汽水性カイアシ類 Sinocalanus tenellus の種々の餌料濃度と水温条件下における産卵速度

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Egg Production of a Brackish-water Calanoid Copepod *Sinocalanus tenellus* in Relation to Food Abundance and Temperature

KATSUNORI KIMOTO, SHIN-ICHI UYE, AND TAKASHI ONBE

Seikai Regional Fisheries Research Laboratory, Kokubu-machi, Nagasaki 850 and Faculty of Applied Biological Science, Hiroshima University, Fukuyama 720

Abstract

The egg production rates of a brackish-water calanoid copepod *Sinocalanus tenellus* were investigated under controlled laboratory conditions. Females laid eggs in discrete egg-masses rather than egg-sacs. In a few minutes after laying, these eggs were separated and shed into the water. Histological examination revealed that this egg-laying pattern was due to the synchronous maturation of a batch of oocytes in the oviducts. Food supply and temperature influenced the spawning interval (i.e. the rate of maturation of oocytes) much more than the clutch size (i.e. the number of eggs in a spawning event). At high food concentrations, the egg production rate became maximal around 20°C at 60 eggs female⁻¹ d⁻¹ or 0.37 d⁻¹ in terms of carbon weight-specific rate. *S. tenellus* was capable of producing more than 2361 eggs during 70 days after molting to adult. Frequent matings (i.e. after every ca. 100 eggs produced) were required to enable the production of fertilized eggs throughout the female’s reproductive period.

*Sinocalanus tenellus* is a brackish-water or mixomesohaline calanoid copepod distributed in the Far East (BRODSKY 1967, MIZUNO 1984). In a preceding study by HADA et al. (1986), it has been demonstrated that *S. tenellus* is the only calanoid copepod in a brackish-water pond in Fukuyama, western Japan, and is continuously abundant except during summer. They inferred that the rapid recovery of the planktonic population of *S. tenellus* after the summer population corruption is due to its high reproductive potential and rapid postembryonic development. We have already shown that *S. tenellus* is a eurythermal and euryhaline copepod with very fast development and high growth potential (KIMOTO et al. 1986). However, information on the egg production of *S. tenellus* is fragmental. MATSUDAIRA (1957) and WATANABE (1961) briefly described the mating and spawning of *S. tenellus*.

The objective of the present study is to understand variations in the egg production rates of *S. tenellus*. Since previous studies indicate that temperature and food abundance are the most important factors influencing the egg production rates in copepods (cf. CHECKLEY 1980, UYE 1981, RUNGE 1984, AMBLER 1985), the effects of these two factors on the reproductive rate of *S. tenellus* were investigated. Detailed observations of spawning behavior and the
histological examination of oogenesis were also made.

**Materials and Methods**

The copepods used in the egg production experiments were laboratory-reared, third generation offspring (F₃) stemming from grandparents (F₁) which were collected from a brackish-water pond in Fukuyama (HADA et al. 1986) between January and March 1983. The stock culture was maintained in 1-liter glass breakers containing standardized culture medium (Chl a concentration: ca. 38 μg L⁻¹), prepared by suspending cultured *Isochrysis galbana* (ca. 5 x 10⁴ cells ml⁻¹) and *Thalassiosira weissflogii* (ca. 5 x 10³ cells ml⁻¹) in glassfiber (Whatman GF/C) filtered water (salinity: 10 %) in a controlled environment room (20.5 ± 0.5°C, 12 h light (L) and 12 h dark (D) photoperiodicity).

We first investigated the cycle of oogenesis to spawning. In preliminary observations, we noticed that *S. tenellus* laid eggs in discrete events in an egg mass. One couple of an adult female and male were introduced into a glass evaporating dish (75 mm diameter, 30 mm deep) containing approximately 50 ml of the standardized culture medium. Five such dishes were prepared and placed in a controlled environment room. Observations were made at least hourly for 48 h to check for mating, maturation of oocytes, spawning and the number of eggs spawned. In addition, a histological study was conducted for more detailed understanding of oogenesis. For this purpose, adult females of different stages of oogenesis were collected from the brackish-water pond, fixed in Bouin’s fluid for 1-2 h, and transferred to 70 % alcohol preservative. After dehydration by alcohol series and embedding in paraffin, the copepods were serially sectioned (6 μm thick) in sagittal dimension and stained following the method of Mallory (SANO 1981).

Second, we investigated the fertility of a female after a single copulation. Three females of copepodite stage V (CV) were individually cultured in glass evaporating dishes under the same conditions as mentioned above. After a female molted to CVI and matured, a newly-molted adult male was introduced into the dish for mating. After mating, the male was removed from the dish. Spawning was monitored at least hourly for 84 h and eggs (fertilized or unfertilized) were counted when spawning occurred.

Third, the effect of food abundance on the egg production rate of *S. tenellus* was examined. One couple of a CV female and male were pipetted into a glass evaporating dish containing approximately 50 ml of the cultured medium with one of 8 different concentrations of food (the ratio of cell concentrations of *I. galbana* and *T. weissflogii* was 10 : 1) ranging from 0.76 to 380 μg Chl a L⁻¹. Five dishes were prepared for each food concentration and placed in a controlled environment room. During the experiment, the copepods were transferred to newly prepared medium every 24 hours. At the same time, the number of eggs remaining in each dish were counted (the first spawning occurred within 2 days of the experiment). Mean daily egg production at each food level was calculated by averaging the daily egg production during the 5-day period following the first spawning event, since the egg production was constantly maximal during this period.
Fourth, the effect of temperature on the egg production rate of *S. tenellus* was investigated. One couple of a CV female and male, reared at 9.9, 20.5 and 30.6°C from eggs spawned by F2 females, were introduced into a dish containing the standardized medium. Five dishes were incubated at each of 9 different temperatures ranging between 6.5 and 35.0°C. In this case, illumination was not specifically controlled and only direct sun light was avoided. The daytime light intensity was 100-500 lx. The copepods were transferred to a new medium every 24 hours and the number of eggs produced in the previous 24 hours was counted. This procedure was conducted for up to 70 days at 20.6°C and for 22 days at the other temperatures. Mean daily egg production at each temperature was determined in the same way as in the previous experiment. The diameters of eggs spawned at the respective temperatures were measured and the average carbon content of an egg was measured for a batch of ca. 400 eggs spawned at 20.5°C (Oceanography International Co. total carbon analyzer, model 524).

In the present experiments, the number of eggs produced might be underestimated especially in experiments with inspection every 24 hours, due to cannibalism. This effect might be minimal in experiments with inspection less than hourly.

**Results**

1. **Oogenesis to Spawning**

Adult female of *Sinocalanus tenellus* has a pear-shaped ovary dorso-medially in the cephalosome and first thoracic segment. Two oviducts originate from the anterior end of the ovary, run forward first, turn ventrally, and then extend posteriorly parallel to the gut (Fig. 1, Pl. I, A). Oogonia are proliferated in the posterior part of the ovary and move forward as they develop. The oocytes from the ovary pass forward first, then ventrally and back into the oviducts proper. We classified the maturation stages of oocytes in the oviducts into 3 categories: "immature," "developing" and "mature," based on the size of oocytes and the accumulation of yolk granules in oocytes. Immediately after spawning, females had only "immature" oocytes (Fig. 1, a, Pl. I, B) in relatively slender and transparent oviducts. At 20.5°C, 1-2 h after spawning, a row of oocytes at the inner side of the oviducts began to enlarge and then grew up to the "developing" stage, but remaining oocytes were "immature" (Fig. 1, b, Pl. I, C). Three to 4 h after spawning, "developing" oocytes began to accumulate yolk granules and became larger (Pl. I, D). Five to 6 h after spawning, these oocytes possessed dense yolk granules, appeared opaque-green, and developed into the "mature" stage (Fig. 1, c, d, Pl. I, E, F). However, remaining oocytes were still in "immature" stage (Pl. I, F). All "mature" oocytes were then squeezed out through the narrow genital openings (Fig. 1, e). Eggs emerged were roughly pear-shaped and adhering to one another in an egg mass which remained attached to the ventral side of the genital segment. In a few minutes after laying, the eggs separated and were shed into the water.

Fertilized eggs were spherical (Pl. II, A) with firm fertilization membrane and perivitelline space. Short hairs of 1 µm length covered the egg surface, as observed by scanning electron microscopy (HAYASHIDA pers. comm.). Unfertilized eggs (Pl. II, B) were not perfectly spherical.
Fig. 1. Schematic illustration of the oogenesis of *Sinocalanus tenellus*.

a: with "immature" oocytes in the oviducts, b: with "developing" oocytes in the oviducts, c: with "mature" oocytes in the oviducts, d: dorsal view of c, e: during spawning; ov.: ovary, ovd.: oviduct, sp.: spermatophore, i.o.: "immature" oocyte, d.o.: "developing" oocyte, m.o.: "mature" oocyte.

and the fertilization membrane and perivitelline space were absent. They were easily broken by handling.

The spawning events of *S. tenellus* were discrete with an interval of $7.7\pm1.9$ h (mean±S.D.) for 5 pairs of copepods at $20.5^\circ C$ (Fig. 2). No diurnal rhythm in egg laying was
During the 48 h observation, mating was often observed, ranging from 3 to 6 times a day (Fig. 2). Almost every time after spawning, mating occurred and a spermatophore was attached to the ventral side of the female's genital segment, covering the genital operculum, and usually remained attached until the following spawning event.

2. Fertility of a Female Following a Single Mating
Mating was achieved soon after the introduction of a male into a dish and the first spawning event was observed within 12 h after a female molted to CVI. Figure 3 shows the times of spawning as well as the quality (fertilized or unfertilized) of eggs produced by 3 females. The first mating failed since these females produced largely, if not entirely, unfertilized eggs. Then, another male was introduced for remating. After the second mating, females produced fertilized eggs. Each female was able to produce at least 4 clutches (probably 5-6 clutches, because hourly inspection was impossible for 20 h) of fertilized eggs by a single mating, with a total of ca. 100 fertilized eggs produced. Thereafter, eggs spawned were unfertilized.

3. Effect of Food Abundance on Egg Production Rate
The daily egg production of *S. tenellus* at various food levels and 20.5°C is shown in Fig. 4. No eggs were produced at an average chlorophyll concentration of 0.76 µg Chl a l⁻¹, and the egg production rate increased from 7.2 eggs female⁻¹ d⁻¹ at 1.9 µg Chl a l⁻¹ to a maximum of 65.6 eggs female⁻¹ d⁻¹ at 38 µg Chl a l⁻¹. The egg production rates were not significantly different at Chl a concentrations between 19 and 380 µg l⁻¹ (overall mean: 58.1 eggs female⁻¹)
no hourly observations were made

Fig. 3. Mating and spawning of *Sinocalanus tenellus* at 20.5°C in the experiment to examine the production of fertilized eggs after a single mating. Shaded vertical column: fertilized eggs, unshaded column: unfertilized eggs, $\S$: the time when mating was observed.

Fig. 4. Effect of food abundance on the daily egg production of *Sinocalanus tenellus* at 20.5°C. Vertical lines: ±S.D. The curve is eyefitted one.

The clutch size was occasionally determined by chance. It was smallest (mean: 11.9 eggs) at 1.9 µg Chl a l$^{-1}$ and increased to 15.9 eggs at 3.8 µg Chl a l$^{-1}$. At food concentrations higher than 19 µg Chl a l$^{-1}$, the clutch size was uniform (ca. 18 eggs).

4. Long-term Pattern of Egg-laying

Before examining the effects of temperature on the fecundity of *S. tenellus*, we monitored the daily egg production of 5 pairs of copepods at 20.5°C for 70 days after females molted to CVI. The pattern of the daily egg production by an individual with the maximum fecundity is shown in Fig. 5. This copepod produced a total of 2531 eggs during 70 days and was still alive.
During the initial 20 days, the egg production rate was maximal (mean: 59.5 eggs female\(^{-1}\) d\(^{-1}\)), and gradually decreased thereafter, with a small increase observed between the 42nd and 50th days.

At seven other different temperatures, the examination of egg production was conducted for 22 days. The case of an individual with the maximum fecundity at each temperature is...
shown in Fig. 6. Daily egg production was constant during the study period, except for copepods at 14.9 and 18.7°C, in which the egg production rate decreased considerably at the end of the period.

5. **Effect of Temperature on Egg Production Rate**

The daily egg production of *Sinocalanus tenellus* increased with the increase of temperature up to ca. 20°C, beyond which it was stable (Fig. 7). At 35.0°C, however, no eggs were produced and females died within 3 days. Females grown at 20.5°C (mean prosome length: 1037 µm) and 9.9°C (1093 µm) produced eggs at similar rates at 6.5, 9.9 and 20.6°C. However, the daily egg production of females originally from 30.6°C (907 µm) was significantly (p<0.05, Student t-test) lower at 20.6 and 31.5°C. There was no significant difference in the egg production rate of females grown at 20.5°C for temperatures between 18.7 and 31.5°C (overall mean: 54.9 eggs female⁻¹ d⁻¹). Since no frequent observations were made in this experiment, data on the clutch size were available only for 9.5 and 6.5°C; these were 17.9±3.0 eggs and 15.5±3.3 eggs, respectively. The latter was significantly (p<0.05) smaller than the former, which was similar to the clutch size at 20.5°C (17.5 eggs), as mentioned before.

The carbon content of an egg was 0.0357 µg for eggs produced at 20.5°C (diameter: 85.1 µm, volume: 323×10⁶ µm³). Carbon contents of eggs spawned at other temperatures were calculated from egg volume. There was no apparent difference in egg diameter at temperatures higher than 14.9°C, but it was larger at lower temperatures (Fig. 8). No significant difference was noted in the diameter of eggs produced by females grown at 9.9 and 20.5°C. However, the diameter of the eggs produced by females grown at 30.6°C was significantly (p<0.05) smaller at 20.6°C and larger at 31.5°C than for females grown at 9.9 and 20.5°C.
Fig. 8. Effect of temperature on the egg diameter of *Sinocalanus tenellus* grown at 9.9, 20.5 and 30.6°C.

Fig. 9. Effect of temperature on the specific rate of the daily egg production of *Sinocalanus tenellus* grown at 9.9, 20.5 and 30.6°C. Vertical lines: ±S.D.

The specific rate of the daily egg production of *S. tenellus* at various temperatures was calculated on carbon basis (Fig. 9). In this case, the carbon content of a female was determined from the prosome length-carbon weight relationship (KIMOTO et al. 1986). The specific rate increased exponentially with the increase of temperature up to ca. 20°C, beyond which it was stable. There was again no difference in the specific egg production rate be-
tween females grown at 9.9 and 20.5°C, and incubated at 6.5, 9.9 and 20.6°C. But the rate at 31.5°C was equal for females grown at 20.5°C and 30.6°C, because larger eggs were produced by the latter (Fig. 8). For animals grown at 20.5°C, the specific rate of daily egg production at temperatures between 6.5 and 20.6°C is expressed as:

\[ F = 0.00129 (T + 2.0)^{1.83} \ (r=0.956) \]

where \( F \) is the specific rate of daily egg production (d\(^{-1}\)) and \( T \) is temperature in \(^\circ\)C. At temperatures between 20.6 and 31.5°C, the rate was 0.359 d\(^{-1}\).

**Discussion**

The pattern of egg-laying of *Sinocalanus tenellus* is unique; it lays eggs in an egg mass, but not in egg-sacs, at a very rigid interval. A similar spawning behavior has been observed for *Calanus finmarchicus* (MARSHALL & ORR 1952), *C. pacificus* (RUNGE 1984) and *C. sinicus* (UYE unpublished). Although CORKETT & ZILLIOUX (1975) only stated that *Temora longicornis* laid eggs singly or in groups in their laboratory experiments, the synchronous development of a batch of oocytes in the oviducts (RAZOUS 1974) indicates that the spawning behavior of this species is similar to that of *S. tenellus*. The characteristics of egg production in copepods are determined primarily by the processes of oogenesis. In *S. tenellus*, oocytes in an entire row on the inner side of each oviduct mature synchronously. This suggests that the transfer of material, especially yolk, through the walls of the oviducal diverticula and oviducts to oocytes also occurs synchronously. By contrast, BLADE-ECKELBARGER & YOUNG-BLUTH (1984) demonstrated that the maturation of oocytes is a continuous process for *Labidocera aestiva*, which implies the spawning of this species to be continuous (see continuous egg production under constant illumination, MARCUS 1985). Continuous egg production was also found for *Acartia tonsa* (PARRISH & WILSON 1978) and *A. clausi* (=A. omorii, UYE 1981).

The egg production rate of *S. tenellus* is the product of two components: the clutch size and the frequency of clutch production. Food supply primarily determines the rate of transfer of nutritive matter to commence vitellogenesis and oogenesis. In the present study, at low food concentrations, the clutch size was significantly reduced (mean: 11.9 and 15.9 eggs at 1.9 and 3.8 \( \mu \)g Chl a l\(^{-1}\), respectively) and the interval of clutch production was prolonged (mean: 40 and 21 h at respective Chl. a concentrations) at 20.5°C. This suggests that under food-limited conditions the time necessary for the accumulation of matter above the threshold level and hence the initiation of synchronous maturation of a batch of oocytes is longer and, concurrently, the number of oocytes per batch decreases. At food concentrations higher than 19 \( \mu \)g Chl a l\(^{-1}\), 18 eggs were produced during every ca. 8 h interval. From these results, it can be concluded that the food supply influences the clutch size much less than the spawning interval for *S. tenellus*, as for *Pseudocalanus minutus* (CORKETT & McLAREN 1969) and *Calanus pacificus* (RUNGE 1984). The egg production rate is a function of temperature when food supply is adequate. Temperature primarily influences the spawning frequency (i.e. the rate of maturation of oocytes), rather than the clutch size, as commonly observed for egg-sac
carrying copepods such as *Tigriopus brevipennis* (COMITA & COMITA 1966) and *Pseudodiaptomus marinus* (UYE et al. 1982).

On the basis of specific rate, SEKIGUCHI et al. (1980) reported that the egg production rate was equivalent to the growth rate of the copepodite stages for *Acartia hudsonica*. However, the egg production rate of *S. tenellus* (e.g. 0.369 d\(^{-1}\) at 20.0°C) was lower than the somatic growth rate of the copepodite stages (0.718 d\(^{-1}\) at 20.0°C, KIMOTO et al. 1986). Inconsistencies between the rates of egg production and somatic growth were also observed for *A. clausi* (LANDRY 1978, UYE 1982) and *P. marinus* (UYE et al. 1982), indicating that the processes controlling oogenesis are different to some extent from those controlling somatic growth in these species.

Although the physiological longevity of *S. tenellus* was not determined in the present study, it was demonstrated that this species could live more than 70 days after molting to CVI and produce more than 2531 eggs at 20.6°C. To our knowledge, *A. tonsa* is the most fecund copepod species; it lays as many as 2980 eggs at 18°C (PARRISH & WILSON 1978). The maximum egg production attained by *S. tenellus* is slightly lower than that by *A. tonsa*, but much higher than that of *A. clausi* (1281 eggs, UYE 1981) and *Temora longicornis* (411 eggs, CORKETT & ZILLIOUX 1975). We, therefore, conclude that *S. tenellus* is one of the most fecund planktonic copepods.

To ensure the fertilization of eggs throughout the reproductive period of a female, frequent matings are necessary in *S. tenellus*; a single mating enabled only ca. 100 eggs (i.e. egg production during ca. 1.5 day period at 20.5°C) to be fertilized. While multiple matings were required to fertilize all the eggs produced during the lifetime of *A. tonsa* (WILSON & PARRISH 1971, PARRISH & WILSON 1978) and *A. clausi* (UYE 1981), females of these species could produce as many as 600 fertilized eggs after a mating (PARRISH & WILSON 1978). It is notable that adult males of *S. tenellus* are capable of producing spermatophores very frequently (see Fig. 2). The natural population of *S. tenellus* in the brackish-water pond in Fukuyama contains adult males and females in equal abundance during most of the year (HADA et al. 1986), indicating that frequent matings may occur. This indicates that there is little chance of infertile eggs being produced in nature.

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**Literature Cited**


Explanation of Plates

Plate I. Sagittal sections of adult female *Sinocalanus tenellus*.
A: ovary and "developing" oocytes in the oviduct,
B: "immature" oocytes in the oviduct,
C: oogonia in the ovary and "developing" oocytes in the oviduct,
D: "developing" oocytes in the oviduct,
E: "mature" oocytes in the oviduct,
F: "mature" oocytes and remaining "immature" oocytes in the oviduct.

Plate II. Eggs of *Sinocalanus tenellus* released into water.
A: fertilized egg, with fertilization membrane and perivitelline space,
B: unfertilized egg, without fertilization membrane and perivitelline space.