Suspension feeding and growth of juvenile Manila clam *Ruditapes philippinarum* reared in the laboratory

YASUO NAKAMURA*

National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan

**ABSTRACT:** Feeding and growth of the Manila clam *Ruditapes philippinarum* fed on diatom *Thalassiosira* sp. were examined in the laboratory. The specific clearance rate (CR) relative to soft-body dry weight (w) of clams (shell length: 8–28 mm) followed a power function of w with an exponent of -0.32. The CR for the juvenile clams (shell length: 9–12 mm; w: approx. 10 mg) increased from 8 to 20 L/g per h in the temperature range 12–21°C, and then decreased gradually in the range 21–30°C. Growth rates (G) of juveniles (shell length: 10–12 mm) in terms of ash-free dry weight of the soft body were measured at 9°C and 20°C and increased linearly with weight-specific daily ration (Q) at both temperatures. G values were higher at 9°C than at 20°C when Q was fixed, probably owing to the lower catabolic rate at 9°C. The slope and y-intercept of G against Q gave an assimilation efficiency (0.62) and daily loss of body weight by catabolic processes (1.4%/day) at 20°C.

**KEY WORDS:** bivalves, clearance rates, daily ration, growth, *Ruditapes philippinarum*.

**INTRODUCTION**

The Manila clam *Ruditapes philippinarum* lives in the sandy sediments of temperate coastal areas (<5 m in depth) along the eastern coastlines of Asia, the north-west coast of North America and the northern coastline of the Mediterranean. The clam is an important fishery resource in all these regions, and is suggested to play substantial roles in material cycling through its activities of suspension feeding, growth and nutrient regeneration.

The suspension feeding of *R. philippinarum* has been examined in the laboratory as a function of temperature, concentration of suspended materials, and size of prey items. Rates of feeding by the clam *in situ* have also been examined. However, these studies were conducted using adult clams and, to the author's knowledge, studies on the feeding of juveniles as a function of environmental variables have not yet been clarified.

The growth of *R. philippinarum* has been examined by many workers. However, because most studies were conducted in the field, the relationships between growth and variables such as temperature and daily ration have not yet been clearly established; studies conducted under controlled conditions are restricted to those by Laing et al. and Coutteau et al. The former group examined the effects of temperature and diet species on the growth of early juveniles (<3 mm shell length), but the effects of daily ration were not considered. Conversely, the latter group examined the effect of daily ration on the growth of early juveniles (<5 mm shell length), but not the effect of temperature.

In the present study the suspension feeding and growth of juvenile *R. philippinarum* under controlled conditions were examined with special reference to the effects of temperature and daily ration. The aim was to establish a basis for assessment of the roles of the clam in material cycling in shallow coastal ecosystems.

**MATERIALS AND METHODS**

**Materials**

Clams were collected from a tidal flat in Tokyo Bay (Oi Flat) and were taken back to the laboratory in aerated seawater.

A diatom, *Thalassiosira* sp. (cell diameter approx. 5 μm) in late exponential phase was used as an algal diet. The alga was cultured in f/2 medium (salinity: 30 PSU; NO₃⁻ concentration: 200 μM; volume: 600 mL) at 20°C and a light intensity of 150 μmol/m² per s under a 12 h light : 12 h dark regime.
Analytical procedures

Clams were labeled with numbers using a felt-tip pen. The length \( (l) \), height \( (h) \) and width \( (d) \) of the shells were measured to the nearest 0.1 mm with a digital caliper. The soft-body dry weight of each clam \( (w) \) was measured with an electric balance (detection limit: 0.1 mg) after the soft body had been dried for 2 days at 80°C. Fatness index \( (FI) \) was defined as the ratio of \( w \) to the ‘volume’ of the shell \( (v = l \cdot h \cdot d) \):

\[
FI = \frac{w}{v}.
\]

The ash-free dry weight (AFDW) of the soft body \( (b) \) was measured by heating the dried soft body at 450°C for 6 h. The ratio of \( b/w \) was 0.86 ± 0.04 (mean ± SD; \( n = 7 \) for clams with \( l \) of 9–12 mm).

The chlorophyll-\( a \) (Chl-\( a \)) concentration of the algal diet was measured fluorometrically following filtration of the sample through a glass fiber filter (GF/C). For measurements of dry weight (DW) and AFDW of \( \text{Thalassiosira sp.} \), algae in the late exponential phase (200 mL) were collected on a GF/C filter (pretreated at 450°C for 4 h), desalted with 0.5 M ammonium formate, and dried at 80°C for 2 days. The DW and AFDW were then measured on an electric balance (detection limit: 1 mg) before and after heating of the filter at 450°C for 6 h. The ratio of AFDW/DW and AFDW/Chl-\( a \) for \( \text{Thalassiosira sp.} \) were 0.68 ± 0.07 and 40 ± 6 (average ± SD; \( n = 15 \)) by weight, respectively.

Statistical analysis was conducted with the aid of a statistical package in Excel (Microsoft, USA).

Culture of the clam

Clams were cultured in a temperature-controlled room (20°C or 9°C) or an incubator under a dim light for 2–24 days, and experiments were conducted during the incubation. The conditions for each experimental culture (runs 1–4) are summarized in Table 1.

Clams brought to the laboratory were labeled with numbers and \( l, h \) and \( d \) were measured for each individual. They were then placed in plastic chambers (11 cm inner diameter by 20 cm high) that contained sand (particle size: 200–500 μm in diameter) with a depth of approximately 4 cm and 1000 mL of GF/C-filtered seawater (30 PSU), and preconditioned with aeration until the next day (day 1). In the experimental culture of runs 2, 3A and 3B (Table 1), some of the preconditioned clams were killed on day 1 and \( w \) was measured \( (n = 22–33 \) for each run) to estimate the initial values of \( FI \).
On the morning of day 1, each clam was transferred to a beaker with approximately 100 mL of the seawater from the chamber. The remaining seawater was discarded, the sand was washed with filtered seawater, and 1000 mL of culture medium preconditioned at the incubation temperature was added to the chamber. The clams were then returned to the chambers and the incubations were continued with aeration. These procedures were carried out daily. The changes in water temperature experienced by the clam in the procedure were within 1°C.

Culture medium was prepared by diluting the algal culture in the late exponential phase with filtered seawater in which the salinity had been adjusted to 30 PSU. Chl-a concentrations in the medium were monitored daily by measuring the Chl-a concentration of the original algal culture and the degree of dilution.

After the incubation, \( l, h, d \) and \( w \) were measured for all clams.

**Feeding experiments**

**Calculation of clearance rates**

Feeding activity of the clams was assessed in terms of weight-specific clearance rates (CR):

\[
CR = \frac{(V/w) \cdot \ln (C_0/C_1) - CR^*}{\Sigma w_i},
\]

where \( V \) is the volume of the medium in a chamber, \( C_0 \) and \( C_1 \) are the Chl-a concentration at the start \( (t_0) \) and at time \( t_s \); \( \Sigma w_i \) is the total soft-body dry weight in a chamber (subscript \( i \) denotes the individual \( i \)), and \( CR^* \) is the apparent clearance rate due to settling of the algae on the floor of the chamber. \( CR^* \) was estimated by monitoring the changes in Chl-a concentration in a chamber without clams (control):

\[
CR^* = (V/w) \cdot \ln (C_0^*/C_1^*),
\]

where \( C_0^* \) and \( C_1^* \) are the Chl-a concentration in the control at the start and at time \( t_s \).

**Time course of feeding**

Just after the clam had dug into the sand following the replacement of the medium (run 1, day 6), the seawater was sampled (50 mL) and filtered through a GF/C-filter for Chl-a measurements. Samplings were continued over a 4 h period at intervals of 1 h. A chamber without a clam was used as the control.

**Effects of clam size on feeding**

Clams of nearly equal size were placed in the experimental chamber (run 4). On day 2, seawater was sampled (50 mL) just after all individuals in the chamber had dug into the sand and 0.75 h later for Chl-a analysis. CR was assessed as a function of the average \( w \) in a chamber \( <w> \); \( <w> = (\Sigma w_i)/n \); \( n \) is number of clams in a chamber.

**Effects of temperature on feeding**

Cultures (run 2) were started at 9°C with eight and six individuals in chambers A and B, respectively. A chamber without a clam was used as the control (chamber C). Then the temperature was increased by 3°C in the afternoon of day 3 \( m = 1, 2, \ldots 7 \). Two or three individuals were removed on the morning of days 7 and 13 to check the changes in FI during the incubation. Shell sizes were monitored at intervals of 6 days or 7 days. The duration of the culture was 18 days for chamber A (final temperature: 24°C, \( n = 3 \) on the final day of incubation) and 24 days for chamber B (final temperature: 30°C, final \( n = 2 \)). Feeding experiments were conducted each day. Seawater was sampled (50 mL) and filtered for Chl-a measurements just after all clams had dug into the sand and 1 h later. Because FI did not change significantly throughout the incubation (Fig. 1; one-way ANOVA \( P > 0.1 \)), values
of $\Sigma w_i$ on each day were estimated from the interpolated $v_i$ and an $FI$ value of 24 mg/mL (equation 1; Fig. 1); $CR$ were then calculated from equation 2.

**Growth experiments**

The effects of daily ration on growth were examined at 9°C (run 3B) and 20°C (runs 3A, 3B). The growth of the clam was assessed on the basis of changes in the total AFDW of the soft body in a chamber ($B$):

$$B = \Sigma b_i = 0.86\Sigma w_i,$$

during the incubation period ($T$). The weight-specific daily increment of $B$ defined here ($G$; %/day) was used as an index of the growth:

$$G = 100 \cdot \frac{\Delta B}{<B>} / T,$$

where $\Delta B$ and $<B>$ are the increment and average, respectively, of $B$ during period $T$. These are calculated as:

$$\Delta B = (B_{\text{fin}} - B_{\text{init}})$$

and

$$<B> = (B_{\text{fin}} - B_{\text{init}}) / \ln (B_{\text{fin}} / B_{\text{init}}),$$

where $B_{\text{init}}$ and $B_{\text{fin}}$ are initial and final values of $B$ during incubation. $B_{\text{init}}$ was calculated from equation 3 with direct measurements of $w$ after incubation. $B_{\text{fin}}$ was estimated from equation 1 and initial estimates of $FI$:

$$B = 0.86 \Sigma w_i = 0.86 \Sigma (FI_i \cdot v_i).$$

Because $FI$ on day 1 had a normal distribution of $N(FI_0, \sigma_0^2)$, with $FI_0$ and $\sigma_0$ of 26.8 and 2.3 mg/mL, respectively, for run 3A, and 30.1 and 3.2 mg/mL for run 3B (data not shown), the 95% confidence interval of $B_{\text{init}}$ is given as:

![Fig. 2](image)

**Fig. 2** *Ruditapes philippinarum*. Time course of chlorophyll-a (Chl-a) concentrations in chambers containing (●) a clam with weight ($w$) of 21 mg; (▲) a clam with $w$ of 17 mg; and (■) no clam (control) in run 1.

![Fig. 3](image)

**Fig. 3** *Ruditapes philippinarum*. Weight-specific clearance rate ($CR$) as a function of soft-body dry weight.

![Fig. 4](image)

**Fig. 4** *Ruditapes philippinarum*. Effects of temperature on weight-specific clearance rates standardized to soft-body dry weight of 10 mg ($CR_0$) in run 2. (a) Effects of the period of time spent at the new temperature ($D$) on $CR_0$ in chamber B. (b) $CR_0$ as a function of temperature (○ chamber A; ● chamber B). The solid line connects the average of $CR_0$ values at each temperature.
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RESULTS

The Chl-a concentration in chambers containing a single clam decreased exponentially with time over the 4 h measuring period (run 1; Fig. 2). In contrast, the changes in Chl-a concentration in the control chamber were negligible and $CR^*$ was 15 mL/h. $CR$ for an individual with $w$ of 21 mg and 17 mg were 9.7 L/g per h and 18.8 L/g per h, respectively.

The $CR$ as a function of clam size was approximated as a power function of $<w>$ (run 4; Fig. 3):

$$CR = 2.41 <w>^{-0.32}.$$  

When the effects of temperature on $CR$ were examined (run 2), $<w>$ increased from 0.007 g to 0.009 g for chamber A and from 0.012 g to 0.017 g for chamber B during the incubation period. To compensate for the effects of $<w>$ on $CR$, the $CR$ values obtained were standardized to a value of 0.01 g of $<w>$ ($CR_0$) based on equation 7:

$$CR_0 = CR \left( \frac{0.01}{<w>} \right)^{-0.32}.$$  

$CR_0$ at each temperature was measured on 3 consecutive days starting 1 day after the cultures were transferred to the new temperature. The effects of the period of time spent at the new temperature ($D$) on $CR_0$ (Fig. 4a) were assessed by Friedman's test: $D$ did not affect $CR_0$ significantly ($P > 0.1$). This indicates that acclimatization to the new temperature was rapid with a time constant of <1 day.

$CR_0$ varied significantly with temperature (Fig. 4b; $P < 0.001$; one-way ANOVA for each chamber). The average $CR_0$ increased from 8 L/g per h to 20 L/g per h in the temperature range of 12–21°C, and then slightly decreased with a temperature range of 21–30°C. $CR^*$ was <30 mL/h throughout the experiment.

The growth rate ($G$) increased linearly with weight-specific daily ration ($Q$) (Fig. 5a). $G$ was consistently higher at 9°C than at 20°C when $Q$ was fixed. Growth of individual shells was assessed in terms of $v_{fin}/v_{init}$, where $v_{init}$ and $v_{fin}$ are the shell 'volume' at the start and end of incubation, respectively (Fig. 5b). Although $v_{fin}/v_{init}$ increased with $Q$ at 20°C in run 3B, increasing trends in $v_{fin}/v_{init}$ against $Q$ at 9°C in run 3B and at 20°C in run 3A were not apparent.

DISCUSSION

Feeding of the Manila clam

In previous experiments using adult individuals of *R. philippinarum*, filtration sometimes ceased for more than an hour. However, clam juveniles were...
not observed to rest from feeding in run 1, and CR was constant for 4 h. In fact, no resting from filtration was observed throughout the present study.

The Chl-\(a\) concentration in the fresh medium was approximately 100 \(\mu g/L\) in run 1 (Fig. 2), corresponding to approximately 6 \(mg/L\) in terms of algal DW (conversion factor given in the Analytical procedures section). Because clams might produce pseudofeces at such high feeding levels,\(^2\) the culture was occasionally checked for production of pseudofeces in run 1. Although pseudofeces production might have been overlooked owing to the difficulties in observing materials deposited on the surface of the sand, none was found. In any case, the contribution of pseudofeces to the total algal diet processed by \(R. \text{ philippinarum}\) is known to be <10% at an algal DW concentration of 7.2 \(mg/L\).\(^5\) Because all of the present experiments were conducted at diet DW concentrations <6 \(mg/L\) (Table 1), it is reasonable to assume that the contribution of pseudofeces to filtration by the clam was not substantial.

The CR-\(<\omega>\) exponent value obtained in run 4 was \(-0.32\). This indicates that the clearance rates of \(R. \text{ philippinarum}\) on an individual basis follow the power function of \(<\omega>\) with an exponent of 0.68, which coincided with the exponents of 0.3–0.8 obtained for other bivalve species (mean: 0.62).\(^20\)

\(CR\) extrapolated to a \(\langle \omega \rangle\) of 1 \(g\) were 2.4 \(L/g\) per h for run 2. This was comparable with those obtained for \(R. \text{ philippinarum}\)\(^8,9\) and for other bivalve species, including the closely related species \(R. \text{ decussatus}\).\(^21,22\)

Goulletquer et al. examined the effect of temperature on suspension feeding of \(R. \text{ philippinarum}\) collected from the Bay of Biscay (France).\(^7\) CR increased with temperature from 5°C to 15°C, and CR at 15°C was 2.5-fold higher than that at 5°C. CR then gradually decreased in the range 15–25°C. This indicates that CR as a function of temperature for the clams from the Bay of Biscay was shifted approximately 5°C lower than that in the present study (Fig. 4b). The cause of the difference is uncertain. However, one possibility is that the Manila clam adapted to the lower temperatures propagated in the Bay of Biscay, because the water temperature in the Bay of Biscay is 5–10°C lower than that in Tokyo Bay throughout the year.\(^3,13\)

The CR of the Manila clam at 21°C was 2.5-fold larger than that at 12°C (Fig. 4b). This indicates that the grazing pressure of the clam on the phytoplankton population changes greatly between seasons. In areas where the clam is abundant, outbreaks or disappearances of phytoplankton blooms might be coupled with seasonal variations in feeding activity of the clam.\(^23\)

### Growth of the Manila clam

Coutteau et al. examined the effect of daily ration on the growth of \(R. \text{ philippinarum}\) juveniles.\(^15\) However, their object was to identify the optimum ration levels for commercial hatchery operations, and they did not relate growth to food ration on an AFDW or carbon basis. Therefore, it is difficult to apply their results to material cycling/energy budget analysis in field populations. In the present paper growth and ration were assessed in terms of AFDW so that the results could be applied in future to field populations.

In the growth experiments, in which three clams were present in each chamber, growth of the clam was assessed in terms of the total biomass present in the chamber (\(B\)), and not in terms of individual biomass (\(b\)). Because CR differed greatly between individuals even when clams were cultured under the same conditions (e.g. \(CR_b\) for one individual (\(w = 21 \text{ mg}\)) and the other (\(w = 17 \text{ mg}\)) were 7.5 \(L/g\) per h and 15.9 \(L/g\) per h, respectively (Fig. 2)), it was difficult to estimate clearance rates and daily ration on an individual basis when several clams were present in a chamber. In this context, growth was assessed in terms of \(B\).

Weight-specific daily ration (\(Q\)) was calculated based on the assumption that all the diet supplied was processed by the clams (equation 6). Strictly, this assumption causes overestimation of \(Q\) because (i) some diets remained in the water just before the daily replacement of the medium; and (ii) others settled on the floor of the chamber. However, measurements done once a week indicated that Chl-\(a\) concentration just before the replacement of the medium was <5% of that of fresh medium (data not shown). In addition, \(CR^w\) (\(-20 \text{ mL/h}\); the volume cleared through the settling of the algae; equation 2) was much lower than the volume of water processed by the clams (\(-600 \text{ mL/h}\); \(CR = 20 \text{ L/g per h}\) and \(\Sigma \omega_i = -0.03 \text{ g}\)). Thus, overestimation of \(Q\) in the present study was not substantial (<10%).

The relationship between growth of the clam and daily ration (Fig. 5a) was connected to feeding, assimilation, and catabolic parameters as follows: temporal changes in \(B\) are related to feeding rate (\(f\)) and catabolic rate (\(r\)) in terms of AFDW as

\[
dB/dt = AE \cdot f - r,
\]

where \(AE\) is the assimilation efficiency.\(^24\) Assuming that \(AE\) was approximated as a constant and \(r\) is proportional to \(B\) with a constant \(R_0\) \([r = (R_0/100) \cdot B; \text{ unit of } R_0 = \%/\text{time}]\),\(^25\) the aforementioned equation is integrated over time throughout the incubation period (\(T\)):
\[
\frac{\text{dB/dt}}{\text{dt}} = AE \left[ f(t) - (R_0/100) \right] B(t) \text{dt}.
\]

This is transformed to: \( B_{\text{fin}} - B_{\text{init}} = AE \sum f_j - (R_0/100) < B > T, \) where \( f_j \) is the daily ration on day \( j \), and then

\[
G = AE \cdot Q - R_0, \quad (8)
\]
is obtained. This indicates that the slope and y-intercept of \( G \) against \( Q \) coincide with assimilation efficiency and weight-specific catabolic rate, respectively.

The \( AE \) values of \( R. philippinarum \) obtained at 9°C and 20°C were 0.52 and 0.62, respectively (Fig. 5a), and were comparable with values previously reported for \( R. philippinarum \) and \( R. decussatus \) fed algal diets. The \( AE \) values estimated previously were not based on growth experiments but on the measurements of organic contents in the feces and algal diets.

Although the \( R_0 \) value at 9°C was not determined accurately owing to the scarcity of data points, that at 20°C was 1.4%/day (Fig. 5a). The respiration rate of the clam at 20°C (0.8 mL O_2/g soft-body dry weight per h)\(^{26} \) was converted to an \( R_0 \) value using values of 0.45 for the C/AFDW ratio of the clam (Hagiwara T and Kohata K, unpubl. data, 2000) and 1 for respiration quotient (assumed). The value calculated from the respiration rate was 2.5%/day; the order of magnitude of this value was the same as that obtained in the present study.

\( G \) at 9°C was higher than that at 20°C when \( Q \) was fixed (Fig. 5a), probably due to the fact that weight loss by respiration at 9°C was lower than that at 20°C.\(^{3} \) In contrast, a previous study of the growth of juvenile \( R. philippinarum \) by Laing et al. showed that growth was more rapid at 20°C than at 10°C.\(^{14} \) This apparent inconsistency stems from the fact that Laing’s group cultured the clam under food-satiated conditions; the algal diet was never depleted before the daily exchange of the medium. Under these conditions, the daily ration for the clam depends directly on clearance rates, and thus the ration and growth at 20°C would be larger than those at 10°C (Fig. 4b). In other words, they compared growth between clams at 20°C with a larger ration and those at 10°C with a smaller ratio.

The \( x \)-intercept of \( G \) against \( Q \) (2.5%/day at 20°C; Fig. 5a) is the level of food ration at which the clam can sustain its life without decreasing its body weight (minimum ration). Assuming an AFDW/Chl-a ratio of 60 for a natural algal diet,\(^{27} \) the minimum ration in terms of Chl-a for an individual with a \( w \) of 12 mg (average size of the clams used in the present growth experiments) is 4.3 \( \mu \)g/day. Because a clam of this size filters seawater at 4.8 L per day (\( CR_0 = -20 \) L/g per h at 20°C; Fig. 4b), the Chl-a concentration that the clam can take as a minimum ration is approximately 1 \( \mu \)g/L. This indicates that the clam can propagate only in mesotrophic/eutrophic areas.

The \( B_{\text{fin}} \) value at a \( Q \) of 6.4%/day (9°C) was 1.8-fold higher than the \( B_{\text{init}} \) value (data not shown). However, the shell volume in the corresponding condition increased to only 1.1-fold of the starting value during incubation (Fig. 5b). Such lack of association between the growth of the shell and soft body was also observed at 20°C in run 3A. The cause of the uncoupling has not yet been identified, but we should be careful if we assess the growth of natural populations only in terms of shell sizes.

In the present study the effects of temperature, clam size and daily ration on the feeding and growth of the Manila clam were established. Based on these results, more realistic ecosystem models can be constructed for the assessment of the material cycling in the shallow coastal area.

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