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Effects of algal food concentration and body size on the ingestion rates of *Ruditapes decussatus* (Bivalvia) veliger larvae

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ABSTRACT: Feeding rates of *Ruditapes decussatus* clam larvae from the D-shaped stage to metamorphosis were recorded at algal food concentrations ranging between 10 and 300 *Isochrysis galbana* cells μl^{-1}. The ingestion rate was an allometric function of the organic weight of larvae ($IR = a AFDW^b$) with exponents between 0.40 and 0.88, increasing with concentration. The ingestion rate was also directly related to food concentration up to an optimum at 200 cells μl^{-1}, beyond which it remained almost constant. The response of the ingestion rate to particle concentration could be described by assuming increasing interference among particles that reduces the efficiency of capture by *R. decussatus* of algal cells. Considering the unique mechanism of feeding in veligers, maximum ingestion capacity (cells h^{-1}) — rather than maximum clearance rate ($\mu l\ h^{-1}$), widely studied in physiological energetics of adult molluscs — is proposed as a more adequate indicator of maximum energy gain in bivalve larvae.

KEY WORDS: Veliger larvae · Food concentration · Ingestion rates

INTRODUCTION

The application of physiological energetics methods to bivalves enables us to predict or explain the growth of specimens exposed to known environmental conditions on the basis of their energy balance (e.g. Dame 1972, Thompson & Bayne 1974, Newell et al. 1977, Griffiths & King 1979, Bayne & Worrall 1980, Navarro & Winter 1982, Widdows & Johnson 1988, Beiras et al. 1993, 1994a). One of the environmental factors exerting a leading influence on the growth of bivalves is ingested food, which is directly related to its availability (e.g. for larvae see Walne 1965 and Pérez-Camacho et al. 1977; for adults see recent review by Griffiths & Griffiths 1987). Physiological energetics studies on bivalve larvae have focused on oyster (Wilson 1979, Crisp et al. 1985), mussel (Sprung 1984a, b), and pectinidae (MacDonald 1988), whereas information on the ecologically and commercially relevant Veneridae family is still lacking. In a previous study, we evaluated the influence of the other major environmental factor, temperature, on the energetics of *Ruditapes decussatus* larvae (Beiras et al. 1994b). Here we explore the interrelations among food concentration, larval size and larval feeding rates throughout the complete larval development of this species.

MATERIAL AND METHODS

Eggs and sperm were obtained by thermal induction of spawning on adult (4 to 5 cm) *Ruditapes decussatus* clams conditioned in our laboratory at 20 °C and with abundant algal food (a mixed diet of *Isochrysis galbana*, *Tetraselmis suecica* and *Phaeodactylum tricornutum*). Fertilization was conducted using 10 sperm per egg. Fertilized eggs were incubated in 50 l flat bottom tanks at 20 °C and 1000 eggs cm^{-2}. Seawater used was prefiltered (1 µm), UV sterilized, and previously dissolved chloramphenicol (10 mg l^{-1}) was added to further retard bacterial growth (Pérez-Camacho & Román 1973). After 48 h, swimming D-stage larvae were collected from the surface of the tanks and transferred to strongly aerated, cylindro-conical fiberglass tanks of 400 l, where larvae were reared at 10 ml^{-1} in treated seawater at 19 ± 1 °C. Larvae were routinely

fed on *I. galbana* (100 cells μl^{-1}) every 2 d, after the water was changed.

Triplicate samples of larvae were transferred at 4 developmental stages (Days 4, 11, 17 and 21) to 1 l beakers and fed on 10, 50, 100, 200 and 300 *Isochrysis galbana* cells μl^{-1}. A control beaker without larvae was also set up. The decrease in cell concentration after a few hours was then recorded by using a TAII Coulter Counter fitted with a 100 µm aperture sampling tube. Experiments were terminated before the cell concentration decrease exceeded 25%. With these data, ingestion rate (IR, cells h^{-1}) and clearance rate (CR, $\mu l\ h^{-1}$) were calculated following the equations (Beiras 1992):

$$IR = V/n\,t\,[(c_0 - c_1) - (c'_0 - c'_1)]$$

$$CR = V/n\,t\,[\ln(c_0/c_1) - \ln(c'_0/c'_1)]$$

where V is volume, n is the number of larvae, t is time, c_0 and c_1 are the initial and final cell concentrations respectively and c'_0 and c'_1 are the initial and final cell concentrations in the controls.

Larval length (anterior-posterior axis) was recorded on a sample of larvae by using a microscope with a graduated eyepiece. The weight of individual larvae was obtained by counting samples, under the binocular microscope, in a 1 ml counting cell, transferring them to Whatman GFC filters, drying them at 100 °C to constant weight and ashing them in a furnace at 450 °C until a constant weight was obtained (see further details in Beiras et al. 1990).

Multifactor analysis of variance (ANOVA) (Sokal & Rohlf 1969), type I, was used to test the effect of larval size and food concentration (both factors were fixed) on the feeding rates. The ingestion and clearance rate data were also logarithmically (base e) transformed and fitted to multiple regression models using a stepping procedure that excluded variables not reaching the level of statistical significance ($p = 0.05$). Statistical analyses were performed using Statgraphics computer software.

RESULTS

Larval size

Allometric growth of clam larvae could be described by the equations (SE of the fitting parameters in parentheses):

$$DW = 1.66 \times 10^{-7}(0.039)L^{3.01\,(0.078)},\ r = 0.999,\ p < 0.001$$

$$AFDW = 0.89 \times 10^{-7}(0.023)L^{2.95\,(0.079)},\ r = 0.999,\ p < 0.001$$

where L is length (µm), DW is dry weight (µg) and AFDW is ash-free dry weight (µg).

Both ingestion and clearance rates were power functions of larval size, expressed as ash-free dry weight, with exponent $b < 1$ (Table 1). At lower algal cell concentration, ingestion rate exponent is very low ($b = 0.40$) indicating higher weight-specific ingestions in smaller larvae. Increasing concentration caused not only an increase in the intercepts, i.e. higher ingestions, but also an increase in the slopes which approach to $b = 1$, i.e. constant weight-specific ingestion rates. A similar pattern is reflected by the exponents of the allometric clearance rate equations, but higher concentrations caused lower intercepts (lower clearance rate values).

Table 1. *Ruditapes decussatus*. Parameters of the allometric equations express ingestion rate (IR, *Isochrysis galbana* cells μl^{-1}) and clearance rate (CR, $\mu l\ h^{-1}$) as a function of ash-free dry weight (AFDW, µg) for larvae feeding on different *I. galbana* concentrations (C, cells l^{-1}); a: intercept (±SE); b: slope (±SE) r: correlation coefficient; p: probability level

C	ln$a$	b	r	p
IR = a AFDW^b				
10	4.04 ± 0.140	0.40 ± 0.103	0.775	<0.005
50	5.37 ± 0.161	0.59 ± 0.118	0.846	<0.001
100	5.74 ± 0.122	0.80 ± 0.090	0.942	<0.001
200	6.43 ± 0.126	0.81 ± 0.093	0.940	<0.001
300	6.45 ± 0.153	0.88 ± 0.125	0.919	<0.001
CR = a AFDW^b				
10	1.76 ± 0.194	0.37 ± 0.142	0.638	<0.05
50	1.73 ± 0.200	0.68 ± 0.147	0.825	<0.001
100	1.42 ± 0.150	0.85 ± 0.110	0.926	<0.0001
200	1.23 ± 0.148	0.86 ± 0.109	0.929	<0.0001
300	0.79 ± 0.249	0.94 ± 0.203	0.839	<0.005

Food concentration

The ingestion rate (Fig. 1a to d) increased with food concentration up to an optimum reached at 200 cells μl^{-1}. Further increases of food failed to enhance the ingestion rate except for 196 µm larvae, which showed maximum ingestion at 300 cells μl^{-1} (Fig. 1c). In general, clearance rate decreased when food concentration increased within the experimental range at all developmental stages tested (Fig. 1a to d). However, this decrease is less marked in larger larvae.

ANOVA showed highly significant differences ($p < 0.0001$) in the ingestion rate between sizes and food concentrations, as well as for the interaction between both factors ($p < 0.001$). There also were significant differences ($p < 0.0001$) in clearance rate between larval sizes and food concentrations, whereas the interaction of both factors was not significant ($p > 0.05$).

Feeding rates (IR and CR) could be described as functions of food concentration (C), dry weight (DW) and their interaction by means of the following

multiple regression equations, where all variables are highly significant ($p < 0.01$):

$$\ln IR = 3.70 + 0.29 \ln DW + 0.016C - 0.42 \times 10^{-4}C^2 + 0.0029 DW\,C \quad (r = 0.913,\ p < 0.0001)$$

$$\ln CR = 2.65 + 0.62 \ln DW - 0.49 \ln C + 0.0013 DW\,C \quad (r = 0.738,\ p < 0.0001)$$

DISCUSSION

The decrease in the rate of capture of suspended particles by filter feeders at increasing concentrations has been interpreted as an active mechanism-denominated functional response (recently reviewed by Hawkins & Bayne 1992). Winter (1978) stated that this response occurs once ingestion capacity of the animal reaches saturation. Following this model, clearance rate is maximum and constant from a very low concentration threshold up to a seston load of 2 mg l^{-1}, which includes the range of variation usual in the wild. This model does not seem to apply to veliger larvae, since clearance rate has been proven to decrease as soon as particulate food concentration exceeds 0.1 mg l^{-1} for *Mytilus edulis* (Sprung 1984a), 0.2 mg l^{-1} for *Patinopecten yessoensis* (MacDonald 1988) or 0.6 mg l^{-1} for *Ostrea edulis* (Crisp et al. 1985). In the present experiment with *R. decussatus*, clearance rate decreased at any concentration higher than the minimum assayed (0.2 mg l^{-1}), especially in younger larvae, whilst ingestion reached saturation at much higher concentrations (4 mg l^{-1}). Therefore, the so-called functional response causing a decrease in clearance rate would appear, in any case, well before larvae meet their ingestion capacity.

Previous workers on bivalve larvae agree that ingestion rate increases linearly with food concentration until saturation. However, controversy has arisen regarding the concentration at which the plateau is reached. Thus, Sprung (1984a) and MacDonald (1988) showed saturation of ingestion at relatively low concentrations of food (5 to 20 *Isochrysis galbana* cells μl^{-1}). In contrast, Wilson (1979) and Crisp et al. (1985) found saturation of ingestion at much higher concentrations (250 to 300 cells μl^{-1}). Experimental evidence is presented here supporting high ingestion capacity by veliger larvae. Nevertheless, differences in test species, larval sizes and culture conditions (such as larval density) may explain this discrepancy.

Some previous studies have related the decrease in clearance rate at higher algal concentrations with filling of the gut and saturation of the digestive capacity (Riisgard et al. 1980, Riisgard 1988). As mentioned above, however, food concentrations causing this decrease are considerably lower than those saturating the ingestion capacity of the larvae.

An alternative model to describe the relationship between food concentration and larval feeding rates is presented here, based on the following. (1) The effi-

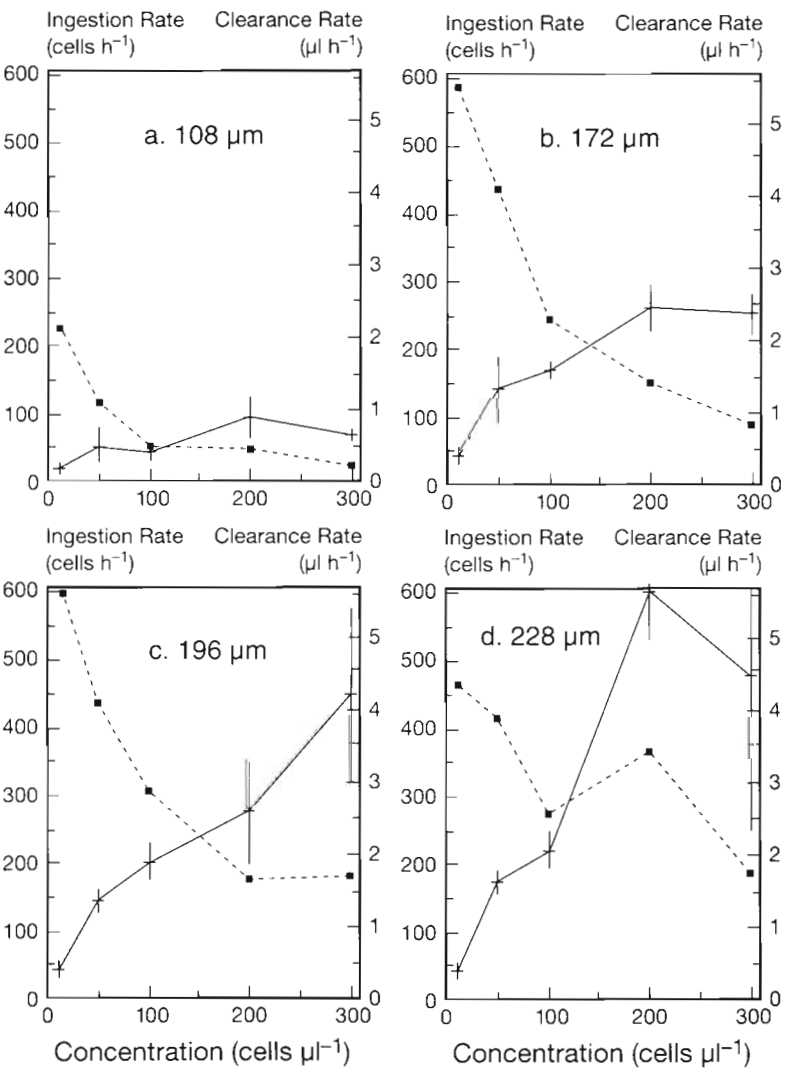

Fig. 1. *Ruditapes decussatus*. Ingestion rate (——) and clearance rate (-----) for larvae of (a) 108 µm, (b) 172 µm, (c) 196 µm and (d) 228 µm shell length fed on different *Isochrysis galbana* cell concentrations. Values are means ± SD of 3 replicates. Error bars represent ± SD

ciency of the process of feeding in veliger larvae is very low, i.e. only a small proportion of the particles moved by the water currents caused by the velar cilia are actually captured and ingested. (2) As a consequence of interference among food particles, the efficiency of capture of these particles decreases when concentration increases. (3) At a certain particle concentration which is a function of the larval size, saturation of the ingestion capacity is reached, due to either excessive interference among particles, limited gut volume and/or limited food processing ability. (4) Maximum ingestion capacity, and not maximum clearance rate, is the relevant parameter from both ecological and zootechnical standpoints, since this is the parameter upon which maximum larval growth depends.

Veliger feeding mechanism has little in common with the highly efficient mechanism of particle retention in the gills of adult bivalves. Gallager (1988) calculated that the water flow through the effective capture zone of veliger larvae constituted only between 15 and 30% of the total flow through the velum and that not even all the particles entering this area were ultimately captured. Further, there was no significant difference in cirral beat frequency or velocity for larvae exposed to different *Isochrysis galbana* concentrations ranging from 0.1 to 1000 cells μl^{-1}, i.e. water flow through the velum was independent of algal cell concentration (Gallager 1988). On the other hand, concentration did affect the efficiency of capture of food particles from the water current caused by the velum, which was 17% at low algal concentrations and markedly decreased at concentrations higher than 100 cells μl^{-1} (for 259 µm length *Mercenaria mercenaria* larvae; Gallager 1988).

Therefore, ingestion rate can be expressed as a function of flow through the velum (F, $\mu l\ h^{-1}$), particle concentration (C, cell μl^{-1}) and efficiency of capture (E, %);

$$IR = FCE/100$$

where, due to interference among particles, E is inversely related to C. By using this equation on the present data, this efficiency of capture can be calculated as a function of cell concentration. For example, assuming larvae of 172 µm length, an approximate value of water flow of 50 $\mu l\ h^{-1}$ through the velum of larvae of this size (Strathmann & Leise 1979, Gallager 1988), and ingestion rate from Fig. 1b, the efficiency values at 10, 50, 100, 200 and 300 cells μl^{-1} were 8.4, 5.6, 3.3, 2.6 and 1.7% respectively. Hence, the efficiency of particle capture can be described as an inverse function of particle concentration within the experimental range (Fig. 2), following the equation (SE of the fitting parameters in parentheses):

$$100/E = 10.8(2.15) + 0.158(0.0128)\,C; \quad r = 0.990,\ n = 5$$

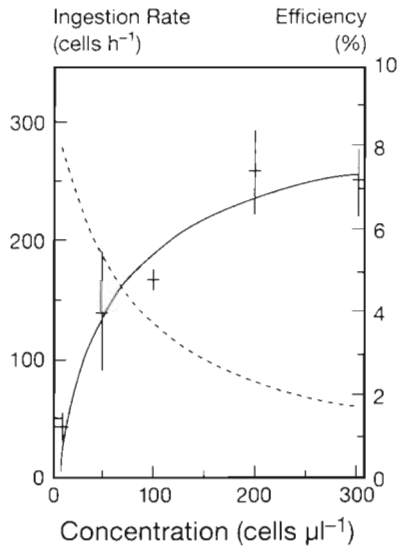

Fig. 2. *Ruditapes decussatus*. Ingestion rate (IR, ——) expressed as a hyperbolic function of particle concentration and efficiency of capture (-----) of food particles (*Isochrysis galbana* algal cells) for larvae of 172 µm shell length. The theoretical model for IR plotted assumes an increasing degree of interference among particles which progressively reduces the efficiency of capture of food particles. Actual IR values (mean ± SD) are also plotted for comparison

Therefore, following this model, the theoretical IR of a 172 µm larvae results in a hyperbolic function of C:

$$IR = 50C/(10.8 + 0.158C)$$

As illustrated in Fig. 2, there was good agreement between the theoretical ingestion rate derived from the model and the actual values recorded.

Some authors have resorted to the study of larval clearance rates at very low algal concentrations in an attempt to meet more ecologically realistic conditions (Riisgard et al. 1980, 1981, Jespersen & Olsen 1982, Sprung 1984a). However, it has been shown that individual adult molluscs feed on monoalgal cultures at rates that differ from their response to the concentration of natural seston (e.g. Winter 1976). The ecological relevance of maximum filtration capacity found at 2 cells μl^{-1} of *Isochrysis galbana* (or *Monochrysis lutheri*) (Riisgard et al. 1980, 1981, Sprung 1984a) is further questioned by the experimental evidence that these low concentrations are insufficient to support optimal growth, or indeed any growth at all (Jespersen & Olsen 1982, Sprung 1984b, Crisp et al. 1985, Pechenik et al. 1990, Beiras 1992). According to the energy balance, faster larval growth is achieved at environmental conditions supporting higher rates of energy acquisition. The food ration meeting this requirement is the ingestion capacity (Sprung 1984a), which is equivalent to the maximum ingested ration

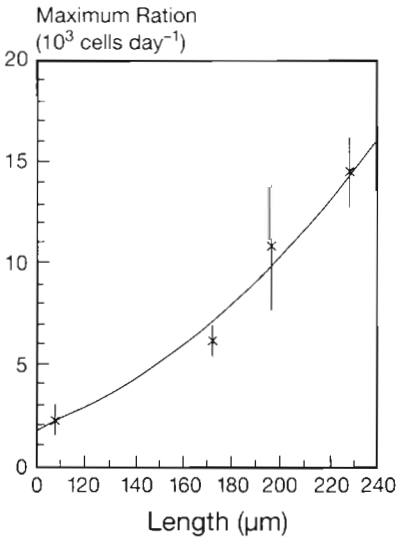

Fig. 3. *Ruditapes decussatus*. Maximum ration ingested daily by a larva fed on *Isochrysis galbana* as a function of larval length. Values are mean ± SD of 3 replicates. See equation of the logarithmic model fitted in the text

(MR), a parameter commonly used in nutrition studies of marine animals (reviewed by Brett 1979). In the present study, the maximum ration varied throughout larval development, as illustrated in Fig. 3, according to the equation:

$$\ln MR = 6.06(0.229) + 0.0158(0.00126)\,L;\ r = 0.994,\ n = 4$$

where, for practical reasons, larval development is presented as length (L, µm) and maximum ration as *I. galbana* cells ingested daily per larvae. Equivalences for other food sources can be calculated from the organic weight of *I. galbana*: 10^6 cells = 20 µg organic matter (authors' unpubl. data).

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