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Relationship of egg production rates of the planktonic copepod *Calanus sinicus* to phytoplankton availability in the Inland Sea of Japan

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Abstract: Egg production rate, clutch size and spawning frequency of the planktonic copepod *Calanus sinicus* were investigated in relation to phytoplankton availability in the Inland Sea of Japan. Egg production rate increased asymptotically with increasing chlorophyll-*a* concentration in various size categories of phytoplankton except for the 1–5 μm fraction, which may be unutilizable by female *C. sinicus*. The egg production rate was best estimated by the concentration of chlorophyll *a* retained on a 5-μm screen. The >5 μm chlorophyll-*a* concentrations which allowed 90% maximum fecundity were 2.26 $\mu g\ l^{-1}$ in April and 1.72 $\mu g\ l^{-1}$ in June. However, these concentrations were rarely met during our study period, demonstrating that the fecundity of *C. sinicus* is food-limited in the Inland Sea of Japan. Decreases in food availability reduced both clutch size and spawning frequency.

Key words: *Calanus sinicus*, egg production, food availability, Inland Sea of Japan

Introduction

The calanoid copepod *Calanus sinicus* is common in the shelf waters around China, Korea and Japan (Hulsemann 1994), and is one of the most important macrozooplankters in terms of biomass in the Inland Sea of Japan (Uye et al. 1987). Compared with other common copepod species in the Inland Sea of Japan, *C. sinicus* has two characteristic attributes: large body size (prosome length of adult females: ca. 2 mm) and a high specific growth rate (average during copepodite stages: 0.8 d^{-1} at 20°C, Uye 1988). Hence, this species is regarded as one of the key secondary producers, linking phytoplankton and higher trophic levels, like other *Calanus* species in many temperate and boreal waters (Marshall & Orr 1955, Conover 1988). Huang et al. (1993a) have demonstrated that although *C. sinicus* reproduces continuously throughout the year, the population shows remarkable seasonal variations in Harima Nada and Kii Channel, the eastern part of the Inland Sea of Japan, indicating that the reproductive rate of this species varies seasonally.

Although a variety of physical and biological factors affect the population dynamics of copepods, the variation in egg production rate is a primary determinant of population abundance, since it often determines the birth rate and recruitment rate of the population unless egg mortality is high. Amongst the several variables (e.g. temperature, food quality, season and lipid storage) influencing the egg production of copepods of the genus *Calanus* (Marshall & Orr 1952; Runge 1985a, b; Lin & Li 1986; Hirche & Bohrer 1987; Peterson 1988; Mullin 1991), food availability is the most important factor, particularly in temperate coastal waters where spatio-temporal variability of the environment is generally largest. The food of particle-grazing copepods is often difficult to identify in nature. Phytoplankton may be the major food for *C. sinicus* in the Inland Sea of Japan because of the high content of pigments derived from phytoplankton in the guts (Uye & Yamamoto 1995), although our personal observations reveal that this species also ingests various forms of microzooplankton (e.g. tintinnids, rotifers, copepod nauplii), as found for other *Calanus* species (Corner et al. 1976; Landry 1981; Ohman & Runge 1994).

In the present study, we investigated the fecundity of *C. sinicus* by incubating females in ambient seawater from the Inland Sea of Japan, and analyzed their fecundity in relation to the availability of phytoplankton food of various size categories. Based on the analysis, we determined the phytoplankton size categories utilized by female *C. sinicus*, and assessed the availability of their food in the Inland Sea of Japan.

Materials and Methods

Egg production rates of *Calanus sinicus* were determined during 3 cruises (April 1994, June 1994 and June 1995) of the T&R/V *Toyoshio Maru*, Hiroshima University, in the Inland Sea of Japan (see Table 1 for areas and numbers of stations). At each station, vertical profiles of temperature, salinity and in vivo fluorescence were monitored using a Sea-Bird CTD with a Sea-Tech fluorometer attached. Seawater was collected using a pair of 10-liter Van Dorn bottles at 4 or 5 depths (0.5, 5, 10, 20 m and 2 m above the sea-bottom), and 200 ml of water from each depth was poured onto a series of screen (20 μm Nitex) and filters (5 μm Nuclepore fil-

Table 1. Water temperature at 3 m and depth-weighted average chlorophyll concentration in various size fractions at stations in various areas of the Inland Sea of Japan where *Calanus sinicus* females were collected for spawning experiments.

Dates	Areas	No. of stations	Temperature (°C)	Chlorophyll (μg l^{-1}) 1–5 μm	5–20 μm	>20 μm
12–21	Kii Channel	2	14.0–15.1	0.53–0.77	0.16–0.33	0.10–0.14
Apr.	Osaka Bay	5	12.2–13.2	0.06–0.77	0.18–1.01	0.46–10.2
1994	Harima Nada	1	11.9	0.43	0.32	1.18
	Hiuchi Nada	2	12.0–13.0	0.64–0.73	0.07–0.24	0.61–1.31
	Hiroshima Bay	2	11.5–12.2	0.43–0.56	0.34–0.59	0.40–0.85
	Iyo Nada	5	12.6–14.0	0.86–1.37	0.16–0.47	0.04–0.33
	Suo Nada	1	13.2	1.09	0.16	0.43
	Bungo Channel	4	12.9–15.1	0.56–0.91	0.09–0.17	0.04–0.16
20–29	Harima Nada	3	17.8–20.5	0.92–2.25	0.30–2.87	0.10–1.37
June	Hiroshima Bay	2	18.4–18.9	0.81–0.88	0.54–0.71	0.85
1994	Iyo Nada	6	18.2–21.5	0.77–1.47	0.34–0.94	0.39–1.07
	Bungo Channel	3	19.8–19.9	0.93–1.41	0.36–0.70	0.28–0.31
12–15	Iyo Nada	3	16.8–18.4	0.61–0.85	0.10–0.25	0.06–0.22
June 1995	Suo Nada	2	18.5–19.8	0.46–0.58	0.15–0.47	0.13–0.21

ter, ca. 1 μm Whatman GF/C) to determine size-fractionated chlorophyll-*a* concentrations. These screens and filters were placed into plastic vials containing 6 ml of dimethylformamide (DMF) and kept in the dark at −20°C until later analysis with a Turner Designs fluorometer.

At each station, immediately after zooplankton were collected by a vertical tow of a plankton net (mouth diameter: 0.45 m, length: 2 m, mesh size: 300 μm, with 1-liter cod-end) from near-bottom depth (<50 m) to the surface, the contents of the cod-end were gently transferred to a 2-liter plastic dish containing ca. 1 liter of seawater. Twenty adult *C. sinicus* females were sorted with a wide-mouthed pipette and transferred individually into Plexiglas tubes (diameter: 7 cm, height: 10 cm) with a 530 μm-mesh screen at the bottom. These were immersed in plastic bottles containing ca. 350 ml of 105 μm-screened seawater from the same sampling site delivered from the ship's built-in pump (sampling depth: ca. 3 m). The great majority of spawned eggs presumably sank through the mesh; thereby egg cannibalism by maternal copepods was assumed to be minimal. These plastic bottles were kept in a tub and maintained at a constant temperature by running seawater delivered from the pump. Incubation ran for exactly 24 h.

After the incubation, the contents of each bottle were recovered on a 40-μm sieve, washed with Whatman GF/C filtered-seawater into a small plastic bottle, and preserved by adding neutralized formalin. Later, eggs and nauplii were counted under a compound microscope. When a single female produced two clutches that could be separated based on developmental stage, the clutch size was determined separately. Prosome lengths of females were measured using a video-micrometer (Olympus, VM–10).

Results

Egg Production Rates

We grouped our results into two categories, i.e. April experiments (22 stations, temperature range: 11.5–15.1°C) and June (1994 and 1995) experiments (19 stations, 16.8–21.5°C), in order to take into account the seasonal variation in ambient temperature. Fecundity of *C. sinicus* was expressed both as egg production rate (eggs female^{-1} d^{-1}) and specific egg production rate (d^{-1}). For the latter, body carbon weights of females (C, μg) were determined from prosome length (PL, μm) using the relationship, $\log C = -9.416 + 3.378 \log \mathrm{PL}$ (Uye 1988). Carbon content of an egg was assumed to be 0.183 μg (Uye, unpublished). Both egg production rates and specific egg production rates are plotted against food availability. Since adult females can perhaps utilize food resources at any depth through diel vertical migration (Uye et al. 1990; Huang et al. 1993b), we used the average concentrations of chlorophyll *a*, which were calculated by dividing the integrated values throughout the water column by the depth of the water column, in the following size categories: 1–5, 5–20, >20, >5 and >1 μm, (Figs 1, 2).

In April, mean prosome length of female *C. sinicus* at each station varied from 1,900 to 2,360 μm. Mean fecundity at each station varied from 0.5 to 71.6 eggs female^{-1} d^{-1} or from 0.001 to 0.14 d^{-1} (Fig. 1). The fecundity increased asymptotically with increasing chlorophyll-*a* concentration in all size categories but the 1–5 μm fraction. A negative correlation of fecundity to the 1–5 μm chlorophyll level indicates that phytoplankton in the smallest size range are not preyed on by female *C. sinicus*. In the other size categories, the data were fit to the Ivlev function with an intercept:

$$E = a + E_{max}(1 - e^{-bP}) \quad \text{or} \quad E_s = a + E_{smax}(1 - e^{-bP})$$

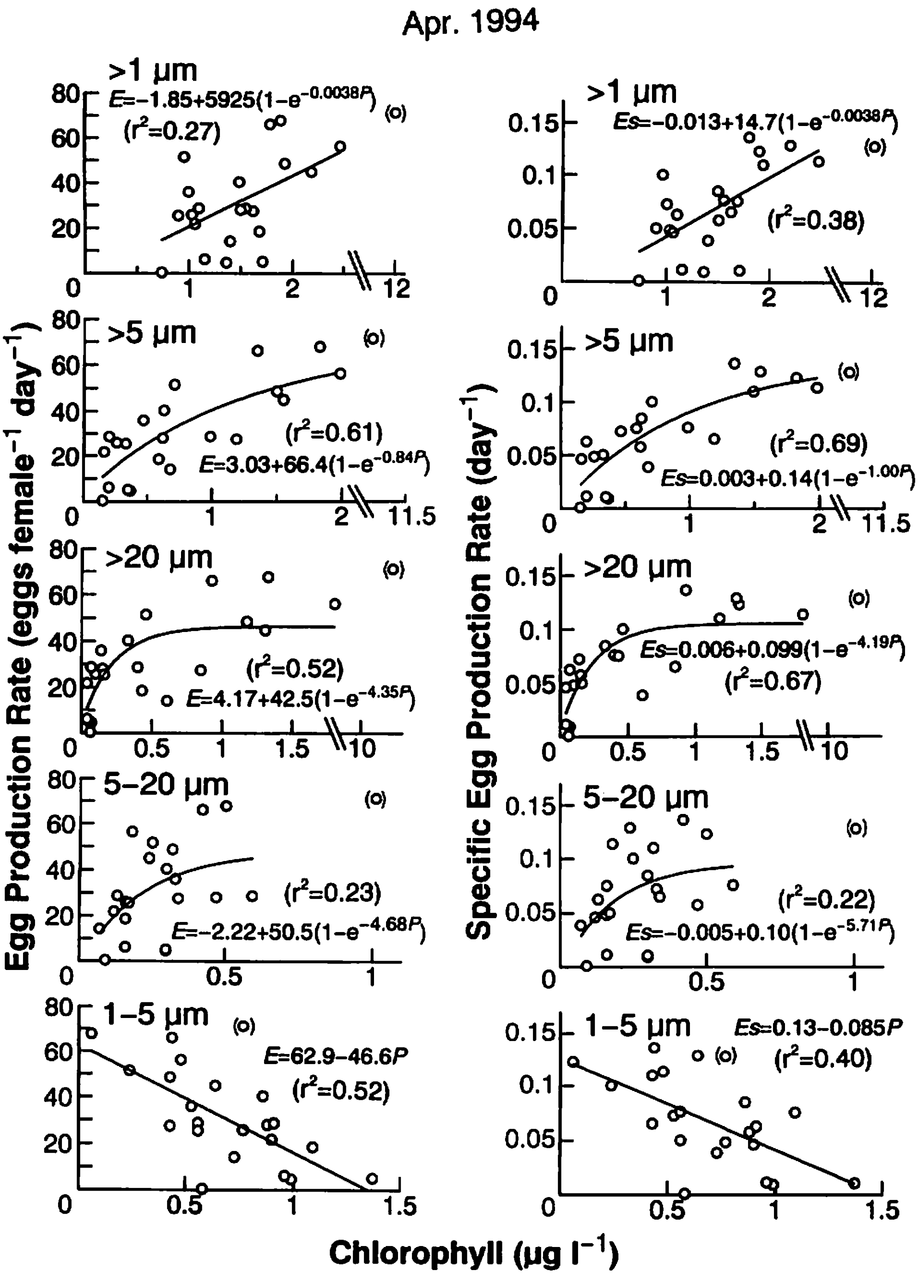

Fig. 1. Relation of egg production rate and specific egg production rate of *Calanus sinicus* to the chlorophyll-*a* concentration in various size fractions (1–5, 5–20, >20, >5 and >1 µm) in the experiments done in April 1994 in the Inland Sea of Japan. Non-linear regression equations for both rates (E, eggs female^{-1} d^{-1}; E_s, d^{-1}) as a function of chlorophyll concentration (P, µg l^{-1}) are given, except for the 1–5 µm fraction, where a linear regression is applied. The values in parentheses are excluded from the calculation.

where E and E_s are egg production rate and specific egg production rate, respectively, E_{max} and E_{smax} are the respective maximum rates, P is the chlorophyll-*a* concentration (μg l^{-1}), and a and b are constants. The correlation coefficients expressed as r^2 were slightly higher when fecundity was expressed in terms of specific egg production rate, as the difference in female body weight canceled out in this expression. The variance in both fecundities was most closely correlated to changes in the >5 μm chlorophyll concentration, suggesting that the phytoplankton in the >5 μm size fraction are the most important as food for this species.

In June, mean prosome lengths of females ranged from 1,980 to 2,140 μm. Egg production rates ranged from 0.1 to 30.1 eggs female^{-1} d^{-1}, and specific egg production rates from 0 to 0.092 d^{-1} (Fig. 2), being considerably lower than those observed in April. The functional response of fecundity to the chlorophyll-*a* concentration in various size fractions was basically similar to that in April. The chlorophyll-*a* concentration of the >5 μm size fraction was again the best estimator of the fecundity of *C. sinicus*.

Clutch Size and Frequency

Calanus sinicus spawned eggs freely into the water in discrete clutches, as already described for other *Calanus* species (Runge 1984, 1985b; Peterson 1988). The number of clutches produced by individual females during an experimental 24-h period varied from 0 to 2. Frequency of spawning at each station was determined by dividing the total number of clutches by the numbers of females (i.e. 20) and assumed that the specimens used in the experiment were representative of the *C. sinicus* population at the sampling site.

In April, mean clutch sizes varied from 10.0 to 67.7 eggs, and clutch frequencies varied from 0.05 to 1.3 d^{-1}. These variables also increased asymptotically with increasing >5 μm chlorophyll concentration (Fig. 3). In June, mean clutch sizes ranged from 7.3 to 23.3 eggs, and showed no significant correlation to the level of >5 μm chlorophyll. Spawning frequencies varied from 0 to 1.5 d^{-1}, and the Ivlev function well described this non-linear relationship (Fig. 3).

Discussion

The correlation between the fecundity of *C. sinicus* and the chlorophyll-*a* concentration of various size categories revealed differences in the utilization of phytoplankton depending on their size. Negative correlation or the absence of a relationship between the two variables showed that the phytoplankton that passed through the 5-μm screen could not be utilized. On the other hand, phytoplankton larger than this were suitable food, since the variance in specific egg production rates was explained most aptly by the change in chlorophyll-*a* concentration in this size category. Total (i.e. >ca. 1 μm) chlorophyll-*a* concentration, which is routinely measured in general oceanographic surveys, is apparently a poor indicator of food availability for female *C. sinicus*. A similar conclusion was arrived at by Runge (1985a) for *C. pacificus* in Puget Sound, USA, from his investigation of the relationship between egg production rate and phytoplankton availability. Although no information is available for *C. sinicus*, the minimum size of particles effectively captured is about 8–10 μm for female *C. pacificus*, based on the laboratory feeding experiments (Frost 1972). This value may also be applicable to *C. sinicus*, as the body size of both species is almost the same.

The concentrations of >5 μm chlorophyll that allowed 90% maximum fecundity (i.e. the asymptote of the Ivlev function, see Figs 1 & 2) of *C. sinicus* were 2.28 and 1.72 μg l^{-1} in the April and June experiments, respectively. Using a conversion factor of 40, these are equivalent

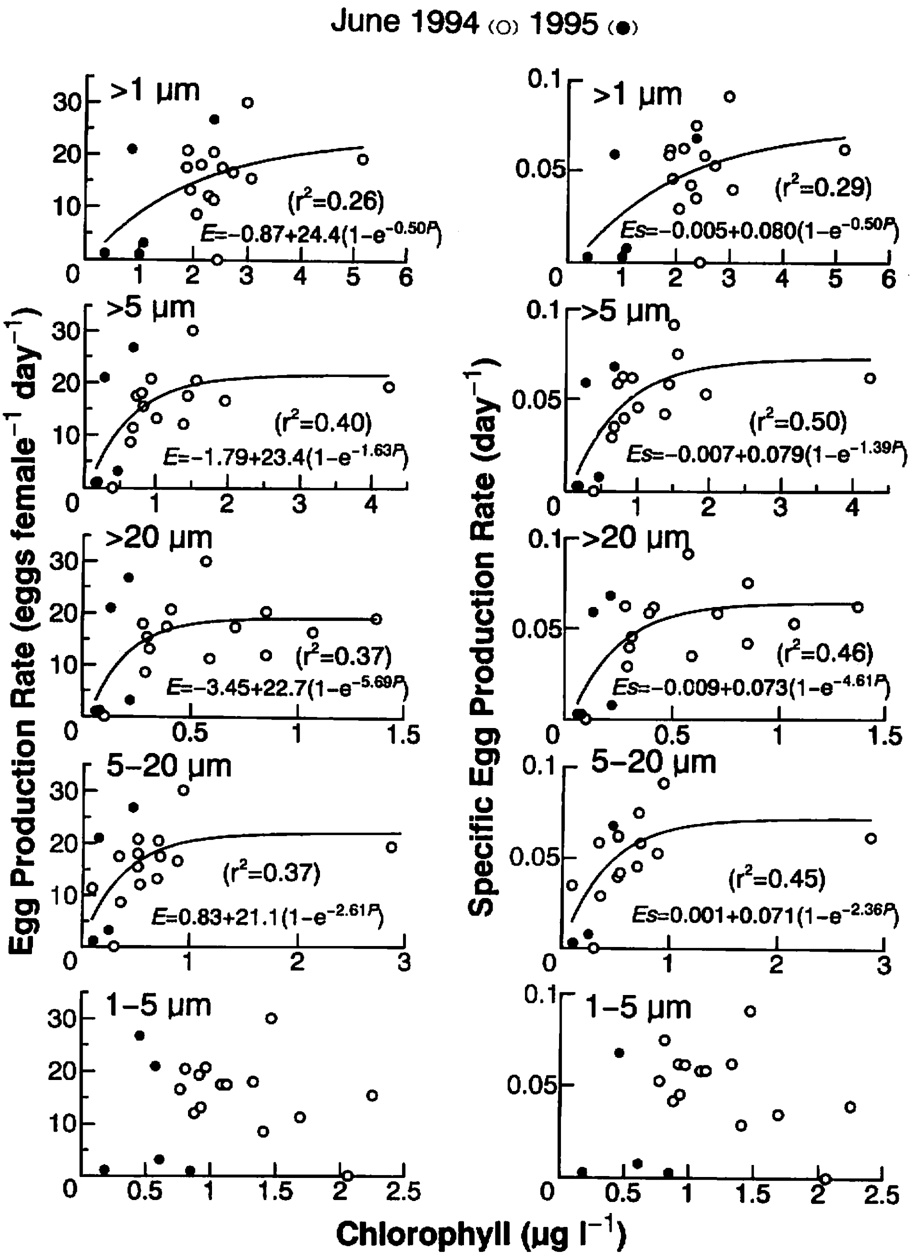

Fig. 2. Relation of egg production rate and specific egg production rate of *Calanus sinicus* to the chlorophyll-*a* concentration in various size fractions (1–5, 5–20, >20, >5 and >1 μm), in the experiments done in June 1994 (O) and 1995 (●) in the Inland Sea of Japan. Non-linear regression equations (see Fig. 1) are given, except for the 1–5 μm fraction, where the regression is insignificant.

to 90 and 69 μgC l^{-1}, respectively, nearly the same or slightly lower than the phytoplankton carbon concentration (ca. 100 μgC l^{-1}) at which the specific egg production rate of *C. pacificus* in Puget Sound approaches its limit (Runge 1985a). Phytoplankton concentrations above

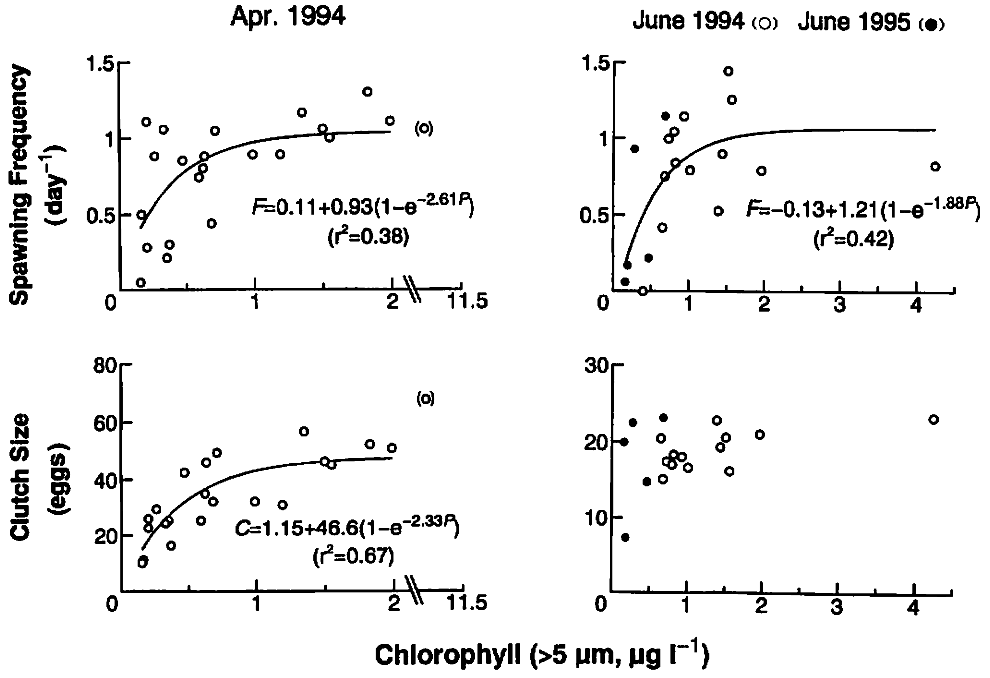

Fig. 3. Relation of clutch size and spawning frequency of *Calanus sinicus* to the >5 μm chlorophyll-*a* concentration in April 1994 and in June 1994 (○) and 1995 (●) in the Inland Sea of Japan. Non-linear equations for clutch size (*C*, eggs) and spawning frequency (*F*, d^{-1}) as a function of chlorophyll concentration (*P*, $\mu g\ l^{-1}$) are given, except for clutch size in June when the regression is insignificant. The values in parentheses are excluded from the calculation.

these were rarely met in our study, at only 1 out of 22 stations in April and at 2 out of 19 stations in June, indicating that the egg production rate of *C. sinicus* is highly food-limited in the Inland Sea of Japan.

We evaluated the contribution of microzooplankton to the diet of female *C. sinicus* during the period of our study. In the Inland Sea of Japan, there are marked geographical and seasonal variations in the abundance and biomass of microzooplankton (i.e. ciliated protozoans and copepod nauplii). The average abundance and biomass over the entire Inland Sea of Japan was 400 indiv. l^{-1} and 2.79 μgC l^{-1} in April 1994 and 272 indiv. l^{-1} and 2.68 μgC l^{-1} in June 1994, respectively (Uye et al. 1996). These carbon biomass levels were much lower than the ciliate biomass (11.74 μgC l^{-1}) in the Gulf of St. Lawrence in summer, when *C. finmarchicus* sustained high egg production rates by ingesting these protozoans (Ohman and Runge 1994). Compared to the >5 μm fraction of phytoplankton biomass (mean: 59.6 and 42.0 μgC l^{-1} in April and June, respectively), the microzooplankton biomass was roughly one order of magnitude lower. We assume that the clearance rate of a female *C. sinicus* is 0.25 l d^{-1}, the mean clearance rate observed for *C. pacificus* when fed with a mixture of phytoplankton and nauplii of its own species (Landry 1981). Calculated ingestion rates on microzooplankton are 0.70 and 0.67 μgC female^{-1} d^{-1} in April and June, respectively, and those on phytoplankton are 14.9 and 10.5 μgC female^{-1} d^{-1} in April and June, respectively. These calculations lead to the conclusion that microzooplankton are unimportant as a food source for female *C. sinicus* in the Inland Sea of Japan, at least during our study periods.

Under laboratory conditions with excess phytoplankton food, the specific egg production rate of *C. sinicus* increases linearly with temperature within the temperature range of 5 to 20°C (Uye, unpublished). The maximum specific egg production rate was much lower in the June experiments (0.072 d^{-1}) than that in the April experiments (0.143 d^{-1}), in spite of the higher temperatures in the former. This may be related to differences in phytoplankton quality (species composition, nutritional content, etc.).

Although more work is necessary for comprehensive modeling of the fecundity of *C. sinicus* depending on biotic and abiotic environmental parameters, the effects of food-particle size and quantity upon the egg production rate, specific egg production rate, clutch size and spawning frequency were determined in this study. The quantity of phytoplankton in the >5 μm size fraction is the principal determinant of these variables for *C. sinicus* in the Inland Sea of Japan.

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