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Note

# Feeding rate of naupliar *Eodiaptomus japonicus*

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Among existing metazoans, the naupliar larva is one of the most abundant forms (Fryer 1986). Copepod nauplii are often dominant zooplankters in aquatic communities, and their activities should have a significant impact on the ecosystem. Nevertheless, our knowledge of their ecology is quite restricted. The feeding rates of copepods, for example, have been measured after molting to copepodids and particularly for the adults. Naupliar stages are usually clumped together even when they are examined, and there are only a few studies on the stage-specific feeding rates (Fernández 1979; Paffenhöfer 1984). For freshwater species, less information is available (e.g. Zánkai 1991).

The planktonic copepod *Eodiaptomus japonicus* (Burckhardt 1913) is widely distributed in the freshwaters of Japan. In Lake Biwa, the largest lake in Japan, *E. japonicus* is the most abundant zooplankter (Kawabata 1987a) and an important grazer on phytoplankton (Nakanishi et al. 1992). For the copepodids and adults of *E. japonicus*, Okamoto (1984) has measured the feeding rates on natural phytoplankton assemblages, and Kawabata (1987b) has analyzed the gut contents. However, the sole knowledge on the feeding ecology of naupliar *E. japonicus* is that it ingests food from the second stage (Kawabata 1987b). *Eudiaptomus gracilis* was also reported to begin feeding in the second naupliar stage (Zánkai 1991).

Measurement of feeding rate in naupliar stages is constrained by the small amounts of food consumption by each individual. Numerous nauplii need to be put into a container to detect change in food concentration before and after feeding. On the contrary, radiotracer techniques permit sensitive measurement of the small intake. Among several kinds of radionuclides, $^{32}$P emits the highest energy and is most practical for Geiger-Müller counting (Peters 1984). In the present study, the feeding rate of *E. japonicus* was measured in the naupliar stages II, III and VI (NII, NIII and NVI) and also in copepodid stage I (CI) using $^{32}$P-labeled *Chlamydomonas reinhardi*.

*Eodiaptomus japonicus* and lake water were collected from the epilimnion in the pelagic area of the north basin of Lake Biwa. For the measurement of feeding rate in NII and NIII, ovigerous females were collected several days before experiments and the hatching nauplii were used. NV and NVI *E. japonicus* were sorted from the plankton sample for measurement of feeding rates in NVI and CI. The total length (mm) was 0.16 in NII, 0.19 in NIII, 0.30 in NVI and 0.42 in CI.

Nauplii were kept in lakewater filtered through a Whatman GF/F filter, and *C. reinhardi* (size, 7.5 $\mu$m×10 $\mu$m; IAM strain C-240) was added as food at a density of about 0.03 mg C l$^{-1}$. Algal cells were counted with a hemacytometer, and the carbon content of a cell was assumed to be 25 pg C (Urabe & Watanabe 1990). Culture and experiments were performed at 25°C, simulating the temperature in the field.

After acclimation for a day, nauplii were sorted under a stereomicroscope about 2 h before feeding trials, and 15–42 individuals of each stage were kept in a 100 ml Erlenmeyer flask filled with GF/F-filtered lakewater.

The cells of *C. reinhardi* in growth phase were filtered through a 10 $\mu$m nylon net, secured from the culture medium by centrifugation, resuspended in the GF/F-filtered lakewater with added $^{32}$P-$H_3PO_4$, and incubated for 18–20 h under fluorescent lamp illumination (about 12 W m$^{-2}$). Then the labeled algal cells were secured by centrifugation and washing, and introduced into each experimental flask containing animals. A part of the inoculated suspension was filtered through a Whatman GF/C filter and algal radioactivity was measured with a gas-flow type Geiger-Müller Counter.

Experimental conditions are shown in Table 1. In standard experiments, nauplii were fed on labeled algae at densities of 0.78–1.05 mg C l$^{-1}$ for 8–17 min under dim light. To examine the time course of food incorporation in NII and NVI, 3 experimental flasks were prepared and nauplii were fed for different durations (8–32 min). The effect of food concentration was examined for NVI using 4 algal densities (0.08–0.79 mg C l$^{-1}$) in duplicate. Before this experiment, nauplii were kept in lakewater filtered through a 40 $\mu$m nylon net instead of fed on *C. reinhardi* so as not to introduce unlabelled algal cells when nauplii were put into experimental flasks with low algal densities.

After feeding, nauplii were fixed with 95% ethanol after

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**Table 1.** Developmental stage of *Eodiaptomus japonicus*, type of experiment (S, standard; D, feeding duration; F, food concentration), food concentration (mg C $l^{-1}$), feeding duration (min), number of animals recovered, and feeding rate (ng C ind. $^{-1}$ $d^{-1}$).

| Stage | Type | Food conc. | Duration | N | Feeding rate |
|---|---|---|---|---|---|
| NII | S, D | 0.94 | 8.0 | 29 | 101 |
| NII | D | 0.83 | 16.5 | 24 | 71 |
| NII | D | 0.84 | 24.0 | 20 | 25 |
| NIII | S | 1.05 | 16.0 | 26 | 94 |
| NVI | S, D | 0.87 | 9.5 | 16 | 220 |
| NVI | D | 0.86 | 21.5 | 23 | 156 |
| NVI | D | 0.88 | 32.0 | 22 | 129 |
| NVI | F | 0.08 | 12.3 | 10 | 6 |
| NVI | F | 0.08 | 10.2 | 9 | 40 |
| NVI | F | 0.15 | 11.0 | 9 | 61 |
| NVI | F | 0.16 | 10.4 | 12 | 141 |
| NVI | F | 0.30 | 10.7 | 11 | 199 |
| NVI | F | 0.41 | 16.0 | 13 | 180 |
| NVI | S, F | 0.78 | 10.0 | 12 | 89 |
| NVI | S, F | 0.79 | 10.3 | 11 | 171 |
| CI | S | 0.98 | 17.0 | 26 | 519 |
| CI | S | 0.79 | 11.0 | 13 | 196 |

anesthesia with cold carbonated water. Fixed animals were washed with the GF/F-filtered lakewater, and pipetted individually onto a Whatman GF/C filter (diameter, 25 mm) recording the number recovered. The filter with nauplii was glued onto a planchet, dried on a hot plate, and examined for radioactivity with a gas-flow type Geiger-Müller Counter.

Feeding rate ($F$, cells ind.$^{-1}$ min$^{-1}$) was calculated from the radioactivities of animals (*Ran*) and algae (*Ral*, cell$^{-1}$): $F=(Ran/N-Rbo)/Ral/t$, where $N$ is the number of animals, *Rbo* is the radioisotope attached to the body surface of an animal, and $t$ (min) is feeding duration. *Rbo* was measured with heat-killed nauplii and was 28% of *Ran*/*N* on average.

The amount of food incorporation increased until 16.5 min in NII *E. japonicus* and until 32 min in NVI (Fig. 1). The feeding rate, which is given by the slope, was largest in the first interval (8.0–9.5 min) in both stages (Table 1). Feeding duration shorter than 10 min is hence preferable and longer durations may underestimate the feeding rate. Nevertheless, the feeding rates in NIII and CI with long durations (16–17 min) were not particularly low. Therefore, the feeding durations of the present standard experiments (8–17 min) were practical though not ideal. Also in *Eudiaptomus gracilis*, it took more than 10 min for particles to pass through the guts of all naupliar stages (Zánkai 1991).

The feeding rate of NVI *E. japonicus* increased with algal density up to about 0.3 mg C $l^{-1}$, and did not exceed 200 ng C ind.$^{-1}$ $d^{-1}$ even at higher algal densities (Fig. 2). Hence, food concentrations used in the standard experiments (0.78–1.05 mg C $l^{-1}$) were higher than the incipient threshold food concentration, 0.3 mg C $l^{-1}$.

The mean feeding rate of NVI *E. japonicus* in the standard experiments was 160 ng C ind.$^{-1}$ $d^{-1}$ (standard deviation=66, $n$=3), being higher than that in NII and NIII (Table 1). The results for CI were variable; one value was similar to results from NVI, and the other was much higher. The maximum feeding rate of early copepodid *E. japonicus* on $^{14}$C-labeled natural algae was 270 ng C ind.$^{-1}$ $d^{-1}$ at a density of 0.4 mg C $l^{-1}$ (Okamoto 1984). This value agrees well with the present results on CI.

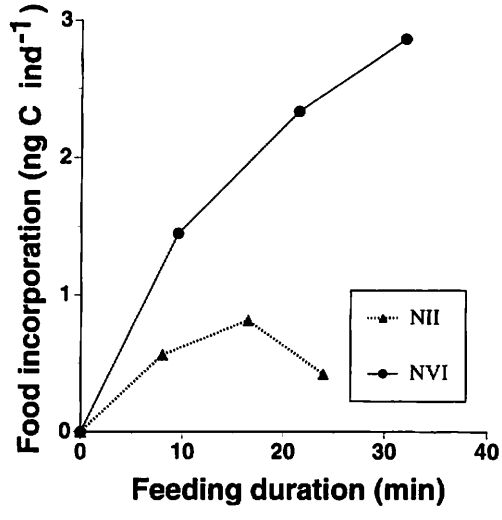


**Fig. 1.** Temporal change in the amount of food incorporation by naupliar *Eodiaptomus japonicus*.

The feeding rate of late naupliar *Diaptomus minutus* (length, 0.21 mm) on $^{33}$P-labeled *C. reinhardi* calculated from the data of Bogdan & Gilbert (1984) using the present conversion factors was about 150 ng C ind.$^{-1}$ $d^{-1}$ at a density of 0.06 mg C $l^{-1}$. This value is higher than the present result for NIII *E. japonicus* (94 ng C ind.$^{-1}$ $d^{-1}$) of similar size (length, 0.19 mm) even though food concentration was lower than in the present

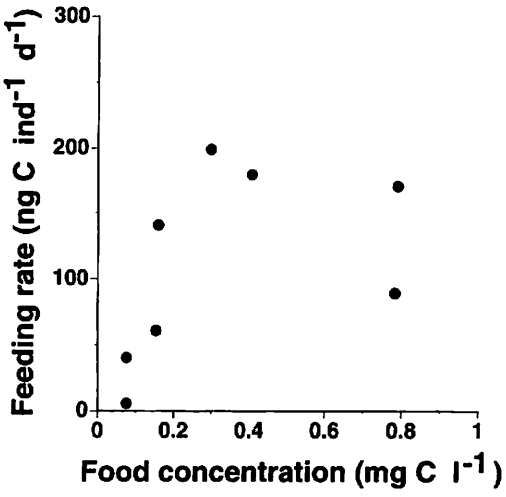


**Fig. 2.** Effect of food concentration on the feeding rate of NVI *Eodiapomus japonicus*.

study (1.05 mg C l$^{-1}$). The feeding rate of NIII *Calanus pacificus* (length, 0.30 mm) attained a maximum of 600 ng C ind.$^{-1}$ d$^{-1}$ at an algal density of 0.8 mg C l$^{-1}$ (Fernández 1979). NVI *E. japonicus*, whose size is similar to NIII *C. pacificus*, showed lower feeding rates at similar food concentrations (Table 1). Thus the feeding ability of naupliar *E. japonicus* was lower than both that of another freshwater diaptomid and a marine calanoid species.

Nakanishi et al. (1992) estimated that naupliar *E. japonicus* consumed 1–9% of daily gross primary production in Lake Biwa. Therefore, precise measurement of the feeding rate of the nauplii is needed for understanding their role in the ecosystem. In addition to the present study, the feeding rate of naupliar *E. japonicus* should be examined in other stages and at other temperatures.

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