

EFFECT OF ALGAL RATION ON FEEDING AND GROWTH OF JUVENILE MANILA CLAM *TAPES PHILIPPINARUM* (ADAMS AND REEVE)

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ABSTRACT Juvenile *Tapes philippinarum* (Adams and Reeve) were reared for three weeks on different rations of *Chaetoceros neogracile* which were either centrifuged and stored or fed together with the culture medium. Algal rations were adjusted daily in order to feed constant weight-specific daily rations. Growth of *T. philippinarum* fed the concentrate of *C. neogracile* was maximal at a daily ration of 1% (algal dry weight per clam live weight). *T. philippinarum* fed single or mixed diets of *C. neogracile* and *Isochrysis* sp. (clone T-Iso) directly from the algal cultures, maximized growth at a ration of 1.3% day⁻¹. Higher growth rates and gross growth efficiencies were obtained with *C. neogracile* fed together with the culture medium. A set of grazing experiments showed that the incipient limiting concentration was about 26 *C. neogracile* cells μl^{-1} . Measurements of cell concentration during the growth tests demonstrated a discontinuous feeding activity in the treatments receiving the optimal ration for maximum growth.

KEY WORDS: algal ration, feeding, growth, bivalve, Manila clam, *Tapes philippinarum*

INTRODUCTION

Earlier work examining the effect of various culture conditions on the growth of bivalve juveniles revealed that the amount of food available per spat was far more important than any other factor explored (Walne and Spencer 1974). Various methods have been used to describe food ration for bivalves. To maintain the link of ration with the concentration at which the food is presented, some authors refer to the cell density upon batch feeding a constant amount of food, independent of the size of the seed (Langton and McKay 1976, Hollett and Dabinett 1989). This method complicates comparisons between experiments which differ in density and size of the seed. For this reason, food rations are preferentially expressed as daily weight-specific rations, such as number of cells (Pruder et al. 1976) or percent dry weight (% DW, Urban et al. 1983) of algae per live weight (WW) of bivalves.

Empirical studies of the relationship between ration size and growth of bivalves weighing less than 1 g are very scarce, although the early juvenile stages are the largest consumers of intensively cultured micro-algae in most commercial hatchery operations (Manzi and Castagna 1989, Helm 1990). Ease of handling has prompted most researchers to use larger juveniles for nutritional studies and, moreover, empirical work has been mainly restricted to oysters. Several equations have been described relating ration size of *Crassostrea virginica* to oyster weight (Pruder et al. 1976, 1977, Epifanio and Ewart 1977, Epifanio 1979). However, these formulas are derived from measurements of maximum filtration rates for oysters mainly in the size range of 10-100 g and predict unreasonably high weight-specific rations for oysters weighing less than 10 mg. By contrast, Urban et al. (1983) concluded that the ration for maximum growth of the American oyster in the size range of 11 to 64 mg was probably greater than that predicted by the equations of Pruder and co-workers.

The optimal ration depends upon the species and culture conditions of algae making up the diet. Enright et al. (1986a) evaluated the nutritional value of 16 phytoplankton species fed individually to *Ostrea edulis* (5-25 mg initial live weight) at rations ranging from 0.1 to 6.0% of the oyster live weight and found that optimal algal rations for growth differed according to the algal species. In the same way, Epifanio and Ewart (1977) demonstrated

that maximum daily rations removed from suspension by *C. virginica* (15 g live weight) varied from 0.4% for *Thalassiosira pseudonana* and *Carteria chuii* to 1.5% for *Isochrysis galbana*. Growth of *O. edulis* juveniles fed nutrient-limited cultures of *Chaetoceros gracilis* was saturated at a ration of $\pm 2.5\%$ DW WW⁻¹ day⁻¹, whereas that of oysters fed algae grown in a complete medium showed a maximum between 2.5 and 4.9% (Enright et al. 1986b). Because optimal rations for maximum bivalve growth will vary according to the culture conditions, they must be determined through empirical growth studies which integrate culture conditions with the physiological, as well as nutritional requirements of the bivalves for maximum growth (Urban et al. 1983). The experimental data reported by Urban and Pruder (1992) showed a linear growth response in *Mercenaria mercenaria* juveniles (initial WW 25 mg) to increased ration from 0 to 1%. The latter authors did not provide the actual daily rations, which should have been lower because rations were adjusted only on a weekly basis. The optimal daily ration for growth of *M. mercenaria* seed (in the size range of 0.4 to 7 mg live weight) fed a mixture of *C. gracilis* and *Isochrysis* sp. (clone T-Iso) was found to be 1.5 to 2% DW WW⁻¹ (Coutteau et al. 1994).

In the present study, the quantitative food requirements for growth of small juvenile *Tapes philippinarum* were determined in small-scale experiments. Grazing tests were performed to determine the critical concentrations for filter-feeding in juvenile Manila clams. Growth data were compiled from seven independent experiments. Growth and feeding rate of clams fed *C. neogracile* either after centrifugation and storage or together with the culture medium was compared. Finally, preliminary tests were run to compare two algal species' optimal algal rations for growth.

MATERIALS AND METHODS

Origin and Acclimatization of the Animals

Juvenile Manila clams *T. philippinarum* (Adams and Reeve) were obtained from commercial hatcheries (see Table 1). The spat were transported in a refrigerated styrofoam box from the hatchery to the lab. Upon arrival they were acclimated gradually to the experimental temperature (temperature increase rate <0.25°C

TABLE I.

Overview of the size and origin of the juvenile *T. philippinarum* used for the various experiments.

Experiment	Initial Mean Unit Weight (mg)	Origin*	Arrival Date
1	5.13	SSW	25/11/89
2	5.65	SSW	26/01/90
3	5.63	GSF	12/03/90
4	1.31	GSF	18/04/90
5	1.35	TM	10/09/90
6	1.32	TM	12/10/90
7	4.91	TM	30/11/90

* SSW: Seasalter Shellfish Whitstable Ltd., UK; GSF: Guernsey Sea Farms Ltd., UK; Tinamenor S.A., Spain.

h⁻¹) and fed *Chaetoceros neogracile* (Vanland. 1968) *ad libitum* for three to seven days prior to the start of the experiment. This species has been referred to previously, and incorrectly, by many authors as either *C. gracile* or *C. gracilis* (VanLandingham 1968).

Culture Systems and Conditions

The experiments were performed in a recirculating system consisting of a 350 µm mesh silo which was submerged partially in a five liter aquarium. An air water lift maintained a flow of about 300 ml min⁻¹ through the silo, which was stocked at the start of the experiment with 0.5 g of spat. An additional aeration point in the aquarium minimized settling of the food. The systems were cleaned and the seawater was renewed three times each week. The seawater was filtered through a 1 µm bag and run through UV prior to use. Cultures were kept in a thermostatic bath at 21 ± 1°C.

Feeding

C. neogracile, which has proven to be among the best algal diets for *O. edulis* juveniles (Enright et al. 1986a) and is used extensively in several commercial bivalve hatchery and nursery operations (Coutteau and Sorgeloos 1992), was selected as the algal control diet. The seed was fed a weight-specific daily ration which was divided between two feedings per day. Rations were adjusted daily for growth of the spat to feed approximately constant weight-specific daily rations throughout the experiment (Urban et al. 1983). The daily ration, calculated as % dry weight (DW) of food per wet weight (WW) of clams, was thus approximated each day by adjusting the amount of food as a function of an assumed growth rate by means of the formula:

$$[\text{DW food day } n] = [\text{DW food day } 1] \times (1 + \text{DGR}/100)^{n-1}$$

where n = day of the week (1 to 7), DGR = daily growth rate (% day⁻¹) measured during the previous week or assumed to be 10% day⁻¹ for the first week, DW food day 1 = initial WW × weight-specific daily ration (%DW WW⁻¹ day⁻¹) × 1/100.

In experiment five, one treatment was fed "on demand" twice daily, i.e. the objective was to keep the *C. neogracile* concentration above 20 µl⁻¹ throughout the experiment.

Calculation of the daily rations was based on dry weight analysis of the algal food species used. Algal dry weights were determined by filtering algae from various volumes of suspension of known concentration. Algae were retained on tared, glass-fiber filters (1 µm pore size) which were subsequently washed with a

solution of ammonium formate (0.5 M) to remove salts. Filters were then dried at 100°C for 4 h to volatilize the ammonium formate, and weighed on an analytical balance (modified from Epifanio and Ewart 1977). The same procedure was followed with control filters on which an equal amount of seawater was filtered. Cellular dry weight was determined from regression analysis of DW retained on the filter versus number of algal cells filtered. Dry weights were 23.8 ± 3.8 and 14.1 ± 0.8 pg cell⁻¹ for *C. neogracile* and *Isochrysis* sp., clone T-Iso, respectively (mean and standard deviation from analysis of five cultures).

Algae were grown semi-continuously in 20 l carboys using Walne medium. Only algal cells in the logarithmic phase of growth were used in the feeding experiments. In the experiments one to five, algae were separated from the culture medium by centrifugation and stored prior to use (Winter and Langton 1976). The algal pellet was resuspended and diluted in filtered seawater to obtain a *Chaetoceros* concentrate of 20 · 10⁶ cells ml⁻¹ using a haemocytometer or Coulter counter, model Zf, and stored in total darkness at 4°C for maximal three days. In experiments five to seven, algal suspensions were drained from the cultures and counted immediately prior to each feeding.

Clearance and Intake Rate as a Function of Food Concentration

During two short term grazing experiments, clearance rates were recorded for *T. philippinarum* (20 mg mean WW) at various concentrations of *C. neogracile*. The animals were derived from a population which was fed *C. neogracile* and transferred to 5 l culture systems which were placed in a thermostatically-controlled water bath at 21 ± 1°C. In order to achieve 10 to 20% decreases of cell concentration over a 2 h period, stocking density was varied between 0.3 and 0.7 g live weight per 5 l system, depending on the food concentration tested. The seed was acclimated to the food concentration for 1 h prior to the experiment (Sprung and Rose 1988). Algal concentration was monitored with a Coulter counter (model Zf) during two consecutive periods of 2 h. A control experiment with no animals present was run in duplicate for all concentrations tested.

Weight-specific clearance rate (CR) was calculated using the equation (Coughlan 1969):

$$\text{CR} = \frac{V}{\text{WW } t} \left[\ln \frac{C_0}{C_t} - \ln \frac{C_0'}{C_t'} \right] \quad [\text{ml g}^{-1} \text{h}^{-1}]$$

where WW = total clam live weight (g), V = volume of the food suspension (ml), C_0 , C_0' = initial, and C_t , C_t' = final concentration of, respectively, the experimental and the control aquarium, and t = elapsed time (h).

The rate at which cells are removed from suspension, to be referred to as intake rate (ir) since pseudofaeces production was not quantified (Foster-Smith 1975), was computed as:

$$\text{ir} = C_m \times \text{CR} \quad [\text{cells g}^{-1} \text{h}^{-1}]$$

where $C_m = (C_t + C_0)/2$ the mean concentration (cells ml⁻¹) encountered by the animals during the measurement of the clearance rate. Clearance and intake rates were graphically presented as a function of C_m .

Average Intake Rate During a Growth Test

During the experiments evaluating the effect of *C. neogracile* ration on growth, food concentration was measured either at short

time intervals of 2 to 6 h (experiment 1) or before and after each feeding (experiment 5). The observed decreases in cell concentration could be related to the total live weight present in the culture system by calculating an average weight-specific intake rate (ir) over the elapsed time interval (t) by means of the formula:

$$ir = \frac{V(C_0 - C_t)}{WW_n t} \quad [\text{cells g}^{-1} \text{ h}^{-1}]$$

where V = volume of the food suspension; C_0 and C_t = initial and final, i.e. after time t (h), concentration; WW_n = total clam live weight (g) present at the moment of the measurement, estimated from the daily growth rate (DGR) and the initial live weight (WW_1 , day 1) from:

$$WW_n = WW_1 (1 + \text{DGR}/100)^{n-1}$$

The intake rates were not corrected for algal growth during the experiment, since the latter was found to be fluctuating in time, but negligible compared to the amount of cells removed by the clams.

Growth Parameters Followed

Animals were selected initially from a single population of juveniles and divided randomly in groups of equal weight, which were distributed among the culture systems. Initial parameters (shell length and individual live weight) were measured on three subsamples.

At 7-day intervals, the seed was removed from the respective silos and the total live weight determined. Enough clams were removed to return the weight to the initial value, and individual live and dry weight were determined on the culled animals.

The total live weight per silo was determined by weighing the clams on a mesh, which was blotted dry on paper towel. To avoid differences in water content between samples due to air-drying, the total biomass was then immediately weighed and reduced to the initial weight prior to returning to the culture systems. The culled animals from each silo were weighed and counted for the determination of the individual live weight. The seed samples were transferred subsequently to Teflon pots, previously dried in an oven at 60°C for 4 h, and weighed. The pots were returned to the oven for 24 h at 60°C and then weighed to give the dry weight. Live weight (WW) of *T. philippinarum* showed a high correlation with dry weight (DW):

$$DW = 0.571 WW + 0.431 \quad (r^2 > 0.99)$$

A constant relationship between live and dry weight was also reported by Urban and Langdon (1984) for *C. virginica* and demonstrated that the drying procedure used for determining the live weight resulted in a constant water content.

Daily growth rate was calculated from the weekly increase of total wet weight per silo (DGR) using the equation:

$$\text{DGR} = \left(\frac{n \sqrt[n]{\frac{WW_n}{WW_0}}}{n} - 1 \right) \times 100 \quad [\% \text{ day}^{-1}]$$

where WW_0 and WW_n are the live weight, respectively, at the start and after n days. Shell length was measured on 30 clams per silo using a dissecting microscope equipped with a calibrated ocular. Live weight (WW, in the range 0.2–32 mg) increased with

increasing shell length (L , in the range 2–5.5 mm) according to the equation:

$$WW = 0.463 L^{2.477} \quad (r^2 = 0.98)$$

Experimental Design

Seven culture tests were performed with *C. neogracile* fed either from a concentrated stock suspension (experiments 1–5) or directly from the algal culture (experiments 5–7). Experiments one and five were run specifically for the study of algal ration, with the latter test comparing feeding and growth on both types of *C. neogracile*. For the other experiments, which were part of a study evaluating algal substitution by a manipulated yeast diet (Coutteau 1992), only growth data from the algal control treatment were used in the present study. In addition, three preliminary experiments were performed to evaluate the effect of ration on growth of *T. philippinarum* fed single and mixed diets of *C. neogracile* and *Isochrysis* sp. (clone T-Iso), both fed directly from the algal cultures.

Data Treatment and Statistical Analysis

Daily growth rates were used to compare the effect of the diets on juvenile growth because this allowed comparisons between experiments that differed in initial live weights of the seed.

Statistical analysis of the growth data included analysis of variance and Tukey HSD multiple range tests. The homogeneity of the variances of means for each experiment was checked by Cochran's C-test and Hartley's test. Because of the limited number of replicates, normality was tested on the deviations $Y_{ij} - Y_i$, which were computed separately for each treatment and pooled per experiment, by means of the Kolmogorov-Smirnov test. Departures from the assumptions of analysis of variance could be rectified in most cases by logarithmic transformation of the data. Inherently heteroscedastic data (Cochran's C-test or Hartley's test, $P < 0.05$, even after transformation) were indicated in the tables of the results with "H.D." and were analyzed using an approximate test of equality of means assuming heterogeneity of variances (MCHETV) or, when only two means were to be tested, an approximate t -test (Sokal and Rohlf 1981).

RESULTS

Effect of Ration Size on Growth of *T. philippinarum* Fed *C. neogracile*

Clam growth increased with increasing daily ration up to a ration of 1% of live weight (Table 2). Further increase of the ration to 1.5% did not result in a significant difference in growth or final size of the clams. The feeding regime was adapted daily to the growth of the clams in the various treatments based on an assumed daily growth rate for each week of the experiment. The actual rations, computed from the feeding regime and the observed growth rates, deviated from the initial ration in the course of each week depending on the accuracy of the assumed growth rate (Fig. 1). To obtain a better estimate of the effective weight-specific ration fed to the clams, the arithmetic mean of the actual daily ration was determined for each week of the experiment. Daily growth rate showed a saturation response around an effective ration of 1%, though growth rate fluctuated between 6.7 and 9.8% day^{-1} according to the week of the test (Fig. 2). Growth of the starved clams declined from more than 2% day^{-1} during the first week to less than 0.5% day^{-1} during the rest of the experiment.

The fluctuation of algal concentration showed a similar pattern in all replicates of each treatment and is represented for one rep-

TABLE 2.

(Experiment 1) Daily growth rate (DGR), final live (WW) and dry (DW) weight, and shell length (L) of *T. philippinarum* fed various daily rations of *C. neogracile*. Data represent mean and standard deviation from four replicates. Like superscripts indicate means which do not differ significantly (ANOVA, Tukey HSD, $P \leq 0.05$, unless stated otherwise).

Treatment§	Week 1	Week 2	Week 3			
	DGR (% day ⁻¹)	DGR (% day ⁻¹)	DGR (% day ⁻¹)	WW (mg ind ⁻¹)	DW (mg ind ⁻¹)	L (mm)
1. Unfed control	2.36 ± 0.30 ^b	0.31 ± 0.50	0.48 ± 0.36 ^c	6.38 ± 0.37 ^e	4.05 ± 0.21 ^c	2.84 ± 0.08 ^e
2. 0.1% Chg	2.91 ± 0.25 ^b	2.05 ± 0.17	1.33 ± 0.26 ^c	8.23 ± 0.28 ^e	5.16 ± 0.17 ^c	3.23 ± 0.15 ^e
3. 0.5% Chg	5.80 ± 0.21 ^a	6.52 ± 0.11	5.74 ± 0.37 ^b	17.99 ± 1.50 ^b	10.82 ± 0.84 ^b	4.37 ± 0.16 ^b
4. 1.0% Chg	6.71 ± 0.67 ^a	9.77 ± 0.09	8.67 ± 0.43 ^a	29.59 ± 3.26 ^a	17.14 ± 1.89 ^a	5.34 ± 0.17 ^a
5. 1.5% Chg	5.84 ± 0.68 ^a	7.85 ± 0.71	9.21 ± 0.53 ^a	25.65 ± 3.27 ^a	14.92 ± 1.89 ^a	5.07 ± 0.18 ^a
ANOVA, F _s	69.0	H.D.*	407.3	88.8	84.4	157.5

§ Chg = *Chaetoceros neogracile* concentrate, rations expressed as initial daily rations (DW WW⁻¹ day⁻¹) initial seed: 5.13 ± 0.58 mg ind⁻¹ (mean ± SD, n = 3).

* Heteroscedastic data, significantly different means separated by / (MCHETV, $P \leq 0.05$): 1/2,3,4,5; 2/3,4,5; 3/4.

licate system per treatment in Fig. 3. By distributing a weight-specific ration of 1% day⁻¹ over two feedings, a peak concentration was attained twice daily of 30–40 *Chaetoceros* μl^{-1} , which was cleared below a level of 10 cells μl^{-1} in the subsequent 5–7 h period (Fig. 3C). The highest ration often resulted in an accumulation of the algae between the periodic renewals of the seawater up to concentrations of 40 (week 3) or even 90 algal cells μl^{-1} (week 2, Fig. 3D). In the cultures fed the lowest rations, the algae were cleared to levels below 1 to 2 *Chaetoceros* μl^{-1} (Fig. 3A and B).

The intake rate, calculated from the decrease of cell concentration over short time intervals during the second and third week of the experiment, was mainly related to the food level present in the culture at the moment of the measurement (Fig. 4). Fluctuation of cell concentration in the cultures fed 1% day⁻¹ thus resulted in a variation of intake rate between 35 and 8.10⁶ cells g⁻¹ h⁻¹, and even lower values may have been observed at the concentrations below 10 *Chaetoceros* μl^{-1} . The rate with which the clams removed the algae from suspension increased linearly up to a concentration of about 30 *Chaetoceros* μl^{-1} . Estimates of intake rate beyond this algal density were mainly derived from the cultures fed 1.5% DW WW⁻¹ day⁻¹ during the second week of the test

and fluctuated strongly irrespective of food concentration. Possibly, the strongly depressed intake rates observed at the high food concentrations were artefacts due to the calculation of intake rates from relatively small decreases of cell concentration. Also, the impact of algal growth, which was not taken into account for the computation of the intake rate, may have been relatively more important at the high algal loads.

Effect of *C. neogracile* Concentration on Clearance and Intake Rate in *T. philippinarum*

T. philippinarum maintained maximum clearance (CR_{max}) and intake (i_{r,max}) rates at, respectively, low and high food concentrations (Fig. 5). The incipient limiting concentration, calculated from the ratio i_{r,max}/CR_{max} (Sprung and Rose 1988), was similar in the two experiments and corresponded with the critical concentrations derived from the intersections of the fitted curves for the second experiment (Table 3). The deviating values obtained for the first experiment by the latter technique, especially for the

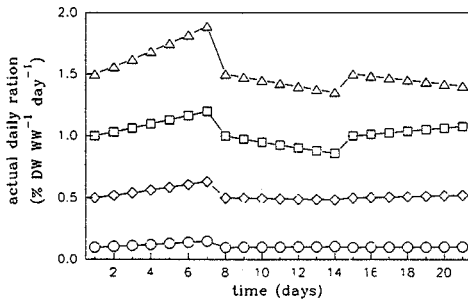


Figure 1. (Experiment 1). Change in actual daily ration over the course of each week of the experiment for *T. philippinarum* fed *C. neogracile* at an initial daily ration of either 1.5% (Δ), 1% (\square), 0.5% (\diamond), or 0.1% (\circ).

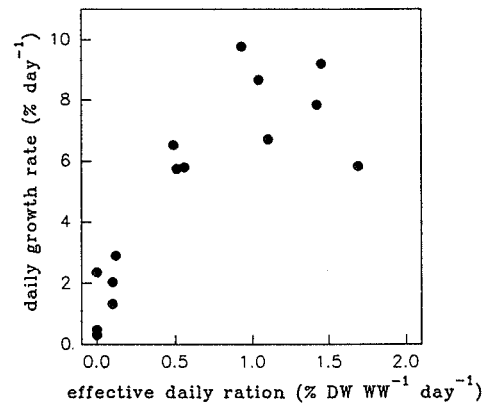


Figure 2. (Experiment 1). Relationship between the effective daily ration and the daily growth rate of *T. philippinarum* fed *C. neogracile*.

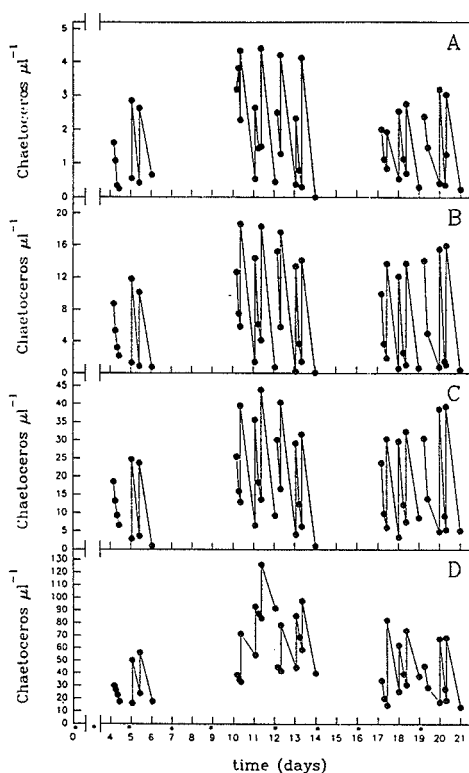


Figure 3. (Experiment 1). Fluctuation of food concentration in one replicate culture of *T. philippinarum* fed *C. neogracile* at a daily ration of either 0.1% (A), 0.5% (B), 1.0% (C), or 1.5% (D). Cell densities measured before and after feeding are connected with a vertical line. Data points are not connected when measurements were discontinued. The periodic renewal of the seawater is indicated on the time axis (*).

critical concentration of clearance rate, may have been due to the insufficient number of data points in the proximity of the incipient limiting concentration. Maximum weight-specific rates of filtration and feeding were about 30% higher during the second test than in the first experiment.

Comparison of Centrifuged Algae and Algal Cultures

Mean daily growth rate of clams obtained during the various weeks of seven experiments for clams fed *C. neogracile* at a daily ration of 1% is presented in Fig. 6. For the five experiments in which concentrated *C. neogracile* was fed, the average daily growth rate of the three weeks did not vary significantly between experiments (ANOVA, $F_{4,10} = 0.780$, $P = 0.56$). For experiments three and five, growth rates measured during the first week were highly deviating from those measured during the following weeks. Growth of clams fed the *C. neogracile* culture, averaged over all experiments, was significantly better than that of spat fed the algal

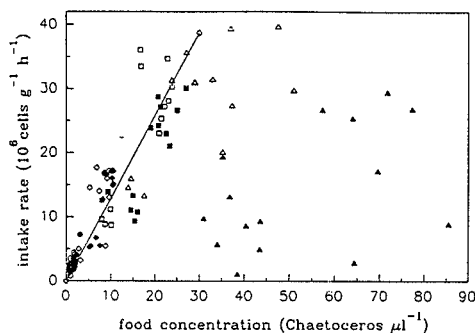


Figure 4. (Experiment 1). Intake rate (ir) as a function of food concentration (C) in *T. philippinarum*. Data are calculated from the decrease of food concentration measured over 2 to 6 h time intervals in the cultures fed various daily rations of *C. neogracile* (0.1%: \circ , 0.5%: \diamond , 1.0%: \square , 1.5%: \triangle) during the second and third week of the experiment (filled and unfilled symbols, respectively). Linear regression equation is given by: $ir [10^6 \text{ cells g}^{-1} \text{ h}^{-1}] = 1.229 C [\text{cells } \mu\text{l}^{-1}]$ ($r^2 = 0.81$).

concentrate at the same daily ration (t -test: $P < 0.05$; $P < 0.001$ if the three aberrant growth rates for week one are excluded).

During the first week of the fifth test the seed exhibited a generally depressed growth, which was less pronounced for clams fed the algae derived directly from the culture. Clams fed the concentrated algae grew at a rate of about 30% of that observed during the subsequent weeks, whereas this was about 60% for seed fed *Chaetoceros* cultures (Table 4). The mean intake rates, calcu-

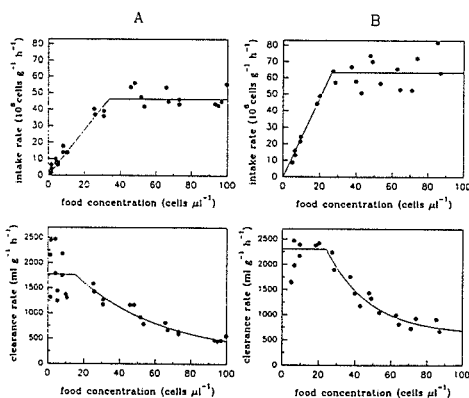


Figure 5. Clearance and intake rates as a function of food density in *T. philippinarum* (20 mg mean WW) fed *C. neogracile*. A and B represent the results of two independent experiments. Maximum clearance and intake rates are calculated from the mean of the data points below and above the critical concentrations, respectively. Curves were fitted to the data points for clearance and intake rate, respectively, above and below the incipient limiting level through, respectively, non-linear ($y = Ae^{-Bx} + Ce^{-Dx} + E$) and linear ($y = Ax$) regression. Derived parameters are presented in Table 3.

TABLE 3.

Maximal clearance (CR_{max}) and intake (ir_{max}) rate, and their critical concentrations for juvenile *T. philippinarum* (20 mg mean WW) fed *C. neogracile* in two independent experiments. Data are derived from Fig. 5.

	Experiment A	Experiment B
ir_{max} (10^6 cells $g^{-1} h^{-1}$)	46.2	63.2
Minimum concentration for $ir = ir_{max}$ (cells μl^{-1})	33.7	26.6
CR_{max} ($ml g^{-1} h^{-1}$)	1771	2301
Minimum concentration for $CR < CR_{max}$ (cells μl^{-1})	15.2	24.1
Incipient limiting concentration [ir_{max}/CR_{max}] (cells μl^{-1})	26.1	27.4

lated from the change in cell concentration between each feeding, were similar throughout the experiment and were not different between the treatments fed 1% DW of *C. neogracile* either as a concentrate or directly from the culture. As a result, gross growth efficiency was higher for clams fed the *C. neogracile* culture, in particular during the first week of the experiment (Table 5). It is of interest that the difference between the gross growth efficiency of clams fed either of the algal types is much reduced during the last two weeks. Possibly, the clams may have needed a longer acclimatization period to adapt to a diet of concentrated algae.

Feeding *Chaetoceros* cultures at daily rations of 1.5% or 1.73% (effective daily ration for treatment fed "on demand") resulted in significantly higher growth compared to the controls fed 1% during the last week of the test (Table 4). This suggests that the optimal ration for maximal growth may be higher for the *Chaetoceros* culture than for the concentrated algae. This was

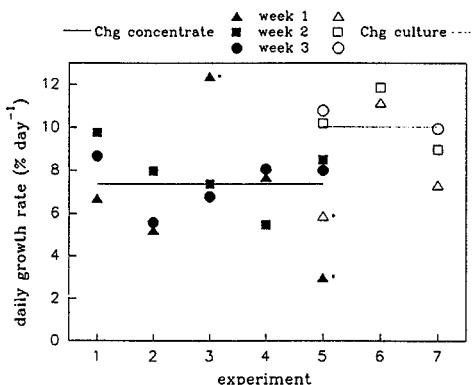


Figure 6. Daily growth rate of *T. philippinarum* fed *C. neogracile* either as a concentrate (filled symbols) or directly from the algal culture (hollow symbols) at a daily ration of 1% in seven independent experiments. Each data point represents the mean daily growth rate of three (four: Experiment 1) replicate cultures for each of the 1-week periods. Lines indicate mean daily growth rate obtained with the concentrate ($7.4 \pm 1.4\%$ day^{-1}) and the culture ($10.0 \pm 1.5\%$ day^{-1}) of *C. neogracile*, respectively (mean \pm SD; aberrant observations from first week, indicated with *; excluded).

TABLE 4.

(Experiment 5) Daily growth rate (DGR) of *T. philippinarum* fed various daily rations of *C. neogracile* (Chg) either as a concentrate or directly from the algal culture. Data represent mean and standard deviation from three replicates. Like superscripts indicate means which do not differ significantly (ANOVA, Tukey HSD, $P \leq 0.05$).

Treatment	Week 1	Week 2	Week 3
1. Chg concentrate 1%	2.98 ± 0.20^b	8.50 ± 0.46^b	8.01 ± 0.37^c
2. Chg culture 1%	5.88 ± 0.45^a	10.20 ± 0.31^a	9.76 ± 0.57^b
3. Chg culture 1.5%	NA	10.41 ± 0.64^a	10.79 ± 0.20^a
4. Chg culture "on demand"	2.69 ± 0.13^b	10.55 ± 0.40^a	10.98 ± 0.19^a
ANOVA, F_x	108.3	12.5	41.1

corroborated by the measurements of cell concentration before and after feeding in one replicate of the algae-fed treatments. Feeding a daily ration of 1.5% maintained the food level above 5–10 *Chaetoceros* μl^{-1} throughout the experiment and resulted in a significantly larger amount of algae removed from suspension than in the treatments fed 1% (Table 5). However, the higher filtered ration was less efficiently converted into clam biomass and resulted only during the last week in a significant growth improvement (Table 4). The high food loads observed in the treatment fed "on demand", i.e. up to 90 *Chaetoceros* μl^{-1} , affected gross growth efficiency only to a small degree.

Clams fed the highest ration (1.73% day^{-1} , treatment fed "on demand") removed the largest weight-specific ration of 1.16% DW $WW^{-1} day^{-1}$ (Table 5), which was equivalent to 67% of the offered ration. Feeding a daily ration of 1% resulted in a clearance of more than 80% of the food offered.

Single and Mixed Diets of *C. neogracile* and *Isochrysis* sp. (Clone T-Iso)

The preliminary series of experiments demonstrated a similar growth response to increasing ration size for *T. philippinarum* (live weight 1.7–6.9 mg) fed either *C. neogracile*, *Isochrysis* sp. (clone T-Iso), or a 50/50 mixture (on DW basis) of both species. Growth increased sharply up to a daily ration of 1%, reached a maximum value at 1.3%, and then decreased with a further increase of algal ration (Fig. 7). Because the experiments were performed with clams of different initial size and previous history, the data do not allow a comparison of the nutritional value of the three algal diets. However, it is interesting to note the consistency of the relationship between growth rate and ration for various algal diets.

DISCUSSION

The present series of experiments demonstrated that the optimal ration of *C. neogracile* for maximal growth of juvenile *T. philippinarum* in the size range of 1 to 40 mg live weight is situated between 1 and 1.5% DW $WW^{-1} day^{-1}$. Growth of clams showed a linear response to increasing rations up to 1% day^{-1} , was not significantly affected by rations ranging from 1 to 1.5%, and eventually decreased with a further increase of the ration. The set of preliminary tests indicated that growth attained a maximum for *T. philippinarum* fed either *C. neogracile*, *Isochrysis* sp. (clone T-Iso), or a mixture of both species, at a daily ration of 1.3%.

In clam cultures fed 1% *Chaetoceros* day^{-1} , the cell concen-

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TABLE 5.

(Experiment 5) Mean intake rate and gross growth efficiency of *T. philippinarum* fed various rations of *C. neogracile*. Data represent mean and standard deviation for week one, and weeks two and three. Like superscripts indicate means which do not differ significantly (week 2 & 3: ANOVA, Tukey HSD test, $P \leq 0.05$).

Type of <i>Chaetoceros</i> → Feeding Regime → (% DW WW ⁻¹ day ⁻¹)	Concentrate 1%	Culture 1%	Culture 1.5%	Culture "On Demand" (1.73% effective DR)
Week 1				
Mean intake rate				
1. (10 ⁶ cells g ⁻¹ day ⁻¹)				
mean ± SD (n = 7)	346 ± 131	346 ± 61		
min.-max. value	121-481	268-434		
CV (%)	38	18		
2. (% DW WW ⁻¹ day ⁻¹)§				
Daily growth rate (% day ⁻¹)	0.82	0.82		
GGE‡	2.98	5.88		
Weeks 2 and 3				
Mean intake rate				
1. (10 ⁶ cells g ⁻¹ day ⁻¹)				
mean ± SD (n = 14)	301 ± 25 ^a	342 ± 56 ^a	457 ± 55 ^b	486 ± 87 ^b
min.-max. value	263-353	219-435	327-552	370-640
CV (%)	8	16	12	18
2. (% DW WW ⁻¹ day ⁻¹)§				
Daily growth rate (% day ⁻¹)†	0.72	0.81	1.09	1.16
GGE‡	8.25	9.98	10.60	10.77
GGE‡	11.5	12.3	9.7	9.3

§ *C. neogracile*: 23.8 pg DW cell⁻¹.

† Average of the mean DGRs for the second and third week of each treatment (see Table 4).

‡ GGE = gross growth efficiency = live weight increase per dry weight of food cleared, estimated as DGR (%WW day⁻¹)/mean intake rate (%DW day⁻¹).

ration was declining rapidly after feeding and was mostly below the incipient limiting level, i.e. about 25 *Chaetoceros* µl⁻¹. As a result, the clams exhibited fluctuating intake rates in the course of the experiment. The higher average food concentration occurring in the cultures that were provided daily rations exceeding 1%, allowed the clams to feed more continuously and maximize their

daily food uptake, but also resulted in a lower efficiency of food utilization. This is in agreement with the observations for other bivalves showing that growth rate is maximized at higher rations than gross growth efficiency (Thompson and Bayne 1974, Goldstein and Roels 1980, Urban et al. 1983).

It is interesting that clams fed the concentrated *C. neogracile* at a higher ration than 1% day⁻¹ showed a tendency towards depressed growth, whereas the animals fed the same alga directly from its culture tended to further increase growth. Apparently, the optimal ration for maximal growth is shifted to lower values, but maximal growth obtained is lower for clams fed the concentrated algae. This is supported by the lower gross growth efficiency observed for the latter.

The periodic feeding activity of the clams fed *Chaetoceros* at 1% day⁻¹ appeared to be mainly imposed by the discontinuous feeding regime which resulted twice daily in a depletion of the food. However, the latter feeding strategy yielded a higher gross growth efficiency and similar growth compared to that of clams fed 1.5% day⁻¹ which filtered the food more continuously and at higher rates. From this it would appear appropriate to apply discontinuous feeding regimes in aquaculture systems in order to maximize the utilization efficiency of the algal food. In the same way, Epifanio and Ewart (1977) observed a discontinuous feeding activity for *C. virginica* in continuously replenished suspensions of algae and proposed to offer algae in pulses rather than maintaining constant food concentrations. This is further supported by the findings of Langton and McKay (1976) who reported better growth of *C. gigas* spat fed discontinuously than when feeding the same amount of food continuously.

The maximal daily amount of *C. neogracile* dry weight re-

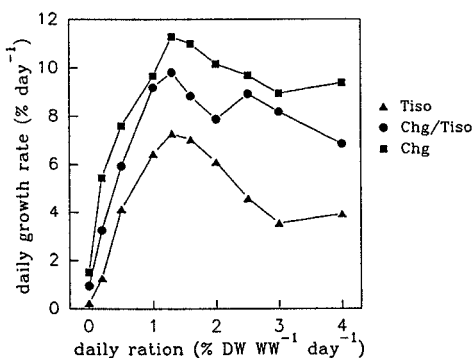


Figure 7. Daily growth rate as a function of daily ration for *T. philippinarum* fed *C. neogracile* (Chg), *Isochrysis* sp. clone T-Iso (Tiso), or a 50/50 (on dry weight basis) of both algal species (Chg/Tiso). Data sets were obtained from independent, one week experiments with one replicate culture per ration. Initial mean individual WW = 1.73 ± 0.03 mg (Chg), 6.94 ± 0.62 mg (Tiso), 3.92 ± 0.15 mg (Chg/Tiso).

moved from suspension averaged 1.16% of the clam's live weight. This is comparable with the weight-specific daily ration removed by *C. virginica* (15 g live weight) which ranged from 0.4% for *T. pseudonana* to 1.5% for *I. galbana* (Epifanio and Ewart 1977). Nevertheless, it appears difficult to explain why the optimal ration for juvenile Manila clams was as low as 1 to 1.5% DW WW⁻¹ day⁻¹ in the present laboratory experiments, whereas the standard regime in a commercial hatchery to feed a mixture of five algal species to seed of the same size and under comparable conditions of temperature and salinity consisted of 4% DW WW⁻¹ day⁻¹ (Albentosa et al. 1989). Also, the scarce literature data with regard to the effect of ration size on bivalve growth indicate higher values than those observed in the present study (Urban et al. 1983, Enright et al. 1986a,b). However, optimal ration differs according to the algal species (Enright et al. 1986a) and may be affected by the algal culture conditions (Enright et al. 1986b). Our data indicate that the optimal ration increases with increasing nutritional value of the algal diet, i.e. in ascending order: *Chaetoceros* concentrate, *Chaetoceros* culture, mixed algal diet used by Albentosa et al. (1989). Furthermore, various methodological factors may affect the value of the estimated daily ration, such as the accuracy of the algal dry weight analysis, the adaptation of the feeding regime to growth during the experiment, the natural food present in the seawater, and the settling and/or growth of the algae in the culture system.

Clam growth in the present experiments was acceptable when compared with the values of 7.6% day⁻¹ and 14% day⁻¹ reported for, respectively, *Mercenaria mercenaria* (initial live weight 25 mg) fed a mixture of *T. pseudonana* and *I. galbana* (Urban and

Pruder 1992), and *O. edulis* (initial live weight 1.14 mg) fed *C. calcitrans* (Laing and Millican 1986). The superior growth obtained when feeding clams with algal culture showed that the nutritional value of *Chaetoceros* decreased due to centrifugation and storage for maximal three days. By contrast, Nell and O'Connor (1991) could not detect any deleterious effects on growth of larvae of the Sydney rock oyster *Saccostrea commercialis* when various species of diatoms were fed after concentration to a paste and storage for 7–14 days at 4°C. The aberrant values observed during the first week of experiments three and five, may have been due to the relatively short acclimatization period prior to the start of the experiment. In this way, a bad initial condition of the spat may have caused difficulties to adapt to the experimental food, resulting in reduced feeding rate and gross growth efficiency. Alternatively, animals that originate from a well-fed population may benefit from their food reserves and initially maintain higher growth rates. Laing and Millican (1986) thus found that greater lipid reserves in *O. edulis* spat were associated with higher growth rates of the seed when transferred to the sea. In this regard, it would be interesting to relate the initial biochemical composition of the seed to its performance in future culture tests.

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