O$	0.04 g/100 ml	5 ml
$Na_2MoO_4 \cdot 2H_2O$	0.025 g/100 ml	5 ml

Reference

Provasoli, L. and I. J. Pintner. (1959) Artificial media for fresh-water algae: problems and suggestions. In *The Ecology of Algae. Spec. Pub. No. 2,,* Eds. by Tryon, C. A., Jr. & Hartmann, R. T., Pymatuning Laboratory of Field Biology, University of Pittsburgh, Pittsburgh, pp. 84-96.