Influence of Temperature on the Sexual Maturation in Manila Clam, *Ruditapes philippinarum*

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Abstract

Wild Manila clam, *Ruditapes philippinarum* collected from Tokyo Bay in four occasions, representing four different stages of gonadal maturation, were exposed to a series of constant water temperatures and abundant microalgal food in the laboratory. The histological development of the gonads and the changes in the condition factor (C.F.) of the clams were examined in relation to the initial gonadal state and rearing temperature.

The clams with gonads in undifferentiated stage (collection in Nov. 1990), in early active stage (Mar. 1991), and in late active stage (Aug. 1991) showed gonadal development at temperatures between 10–27 °C, while those in spawning or spent stages (Oct. 1991) showed gonadal degeneration. The rate of increase of gonadal index \((y)\) was always directly related to temperature. It could be expressed as the function of water temperature \((x)\) \(y = 0.0037x - 0.0168\). The biological zero in gonadal development was estimated to be 4.5 °C. From these results, the environmental temperature is probably not a limiting factor for the reproductive activity for *R. philippinarum*, and the gonadal development occurs between the temperature of 10–27 °C regardless of the initial gonadal condition except immediately after spawning. The wild *R. philippinarum* is considered to be more strongly influenced by food availability than by water temperature (min. 8 °C, max. 27 °C).

For poikilothermic marine bivalve mollusks, temperature is a significant environmental factor regulating the reproductive activity through the determination of the rate of gonadal development and gametogenesis. In a number of bivalve species (e.g., the hard clam, *Mercenaria mercenaria*, soft shell clam, *Mya arenaria*, American oyster, *Crassostrea virginica*, bay scallop, *Argopecten irradians*), gonadal development has been shown to be accelerated by elevating the water temperature\(^{1-3}\).

Additionally, there appears to be a threshold level of environmental temperatures for the normal occurrence of reproductive events, e.g., the initiation of gametogenesis, formation of fully developed gametes, and their release. Although *A. irradians* in North Carolina initiates oogenesis at 15 °C, further gametogenic development does not occur at this temperature. Growth of the oocytes immediately takes place, however, if the scallops are transferred to temperatures higher than 20 °C\(^4\). This indicates that temperature requirements

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for gonadal growth in bay scallop differ with gametogenic stage.

In Manila clam *Ruditapes philippinarum*, Mann (1979) demonstrated in a laboratory experiment that the sexual maturation proceeds more rapidly at higher water temperatures over a range from 12 °C to 23 °C. However, the temperature effects on the different gonadal stages and the threshold levels of the temperature for the gonadal development are still unclear in this species.

As part of a study to clarify the controlling environmental factors of the reproduction of *R. philippinarum*, the authors previously investigated the reproductive cycle of wild stocks in Tokyo Bay (1979), and experimentally demonstrated the importance of food availability for gonadal growth and spawning. This study was conducted to examine the effect of temperature on the gonadal development of *R. philippinarum*.

**Materials and Methods**

Four rearing experiments, *Experiments 1 to 4*, each of 2-3 months duration, were carried out from Nov. 1990, and from Mar., Aug., and Oct. 1991. More than 1000 Manila clams were collected from the coastal zone at Funabashi (Chiba Pref.), Tokyo Bay, a few days before each the four rearing experiments. Collected clams were immediately brought to the laboratory at Futtsu (Chiba Pref.), and placed in running filtered sea water of natural ambient temperature. Clams were not fed until the beginning of the experiment. At each experiment, clams with shell length (longest length of the anterior-posterior axis) between 32.1-40.0 mm were selected and divided into 4 groups of the same size composition, each of 120 individuals.

A diagram of the indoor system for rearing the clams under constant water temperature is shown in Fig. 1. The plastic rearing tank (72 l, 60 cm × 40 cm × 30 cm) received a continuous supply of microalgae-supplemented sea water at a rate of approximately 300 ml/min, and was provided with adequate aeration. Water temperature of the rearing tank was maintained by adding water of the desired temperature to the external water bath tank (200 l, 80 cm × 56 cm × 48 cm). Clams were placed in rearing mesh containers (40 cm × 30 cm × 10 cm) containing a 10 cm-deep layer of sand substrate. Sand substrate was collected from the coastal area nearby the laboratory, sieved to remove any course particles (particle size > 1.0 mm diameter) and sift, washed with tap water, and dried before use.

Sea water was supplemented with a diluted cultured fluid of *Pavlova lutheri* (Haptophycaceae). Density of the

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**Fig. 1.** Rearing apparatus of Manila clam *R. philippinarum* under constant water temperature with a continuous food supply. A, clams; B, inlet of microalgae-supplemented sea water; C, inlet of temperature-controlled sea water; D, diaphragm pump; E, overflow of rearing sea water; F, overflow of temperature-controlled sea water; G, sand substrate; H, water bath tank; I, rearing tank; J, rearing mesh container; K, food reserve tank; L, microalgae-supplemented sea water.
microalgae in the rearing tank was kept below $15 \times 10^4$ cells/ml in order to avoid inhibition of feeding in denser algal suspensions. The amount of algae ingested daily was calculated by multiplying the difference in cell density between the inlet and outlet of the rearing tank by the flow rate of microalgae-supplemented seawater. Measurements of cell density were made using a particle counter (TA-II, Coulter Electronics, Ltd.) with a 100-μm orifice aperture tube. Preliminary observations showed that the diameter of P. lutheri cell ranged between 3.15 μm and 6.35 μm. The rearing tank was drained, the sand substrate was cleaned, and sea water was totally replaced every two days.

Four constant temperature conditions, 10 °C, 17 °C, 23 °C, 27 °C (16 °C, 19 °C, 23 °C, 27 °C in Experiment 3) were chosen after consideration of the seasonal changes in temperature and reproductive activity of Manila clam in the sampling area. According to investigations on the reproductive cycle of natural stocks and water quality at Funabashi, developing gonads are observed in Mar. at water temperature around 10 °C, spring spawning occurs in May at around 17 °C, recovery and growth phases are found in Jul. at around 23 °C, and autumn spawning takes place in late Aug. and early Sep. at temperature of around 27 °C. Clams were acclimated to the experimental temperatures by elevating or reducing the water temperature by 2.0–3.0 °C per day. A single rearing apparatus was assigned to each experimental temperature.

During the experiments, 17–30 clams were sampled four times at intervals of 15–30 days from each of the temperature regimes. Measurements of the shell length (SL), shell height (SH), and shell width (SW) of sampled clams were taken prior to weighing of wet soft tissue weight (STW). The condition factor (C.F.), as a index of nutritional state, was calculated by the following equation:

$$C.F. = \frac{STW (g)}{SL (cm) \times SH (cm) \times SW (cm)} \times 100$$

Ten clams were chosen from among these for histological examination of gonadal condition. The dissected visceral portions were preserved in 10 % formalin, dehydrated, cleared, and embedded in paraffin using standard histological methods. After sectioning at 4–7 μm, specimens were stained with Mayer’s hematoxylin and counter-stained in an aqueous solution of eosin Y and erythrosine.

Gonads were classified into one of eight developmental stages: undifferentiated, early active, late active, ripe, spawning, spent, early regressive, and regressive. The first six divisions were based on the criteria of Ko (1957) 10 and Holland and Chew (1974) 11, and the second two divisions were after Tobs and Miyama (1981) 10.

The gonadal index (G.I.) was estimated for the histologically examined clams in Experiment 1. For this purpose the following scoring system was used: undifferentiated = 0, early active = 1, late active = 2, ripe = 3, spawning or early regressive = 2, spent or regressive = 1. The G.I. for a group of clams was determined by multiplying the number of specimens ascribed to each histological category by the category score described above, summing all of the values, and finally dividing that figure by the number of clams assessed 11.

Survival rate (SR) was estimated as follows:

$$SR = SR_1 \times SR_2 \times \cdots \times SR_n \times R_a$$

where SRa is a survival rate from one sampling to the next, SRa is that from the latest sampling to the day. Number of sampled individuals were excluded from calculations.

Results

Experiment 1 (Nov. 7, 1990—Jan. 9, 1991)

The histological examination revealed that 60 % of the clams were in the undifferentiated stage and the remainder in the spawning or spent stages at the beginning of the experiment (Fig. 2A). After 16 days of temperature exposure, clams showed signs of gonadal development. The rate of gonadal development was faster at higher temperatures, and ripe gonads appeared after 44, 30, 16, and 16 days at 10, 17, 23, and 27 °C respectively. At 23 and 27 °C, most of the clams were in the early regressive to regressive stages by days 44

Fig. 2. Histologically determined gonadal stages in Manila clam *R. philippinarum* reared under a series of constant water temperatures. Experiments initiated on Nov. 2 1990 (Experiment 1), Mar. 14 1991 (Experiment 2), Aug. 2 1991 (Experiment 3), and Oct. 11 1991 (Experiment 4).
and 30, respectively, indicating that the gonads matured and degenerated rapidly without the release of gametes at these temperatures.

The C.F. of the clams at the beginning of this experiment was the lowest (12.9) of all four rearing experiments (Fig. 3A). The C.F. at 10 and 17 °C showed steady increases thereafter and reached 19.2 and 18.9, respectively, by 64 days. Although the C.F. at 23 and 27 °C increased as in low temperatures until day 16, they declined sharply afterwards to approximately 13 on day 44. Cumulative mortality was higher

Fig. 3. Changes in condition factor of Manila clam R. philippinarum under a series of constant water temperatures.

Fig. 4. Changes in the survival rates of Manila clam R. philippinarum under a series of constant water temperatures.
at the high temperatures (Fig. 4A), particularly at 27 °C. At this temperature, a massive death occurred between days 15-20, and survival rate declined to 20% by day 40. In contrast, survival rate at 10 °C and 17 °C remained higher than 95% until the end of the experiment.

The ratio of ingested ration to added ration (I/A ratio) was generally lower than 0.9 (Fig. 5A), indicating that the added rations were above the satiation level. Likewise, the reduction of the I/A ratio with the rearing period reflects the decreasing number of clams due to sampling and mortality. The daily ingested ration (DIR, mean amount of ingested microalgal cells per soft tissue weight per day) did not show any marked differences between the temperatures (4.0 X 10⁶ - 5.8 X 10⁶, 4.2 X 10⁶ - 6.4 X 10⁶, 5.1 X 10⁶ - 5.9 X 10⁶, 5.4 X 10⁶ - 4.7 X 10⁶ cells/gSTW/day at 10, 17, 23, and 27 °C, respectively) (Fig. 6A).

**Experiment 2** (Mar. 14 - May 26, 1991)

The histological observation showed that the majority of the clams were in the early active stage at the commencement of this experiment (Fig. 2B). Similarly to **Experiment 1**, the gonads exhibited developmental activity at all temperature conditions and the rate of gonad development was the fastest at higher temperatures. For temperatures of 17 °C - 27 °C, clams having ripe gonads peaked between days 17-32. However, an increasing number of individuals showed gonads in the early regressive or regressive stage by the end of the experiment.

The C.F. showed a similar trend to the previous experiment, with higher C.F. levels at low temperature (Fig. 3B). The initial C.F. of 15.3 remained largely unchanged or slightly increased until day 32 in all temperature regimes, but rose sharply thereafter to peak values of 18.4-20.7 around days 55-73.

Survival rates at the end of the rearing period were the highest among the four experiments (> 80% in each temperature exposure; Fig. 4B). The I/A ratios were always less than 0.9 (Fig. 5B). The DIR in 10, 17, 23, and 27 °C were 3.4 X 10⁶ - 6.0 X 10⁶, 2.7 X 10⁶ - 5.9 X 10⁶, 4.8 X 10⁶ - 6.8 X 10⁶, 5.8 X 10⁶ - 7.8 X 10⁶ cells/gSTW/day, respectively (Fig. 6B).

**Experiment 3** (Aug. 2 - Oct. 17, 1991)

At the start of this experiment, gonads in both active and regressive conditions were observed in the same sample (Fig. 2C). Gonadal development proceeded at all temperature after the start of the experiment. The rate of gonadal development was faster at higher temperatures, and the proportions of the ripe stages were highest on days 41, 41, 20, and 20 at 16,
The initial C.F. was the highest of all four experiments (16.7; Fig. 3C). The C.F. in all temperatures except 27°C increased after the onset of the experiment, reached a maximum, and decreased thereafter. Maximal values were attained at 20, 41, and 41 days at temperatures of 23, 19, and 16°C, respectively. Higher C.F. were observed at the lower temperatures.

Survival rates at the end of the experiment were 95, 89, 58, and 14% at 16, 19, 23, and 27°C, respectively (Fig. 4C). At 27°C, a high mortality occurred between days 0–15.

The I/A ratios were consistently less than 0.9 throughout the experiment (Fig. 5C). The DIR at 16, 19, 23°C were $2.5 \times 10^8 - 4.3 \times 10^8$, $2.9 \times 10^8 - 4.6 \times 10^8$, $2.8 \times 10^8 - 4.0 \times 10^8$ cells/gSTW/day, respectively (Fig. 6C).


The majority of the clams examined at the beginning of this experiment were either in spawning or in spent stages (Fig. 2D). Except for a slight increase in the number of ripe clams at 17°C and 27°C on day 17, no continuous development of the gonads were observed in any of the temperatures during the experimental period. While the clams maintained at lower temperatures remained in the early regressive or regressive stages throughout the experiment, the gonads of more than half of those kept at 23°C and 27°C showed extensive regression and eventually ceased reproductive activity.

The C.F. of the clams at the beginning of the experiment was 15.7 (Fig. 3D). The C.F. of clams at 10°C and 17°C did not show any marked changes during the experiment whereas at 23°C and 27°C the C.F. declined to 12.9 and 13.1, respectively.

Cumulative mortality was higher at the higher temperatures (Fig. 4D). Survival rates at the end of the experiment were 98, 91, and 80% at temperatures of 10, 17, and 23°C, respectively. Observations for 27°C were discontinued at 33 days when survival rate had dropped to 42%. Feeding activity was reduced at all temperatures and the I/A ratios were usually less than 0.6 (Fig. 5D). The DIR for all temperatures were the lowest among the four experiments: $1.8 \times 10^8 - 3.4 \times 10^8$, $2.0 \times 10^8 - 3.8 \times 10^8$, $1.9 \times 10^8 - 3.5 \times 10^8$, 2.2
\( \times 10^6 - 3.1 \times 10^6 \text{ cells/gSTW/day} \) for temperatures of 10, 17, 23, and 27 °C, respectively (Fig. 6D).

**Relationship between temperature and gonadal development**

The calculated values of G.I. in *Experiment 1*, in which the initial G.I. was the lowest of the four experiments, are shown in Fig. 7. The G.I. data indicate faster development of gonads at higher temperatures excluding 27 °C, at which a large proportion of the clams were in the regressive stage as early as on day 30. At 10, 17, and 23 °C, linear regression lines (least squares) were calculated for the changes of G.I. values from the beginning of the experiment to the time before regressive gonads appeared (64, 44, 15 days, respectively). The rate of increase of G.I. at temperatures of 10, 17, and 23 °C thus calculated were 0.021, 0.044, 0.069/day, respectively. Therefore, the relationship between temperature (x) and the rate of G.I. increase (y) (Fig. 8) is expressed as:

\[ y = 0.037x - 0.0168 \quad (r = 0.98) \]

From this, the biological zero for gonad development, *i.e.*, the temperature at which the rate of increase of G.I. drops to 0, is estimated to be 4.5 °C.

![Fig. 7. Changes in gonadal indices of Manila clam *R. philippinarum* under a series of water temperatures in *Experiment 1*. Gonadal indices are the mean values of the histologically determined gonadal stages in Fig. 2. Calculation factors were: undifferentiated = 0, early active = 1, late active = 2, ripe = 3, early regressive = 2, regressive = 1.](image)

**Discussion**

Gonadal stages of *R. philippinarum* collected from Tokyo Bay were mainly undifferentiated in Nov. 1990 (*Experiment 1*), early active in Mar. 1991 (*Experiment 2*), and late active in Jul. 1991 (*Experiment 3*). Although clams were in different gonadal conditions at the beginning of the above three experimental periods, their gonads developed during the exposure to temperatures between 10 °C and 27 °C (16 °C and 27 °C in *Experiment 3*). However, when the majority of the clams were in spawning or spent stage (*Experiment 4*), the clams did not show development of the gonads and the gametes degenerated in all the temperatures. Therefore, under sufficient food supply, the gonads of *R. philippinarum* develop in a range of temperature of 10-27 °C regardless of the gonadal condition at the beginning of the exposure, except if they have immediately finished spawning.

The observation that gonadal development of *R. philippinarum* took place at a temperature range of 10-27 °C in this study agrees with findings that wild clams at Funabashi, Tokyo Bay were reproductively active almost throughout the year including Feb. (minimum temperature around 8 °C) and Aug. (maximum temperature around 28 °C). Although the biological zero of gonadal development calculated in this study (4.5 °C) requires further examination because of the limited data, it may be good estimation of naturally occurring phenomena. Consequently, the environmental temperature in Funabashi is probably not a limiting factor for
the reproductive activity for *R. philippinarum*.

However, at temperatures higher than 19 °C, extensive degeneration of the gonads was observed simultaneously with the reduction of C.F. and with an increase in mortality in all experiments but in Experiment 2. Except in the growth phase of gonad, temperatures above 23 °C may give a physiological stress to *R. philippinarum*.

The reproductive activity of the wild *R. philippinarum* at Funabashi temporarily ceases during Dec. Likewise, the clams collected in Nov. in this study showed degeneration of the gonads under natural conditions. However, the clams began gonadal growth and eventually some reached the sexual maturation (Experiment 1), after transfer to 10 °C, which is lower than the natural water temperature of Dec. (11-15 °C). Therefore, the seasonal disappearance of reproductive activity in Dec. under natural conditions at Funabashi is not considered to be due to the low temperatures. On the other hand, Chlorophyll a content and phytoplankton production at Funabashi decline markedly in Dec. to the minimal level of the year and as a result environmental food conditions are unfavourable for gonadal development. Since gonadal growth was observed under conditions of low temperature but abundant microalgal food supply in this study, the timing of recrudescence of reproductive activity under natural environmental conditions in winter is probably delayed by adverse food conditions. Sexual maturation of *R. philippinarum* has been experimentally confirmed to be greatly influenced by the amount of food ingested.

This indicates that with a suitable water temperature and adequate food supply, continuous reproduction of *R. philippinarum* throughout the year may occur. Toba *et al.* (1963) have assumed that a second breeding period at Funabashi in the fall, which has similarly been reported from warm climatic regions along the Pacific coast of south Japan, was the result of suitable environmental conditions of water temperature and food. This assumption is supported by the results of this study.

In the American oyster, *C. virginica* mussel, *Mytilus edulis*, and the bay scallop, *A. irradians*, it is known that nutritional reserves stored in the midgut gland and/or adductor muscle during the resting period is utilized for gonadal development and gametogenesis. The storage of nutritional reserves before gonadal development and gametogenesis is one of the characteristics of reproductive physiology in these species. However, in *R. philippinarum*, there is only a limited amount of nutritional storage prior to gonadal development, as indicated by the annual minimal C.F. recorded just before the commencement of gametogenesis. The reason why the C.F. shows a steady increase during the gonadal development in *R. philippinarum* is suggested to be that gametogenesis is mainly dependent upon the food intake during gonadal growth.

In all experiments, the increment and peak value of C.F. were larger at temperatures lower than 23 °C. In the natural population of *R. philippinarum* in Tokyo Bay, the increment and peak value of C.F. recorded during gonadal development under low temperatures in spring are larger than those recorded during the high temperatures of summer-fall. Thus, the steady and continuous assimilation of nutritional substances and their use for gonadal maturation, specially under low temperature conditions, might also be one of the physiological characteristics in *R. philippinarum*.

References


5) Mann, R. (1979): The effects of temperature on


