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

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Introduction

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.

Chapter 1

Effects of temperature on life history traits of *E. japonicus* were examined to evaluate its population dynamics in Lake Biwa. Embryonic and post-embryonic development times and reproduction were determined in the laboratory at four temperature conditions (10, 15, 20 and 25°C) 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 (Fig. 1).

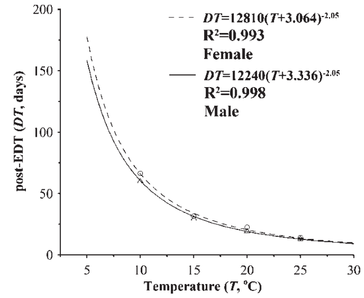

Fig. 1 Relationship between temperature and median post-embryonic development time (post-EDT, days) of *Eodiaptomus japonicus* males (*Open triangles, solid line*) and females (*open circles, dotted line*) reared at four temperature conditions.

Only 15% of the individuals survived until the adult stage at 10°C, while 40% did so at >15°C. 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°C, suggesting that *E. japonicus* retards its growth during winter. The null value obtained at 8.6°C for the computed population growth rate *r* supports the idea of an overwintering strategy (Fig. 2).

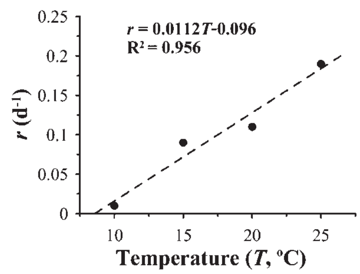

Fig. 2 Population growth rate (r, day^{-1}) of *Eodiaptomus japonicus* reared at four temperature conditions. The linear fit and its coefficient of determination are provided.

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.

Chapter 2

Life history traits of *E. japonicus* from Lake Biwa were examined under 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°C) 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°C, although survival was <42% at all six of the other food-temperature combinations. Post-embryonic development times to adult stage in males and females were affected by both food concentration and temperature; median development times ranged from 28.7–37.3 and 31.4–35.0 days at 15°C and 13.7–23.9 and 14.3–27.7 days at 25°C, 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°C. 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°C and from 0.799 mm to 0.904 mm at 25°C.

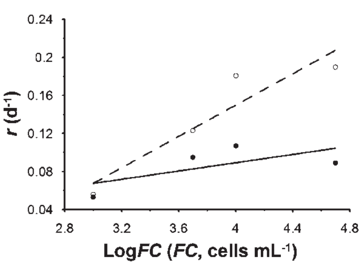

Fig. 3 Estimated population growth rate (*r*, day^{-1}) of *Eodiaptomus japonicus* reared under different food treatments at 15 (*filled symbols* and *solid line*) and 25 °C (*open symbols* and *dashed line*).

Population growth rates calculated from the experimental data increased with food concentration but the increase was more important at 25°C (Fig. 3). This 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°C.

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.

Chapter 3

Oxygen consumption rates (*R*, $\mu L O_2$ mg-dry-weight $^{-1}$ h^{-1}) of *E. japonicus* collected from Lake Biwa were determined in a temperature range of 8−30 °C (i.e. 8, 10, 15, 20, 25, 28 and 30°C) using an optical oxygen meter after two different temperature acclimatizations (Fig. 4).

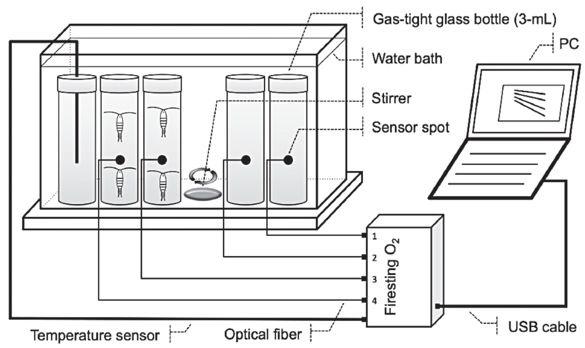

Fig. 4 Schematic diagram on a water-bath and closed-bottle unit for measuring oxygen consumption of copepods using a fiber-optic oxygen meter in an incubator.

Average *R* in adult stage varied 1.64–10.78 and 1.55–9.77 $\mu L O_2$ mg-dry-weight $^{-1}$ h^{-1} with experimental temperatures for males and females, respectively, acclimatized at 15°C, while 1.71–11.13 and 1.98–10.10 $\mu L O_2$ mg-dry-weight $^{-1}$ h^{-1} for those acclimatized at 25°C. *R* exponentially increased with increasing temperature from 8 to 28°C and deviated from the exponential phase at 30°C for the animals acclimatized at both 15 and 25°C (Fig. 5).

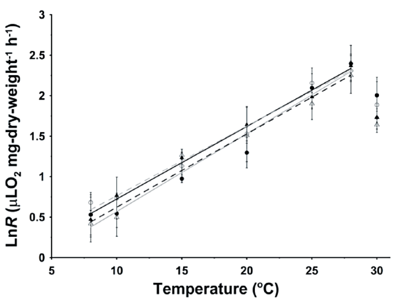

Fig. 5 Ln-transformed weight-specific respiration rates (R, μLO_2 mg-dry-weight^{-1} h^{-1}) of *Eodiaptomus japonicus* acclimatized at 15 °C (T15, *triangles*) and 25 °C (T25, *circles*) at seven experimental temperatures (*solid symbols* in male and *open symbols* in female). The regression lines of R against temperature except for 30 °C (*black* in T15 and *grey* in T25, *solid* in male and *dashed* in female). Error bars indicate standard deviation.

No significant differences of R from acclimatizations and genders were shown without any interactions. Rs in various copepodid stages acclimatized at 15°C were always higher at 25°C than those at 15°C, but not correlated with body weight at both temperatures. According to these results, relationship between R and experimental temperature (T, °C) ranged from 8 to 28°C could be expressed as an exponential function; R = 0.8072 e0.0897T (r^2 = 0.995, P ＜ 0.05) for C1 to C6. Q_{10} between 15 and 25 °C could be calculated as 2.3.

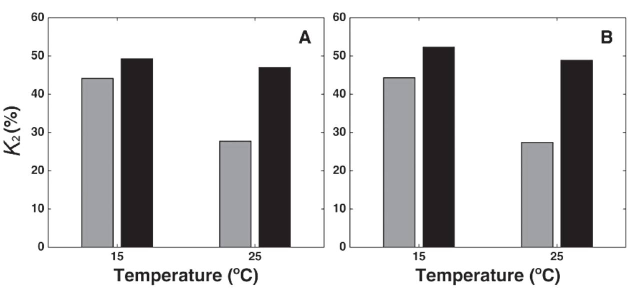

Fig. 6 Estimated net growth efficiencies (K_2, %) for food-limited (*grey bars*) and well-fed (*black bars*) *Eodiaptomus japonicus* males (A) and females (B) at 15 and 25 °C.

Net growth efficiencies (K_2) calculated in well-fed individuals were 47－52% at both 15 and 25°C, whereas those in food-limited ones were 44% at 15°C but decreased to 27－28% at 25°C (Fig. 6). The low K_2 in the food-limited animals at 25°C may imply that the metabolic cost at higher temperatures induces lowering the growth rate under food-limited environment.

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 *E. 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 (Fig. 7).

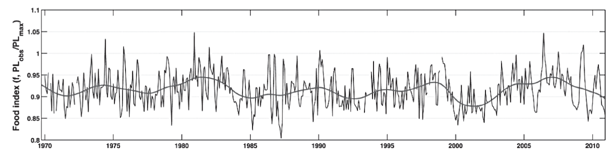

Fig. 7 Monthly variation of food index (f, PL_{obs}/PL_{max}) in *Eodiaptomus japonicus* at St. 4 in Lake Biwa from 1971 to 2010. Regression line showed the fluctuation trend in a ten years window.

Then, the specific growth rate k was also calculated from an equation with multiple factors of f and ambient temperature (T, °C): $k = -1.3607 f - 0.1037 T + 0.1225 f \times T + 1.237$ (n = 8, r^2 = 0.9), showing less oscillation even in the eutrophication period in 1970's and 1980's. Finally, we calculated the annual production (Pa) of *E. japonicus* from the biomass and k during the last 4 decades (Fig. 8). The productions calculated were relatively stable until the late 1990's, but tended to increase after 2000, related to biomass trend.

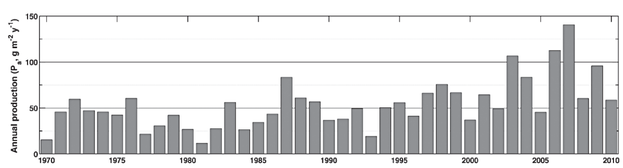

Fig. 8 Year-to-year variation of annual production (P_a, g m^{-2} y^{-1}) in *Eodiaptomus japonicus* at St. 4 in Lake Biwa from 1970 to 2010.

Table 1 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 °C 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 (°C)	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 °C	17.2	0.915	1.15	0.137**	57.5
Average of 1971−2010 + 3 °C & 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 : $k = -1.3607 f - 0.1037 T + 0.1225 f \times T + 1.237$

According to the global warming scenarios, we predicted average Pa at the end of 21st century (Table 1). Average *P*a could increase from 46.9 to 57.5 g m^{-2} y^{-1} due to 3°C 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.