

Nutritional value of algal diets to clam spat *Venerupis pullastra*

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ABSTRACT: Clam spat *Venerupis pullastra* were fed for 4 wk on diets of 2 species of microalgae supplied singly or mixed. Microalgae were *Isochrysis galbana* and *Tetraselmis suecica*, species normally used in bivalve hatcheries. Observed growth responses were high for mixed and *I. galbana* diets and intermediate for the *T. suecica* diet. A slightly higher growth rate was obtained with the mixed diet than with the *I. galbana* diet. Differences in growth rate were explained by differences in rates of ingestion and digestibility and in the biochemical composition of diets. The intermediate growth rate for spat fed on *T. suecica* was related to lower ingestion rates, lower assimilation efficiencies and lower dietary protein and lipid content. Spat biochemical composition was correlated with growth indices. Lower growth indices were related to lower protein and higher lipid content of spat. The high growth response to the mixed diet was not explained by the factors considered.

INTRODUCTION

One of the major operations in bivalve hatcheries is the production of phytoplankton used as food for broodstock, larvae and spat. Algal culture management is the most costly stage in bivalve seed production (De Pauw 1981).

A number of studies have examined the nutritive value to bivalves of a variety of microalgal species (Walne 1970, Epifanio 1979, Webb & Chu 1983, Enright et al. 1986a). Generally, bivalve molluscs fed on diets consisting of more than one species of algae grow faster than those fed on single species diets. Moreover, certain algal species considered as 'poor' diets are better utilized in growth when fed in combination with other species of algae (Romberger & Epifanio 1981).

Although there have been many attempts to correlate differences in mollusc growth with composition of algal species (Wikfors et al. 1984, Enright et al. 1986b), the quantitative and qualitative nutritional requirements of molluscs are still unclear. Some polyunsaturated fatty acids of the ω 3 family have been reported to be essential for the normal growth of certain species of molluscs (Langdon & Waldock 1981).

In the present work, the nutritional value of 2 microalgal species, *Isochrysis galbana* and *Tetraselmis suecica*, to the spat of the bivalve *Venerupis pullastra* was examined. This study included the effect of diet on spat ingestion rates, growth and biochemical composition. Three diets were evaluated, two composed of single species, and the third composed of a mixture of both species.

MATERIALS AND METHODS

Spat. *Venerupis pullastra* spat used in this experiment were obtained from broodstock conditioned in our laboratory. Spawning induction and larval culture were performed following the method described by Pérez Camacho et al. (1977).

From the available stock spat were selected (mean length 1.36 ± 0.28 mm). Live and dry weights of this group were 0.47 and 0.21 mg ind.⁻¹, respectively. Ash-free dry weight of the initial spat was 15.3 % of dry weight.

Spat stock was randomly distributed between the experimental cultures, each one containing an initial biomass of 2 g (live weight).

Experimental conditions of spat cultures. Spat biomass was placed in partly submerged cylinders, 12 cm diameter by 7 cm high, equipped at the bottom with a 400 μm nylon mesh. Each cylinder was hung at the surface of 12 l plastic tanks (40 \times 30 \times 20 cm) to a depth of about 10 cm. Seawater, containing suspended food particles, was circulated over the cylinders by air-lift PVC pipes. To avoid food sedimentation, each experimental tank was equipped with an auxiliary air point.

Experimental tanks were maintained at $20 \pm 1^\circ\text{C}$. Tanks were emptied, cleaned and refilled with freshly filtered seawater, at a salinity of 33 ‰, 3 times per week. Experimental cultures were maintained for 4 wk when differences in growth between diets could be clearly detected.

Each diet was tested in duplicate; a third control tank without animals was used to quantify food sedimentation.

Diets. Two algal species, *Isochrysis galbana* (T-iso) and *Tetraselmis suecica* were evaluated singly and in mixed diets. Diets were always in equal relative-biomass proportions on a dry weight basis. The mixed diet was composed of 50 % of each species.

Microalgae were cultured in 6 l glass flasks, in a temperature-controlled chamber at 18°C with continuous illumination at 9900 lux. Salinity was constant at 33 ‰. The culture medium has been described by Walne (1966). Algae used in feeding experiments were harvested during the initial stationary phase of growth.

Daily feeding ration, expressed as algal dry weight, applied for all diets was about 5 % of clam live weight. Initial algal concentrations never exceeded $10 \mu\text{g ml}^{-1}$, pseudofaeces-free food density (Epifanio & Ewart 1977). Algal cell concentration in the tanks before and after feeding each day was monitored using a Coulter Counter TA II. From these values the number of cells cleared from suspension was calculated so that daily food ration was kept constant. Daily increases in the biomass of the spat were taken into account to adjust food ration. During the first week, daily growth rates were estimated from previous experiments performed in our laboratory.

Dry and organic content of the phytoplankton cells were calculated by filtration through 4.25 cm Whatman GF/C glass fiber filters previously ashed and rinsed with 0.5 M ammonium formate solution. Filters were dried to constant weight at 80°C , ashed at 450°C in a muffle furnace, and weighed again. Variations in algal dry weight during the experimental period were considered when calculating.

Phytoplankton samples were taken during the 4 wk of the experiments for biochemical analysis. Samples were centrifuged, resuspended with 0.5 M ammonium formate solution, freeze-dried and stored at -30°C until analyzed.

Parameters for diet evaluation. Daily ingestion rates were calculated by the number of cells cleared after the feeding period using the following expression:

$$IR = \frac{V}{nt} \left((C_{e_0} - C_{e_t}) - \frac{C_{b_0} - C_{b_t}}{2} \right) \quad (1)$$

where IR is the ingestion rate expressed in cells $\text{ind.}^{-1} \text{h}^{-1}$; V is the volume of the experimental tank (ml); n is the number of clams; and time (t) is expressed in hours. Initial and final concentrations in the experimental tank are given as C_{e_0} and C_{e_t} , and initial and final concentrations in the control tank as C_{b_0} and C_{b_t} .

Specific ingestion rates were estimated as the individual ingestion rate divided by the individual dry weight.

Once a week, spat live weight increases for each experimental culture were measured by drying total biomass on absorbent paper for 10 min to remove surface water and then weighing it. Part of the spat were removed and the remainder replaced in the cylinders in such a way that total biomass never exceeded 2 g (live weight) in any experimental tank. The removed spat were used for dry and organic weight determinations and for biochemical analysis. Dry and organic (ash-free dry weight) weights of spat were determined at 80 and 450°C , respectively.

Spat growth during experiments was fitted to the equation:

$$\text{Dry weight} = e^{kt + \alpha} \quad (2)$$

where dry weight is expressed in mg ind.^{-1} , time (t) in days, and k , α are constants. The constant k , which represents the slope of the line, was considered as a coefficient of growth rate and was used for diet comparisons.

Once a week samples from each replicate were taken for measurement of length using a graduated binocular microscope.

Gross growth efficiency (K_1) was considered as the proportion of the organic weight of the food ingested that is incorporated into organic growth of spat, that is

$$K_1 = \frac{G}{I} \quad (3)$$

where G is the increase in organic weight of the spat over a given time period, and I is the organic weight of the food ingested during the same time interval. Net growth efficiency (K_2) was considered as the proportion of the organic matter assimilated – including organic matter incorporated in growth and organic matter consumed in respiration – that is incorporated into organic growth of spat,

$$K_2 = \frac{G}{G + R} \quad (4)$$

where R is the organic weight equivalent of the oxygen consumption of the spat due to respiration. An equal respiration rate was assumed for all diets using a mean value of $2.27 \pm 0.28 \text{ ml O}_2 (\text{g AFDW})^{-1} \text{ h}^{-1}$ obtained in our laboratory for *Venerupis pullastra* spat of the same size and cultured at the same temperature as used in the present experiment (Albentosa et al. unpubl.). For oxygen consumption, we used the ash-free dry weight equivalent given by Walne (1965), namely 1 mg per 1.2 ml O_2 consumed.

Assimilation efficiency (AE) was estimated as the proportion of the organic matter ingested that is assimilated, as follows:

$$AE = \frac{G+R}{I} \quad (5)$$

Utilization efficiencies of the food protein were calculated following the expression given by De La Higuera et al. (1977),

$$PPV = \frac{P_f - P_i}{P_{ing}}$$

where PPV is the protein productive value, P_f , P_i are the final and initial protein compositions and P_{ing} is the amount of protein ingested.

Analytical methods. Protein was assayed as described by Lowry et al. (1951) after hydrolysis in 0.5 N NaOH for 24 h at 30 °C. Total carbohydrates were quantified as glucose by the phenol-sulphuric acid method (Strickland & Parsons 1968). Lipids were extracted following a modification of the method of Bligh & Dyer (1959) (Fernández-Reiriz et al. 1989). Lipids were first extracted with chloroform-methanol (1:2) and after centrifugation the precipitate was extracted again with chloroform-methanol-water (8:4:3) (Folch et al. 1957). Total lipids were determined by the Marsh & Weinstein (1966) method using tripalmitolein standards.

RESULTS

Growth

Changes in mean individual spat weights during the 4 wk of the experiment are shown in Table 1. Initial weights were equal for all treatments. Analysis of variance indicated significant effects ($p < 0.05$) of diet on ash-free dry weight of the clams after the second week. Differences were detected after the second week between spat fed on *Tetraselmis suecica* (TS) and those fed on *Isochrysis galbana* (ISO) or mixture (MX); however, between the latter 2 diets weight dif-

Table 1. *Venerupis pullastra*. Changes in spat live, dry and ash-free dry weights (\pm standard deviation) during the 4 wk of experimentation. ISO: *Isochrysis galbana*; TS: *Tetraselmis suecica*; MX: *I. galbana* and *T. suecica*

Diet	Live weight (mg ind. ⁻¹)	Dry weight (mg ind. ⁻¹)	AFDW (mg ind. ⁻¹)	%AFDW
Initial	0.47	0.21	0.03	15.3
1st week				
ISO	0.95 \pm 0.06	0.43 \pm 0.02	0.10 \pm 0.01	24.4 \pm 0.7
TS	0.86 \pm 0.01	0.43 \pm 0.00	0.08 \pm 0.01	18.7 \pm 1.8
MX	0.97 \pm 0.01	0.46 \pm 0.02	0.08 \pm 0.01	17.2 \pm 0.6
2nd week				
ISO	2.32 \pm 0.07	1.06 \pm 0.04	0.22 \pm 0.04	20.5 \pm 2.7
TS	2.48 \pm 0.07	1.12 \pm 0.06	0.23 \pm 0.02	20.4 \pm 0.3
MX	2.59 \pm 0.01	1.15 \pm 0.01	0.21 \pm 0.07	21.3 \pm 0.6
3rd week				
ISO	5.05 \pm 0.46	2.17 \pm 0.21	0.57 \pm 0.09	26.1 \pm 1.4
TS	3.50 \pm 0.16	1.49 \pm 0.01	0.33 \pm 0.01	22.0 \pm 0.5
MX	6.43 \pm 0.08	2.71 \pm 0.05	0.74 \pm 0.08	27.3 \pm 3.6
4th week				
ISO	10.39 \pm 0.26	4.46 \pm 0.18	1.22 \pm 0.13	27.4 \pm 1.8
TS	5.59 \pm 0.67	2.46 \pm 0.21	0.57 \pm 0.12	23.1 \pm 2.9
MX	16.43 \pm 3.45	6.87 \pm 1.26	1.65 \pm 0.19	24.1 \pm 1.7

ferences were not significant, even in the third or fourth week ($p = 0.0738$ and $p = 0.1156$, respectively).

Dry weight (DW) growth of the clam spat was related exponentially with time (Fig. 1). Regression lines were fitted in the form:

$$\text{ISO: } DW = e^{-1.63 + (0.114 \pm 0.0024)t} \quad r = 0.99, p < 0.01$$

$$\text{TS: } DW = e^{-1.48 + (0.090 \pm 0.0067)t} \quad r = 0.97, p < 0.01$$

$$\text{MZ: } DW = e^{-1.69 + (0.129 \pm 0.0023)t} \quad r = 0.99, p < 0.01$$

where DW is expressed as mg ind.^{-1} , and t as days. The values in parentheses are k values with their standard errors. Higher values of k represent faster growth

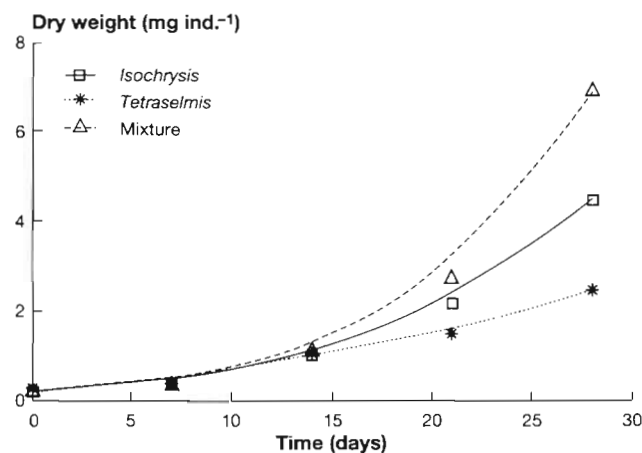


Fig. 1. *Venerupis pullastra*. Dry weight growth rate of spat fed on *Isochrysis galbana*, *Tetraselmis suecica* and a mixture of both microalgae. See the text for regression equations

Table 2. *Venerupis pullastra*. Covariance analysis of spat growth fitted between individual dry weight (mg ind.⁻¹) and time (d) for the 3 diets tested, ISO (*Isochrysis galbana*), TS (*Tetraselmis suecica*) and MX (*I. galbana* and *T. suecica*). Degrees of freedom are shown in parenthesis. * Significant differences between *k* coefficients ($p < 0.05$)

Diet	F_v (df)	F_k (df)	F_a (df)
ISO TS	0.14 (7,7)	11.92 (1,14)*	5.07 (1,16)
ISO MX	0.79 (7,7)	14.85 (1,14)*	12.45 (1,16)
TS MX	5.48 (7,7)	29.36 (1,14)*	10.39 (1,16)

rates. Covariance analyses were performed to compare growth lines; as can be seen in Table 2, *F*-ratios for the growth rate coefficients (*k*) were significantly different for all diet comparisons ($p < 0.05$).

Relationship between length (*L*) and weight of the clam spat was the same for all diets tested ($p > 0.05$), so that the regression equation was calculated with the combined data for the 3 diets,

$$DW = 0.13 L^{2.50} \quad r = 0.96, p < 0.01$$

where *DW* is expressed in mg ind.⁻¹, and *L* in mm.

Gross growth efficiencies (K_1) were different between diets and showed a remarkable decrease during the experimental period, particularly in the last week. As shown in Fig. 2, after the second week higher growth efficiencies were obtained with MX and ISO than with TS. In Fig. 3 growth efficiencies are plotted against spat weights. For the same spat size, growth efficiencies for TS were notably lower than growth efficiencies for the other diets. Thus, for e.g. 2.5 mg spat, growth efficiency obtained with TS was 20 %, whereas with MX it was > 40 %.

Table 3 shows growth and assimilation efficiencies calculated for the overall experiment. Assimilation effi-

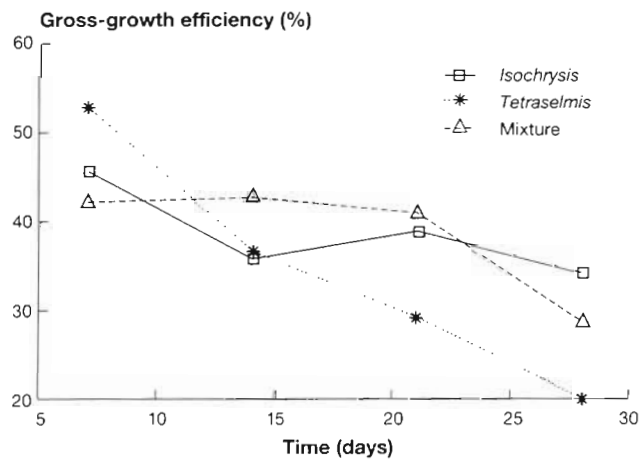


Fig. 2. *Venerupis pullastra*. Gross growth efficiencies of spat fed on *Isochrysis galbana*, *Tetraselmis suecica* and a mixture of both microalgae. Points are the mean values of 2 replicates

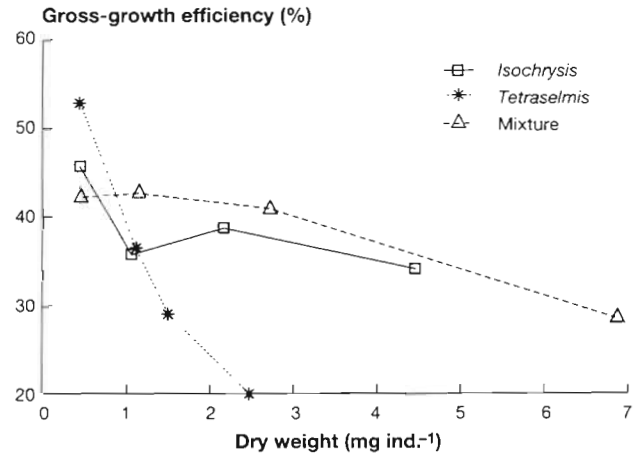


Fig. 3. *Venerupis pullastra*. Relative-weight gross growth efficiencies of spat fed on *Isochrysis galbana*, *Tetraselmis suecica* and a mixture of both microalgae

ciencies ranged from 55 to 70 %, similar values to those described by Romberger & Epifanio (1981) for good and regular diets. In Fig. 4, assimilation efficiencies are plotted against spat dry weight. Assimilation efficiencies for TS decreased during the experiments and were slightly lower than for the other diets, mainly in the last weeks. Between ISO and MX, assimilation efficiencies were quite similar, although in the last week the assimilation efficiency was higher for the ISO diet.

Ingestion rates

Sedimentation rates observed in the control tanks were negligible for all diets (mean sedimentation rate

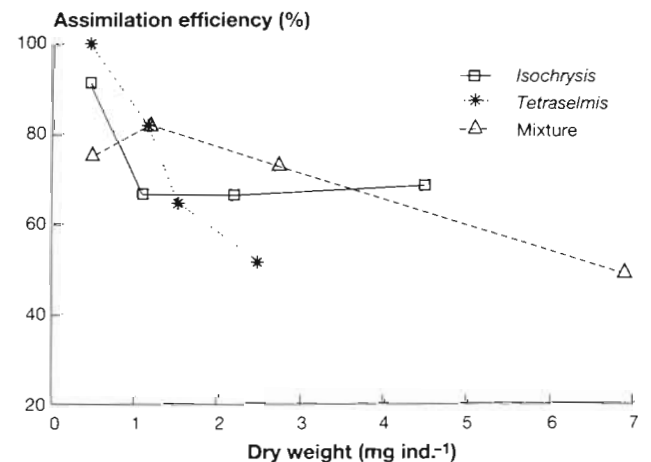


Fig. 4. *Venerupis pullastra*. Relative-weight assimilation efficiencies of spat fed on *Isochrysis galbana*, *Tetraselmis suecica* and a mixture of both microalgae. Assimilation efficiencies were calculated assuming growth increases and respiration costs as assimilated ration

Table 3. *Venerupis pullastra*. Efficiencies calculated for the overall experimental period. *G*: total increase in organic matter per individual; *I*: total ingestion per individual; K_1 : gross-growth efficiency; *R*: respiration cost; K_2 : net-growth efficiency; *AE*: assimilation efficiency

	<i>G</i> (mg AFDW ind. ⁻¹)	<i>I</i> (mg AFDW ind. ⁻¹)	K_1 (<i>G</i> / <i>I</i>)	<i>R</i> (mg AFDW ind. ⁻¹)	K_2 [<i>G</i> /(<i>G</i> + <i>R</i>)]	<i>AE</i> [(<i>G</i> + <i>R</i>)/ <i>I</i>]
ISO	1.20 (0.13)	2.72 (0.05)	44.0 (4.2)	0.67 (0.08)	64.1 (0.28)	68.7 (6.9)
TS	0.55 (0.12)	1.54 (0.35)	35.5 (0.4)	0.39 (0.05)	58.4 (2.26)	60.8 (2.9)
MX	1.62 (0.19)	4.33 (0.33)	37.1 (1.4)	0.86 (0.03)	65.2 (1.9)	57.2 (0.8)

during the 4 wk was below 5 % of the initial cell concentration), so they were omitted from the ingestion rate calculations (Eq. 1).

Ingestion rates of the clams were mainly dependent on spat weight and could be described by the following expressions:

ISO: $IR = 2.92 DW^{1.01}$ $r = 0.97$, $p < 0.01$

TS: $IR = 1.88 DW^{1.09}$ $r = 0.83$, $p < 0.01$

MX: $IR = 3.00 DW^{1.16}$ $r = 0.97$, $p < 0.01$

where *IR* is expressed in $\mu\text{g AFDW ingested ind.}^{-1} \text{ h}^{-1}$, and *DW* as mg ind.^{-1} . From these regression lines (Fig. 5), it can be seen that ingestion rate for TS was slightly lower than for the others. Regression lines for ISO and MX diets were almost congruent. Specific ingestion rates [*sIR*, $\mu\text{g AFDW ingested (mg spat DW)}^{-1} \text{ h}^{-1}$] calculated daily were compared by an analysis of variance. The *sIR* were significantly different ($p < 0.01$) among diets, except for the ingestion rates recorded during the second week of the experiment. These differences were detected between TS and ISO or MX diets; for the latter, significant differences ($p < 0.01$) were only observed in the last week, when *sIR* for MX were higher with a mean value of $4.11 \mu\text{g AFDW (mg DW)}^{-1} \text{ h}^{-1}$ compared to 3.08 for ISO. Mean *sIR* for the 4 wk were: 2.11 for TS, 2.98 for ISO and 3.20 for MX.

In MX, it was observed that the ingestion of *Isochrysis galbana* cells was significantly ($p < 0.01$) higher than that of *Tetraselmis suecica*, except for the fourth week when no selective ingestion was detected ($p > 0.05$). Mean ingestion values for each microalgal species were $1.37 \mu\text{g AFDW (mg DW)}^{-1} \text{ h}^{-1}$ for *T. suecica* and 1.83 for *I. galbana*.

Gross biochemical composition

Results obtained from biochemical analysis of the phytoplankton used as food in the experiments are shown in Table 4. For MX, biochemical composition was considered as the mean composition of the single diets.

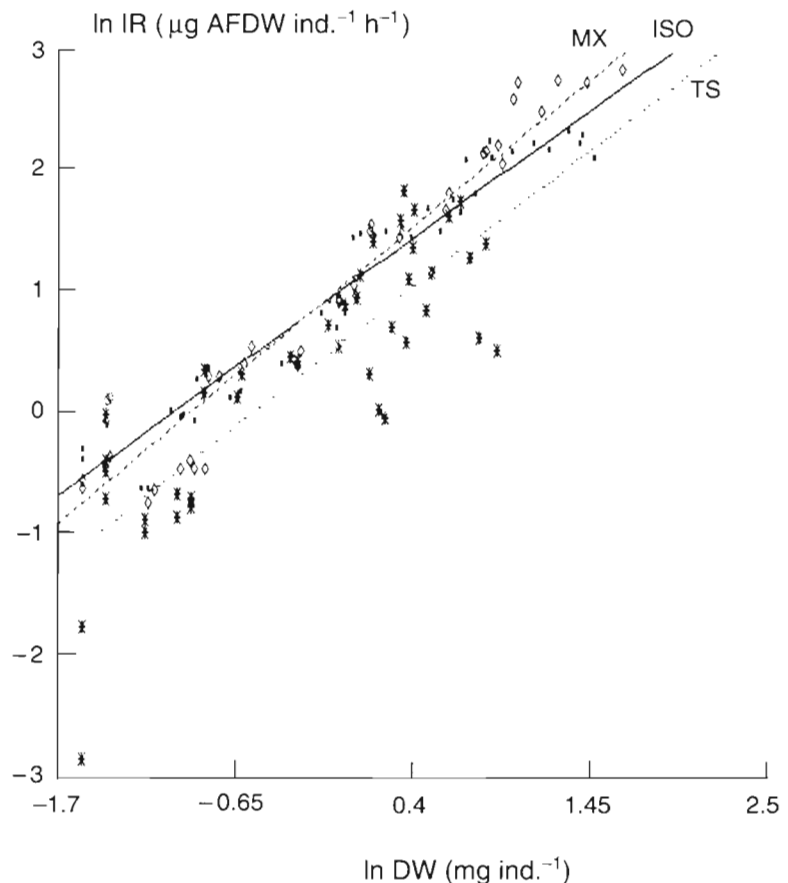


Fig. 5. *Venerupis pullastra*. Regression lines fitted between ingestion rates and individual dry weight of spat. Ingestion rates are expressed as $\mu\text{g AFDW ind.}^{-1} \text{ h}^{-1}$ and dry weights as mg ind.^{-1} . (□) *Isochrysis galbana*; (*) *Tetraselmis suecica*; (◇) mixture-fed spat

Table 4. Biochemical composition of the phytoplankton used as food for the clams. *Standardized percentages

	Ash %DW	Carbohydrates		Proteins		Lipids	
		%DW	%AFDW	%DW	%AFDW	%DW	%AFDW
ISO	15.44	20.23	23.92	23.60	27.91	27.84	32.92
		23.87*	28.22*	27.09*	33.69*	32.85*	38.84*
TS	24.66	20.50	27.21	13.48	17.89	6.03	8.00
		38.60*	51.24*	25.38*	33.69*	11.35*	15.07*
MX	20.05	20.37	25.57	18.54	22.90	16.94	20.46
		31.24*	39.73*	26.61*	33.31*	22.10*	26.96*

The sum of the gross biochemical components did not correspond with the total organic matter estimated by ashing, so biochemical percentages were standardized – noted by an asterisk in the table – in such a way that the sum of the evaluated components was considered the total organic content of the samples. In Fig. 6 percentages of ash-free dry weight of biochemical constituents of the diets are shown together with the percentages of the organic matter not detected by the analysis.

Gross biochemical composition of spat at the beginning and end of the experiment is detailed in Table 5. Increases in the biochemical composition during the experimental period are shown in Fig. 7. The increase in proteins for ISO- and MX-fed spat was twice the increase in lipids or carbohydrates. However, there was a substantial increase in lipids – higher even than the protein increase – for TS-fed spat.

Statistical analysis applied to angular transformations of the biochemical percentages shows that there were significant differences ($p < 0.05$) in protein con-

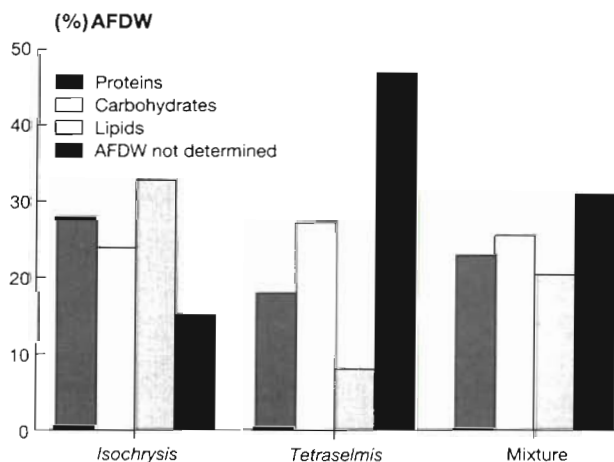


Fig. 6. *Venerupis pullastra*. Gross biochemical composition of the phytoplankton used as food for spat. Mixed diet composition was considered as the mean value of the 2 single-species diets. AFDW not determined by the analytical methods used is also shown

tent of the spat depending on the diet. Thus, protein percentage of TS spat was lower than for the other diets. However, no significant differences were detected in the protein content between ISO and MX spat. Standardization of the biochemical percentages also allowed us to detect significant differences ($p < 0.05$) in the lipid content of the spat. It is assumed that the proportion of the components in the organic matter undetected by the analytical methods is the same as in

the organic matter analyzed. Spat growth differences between diets are highly correlated with the protein and lipid content of the spat. Protein content shows a positive correlation (Table 6) with spat growth, so that in those cultures in which growth rate was high, spat protein content was also high. By contrast, the correlation coefficient detected between growth and lipid content was negative, meaning that low growth rates were associated with high body lipid levels.

Differences in the protein conversion indices were detected between diets (Table 7). The protein conversion index was high (i.e. 76 % and 67 % for MX and ISO diets, respectively) and promoted good growth, whereas for TS the protein conversion index was substantially lower (43 %).

DISCUSSION

Isochrysis galbana is a good food whereas the nutritive value of *Tetraselmis suecica* is moderate in the culture of *Venerupis pullastra* spat. Better results can be obtained with a mixture of both microalgal species.

Traditionally, it has been considered that the following factors are responsible for the nutritive value of the microalgae species used as food for bivalves: cell size, digestibility, toxicity and biochemical composition (Webb & Chu 1983). With regard to algal cell size, the species studied show considerable differences: *Tetraselmis suecica* cells are notably larger than *Isochrysis galbana* cells, with mean cell diameters of $7.64 (\pm 0.36) \mu\text{m}$ and $4.00 (\pm 0.09) \mu\text{m}$, respectively; mean cell volumes were $249.85 (\pm 33.91) \mu\text{m}^3$ and $35.07 (\pm 2.66) \mu\text{m}^3$ respectively. However, both cell sizes can be retained efficiently by the filtration mechanism of molluscs (Jørgensen 1990).

Growth differences observed between treatments can be explained partly by differences in ingestion rates. In fact, specific ingestion rates were slightly higher for MX-fed spat, followed by ISO, and were significantly lower in TS-fed spat. Ingestion of *Isochrysis galbana* was higher than that of *Tetraselmis suecica*

Table 5. *Venerupis pullastra*. Biochemical composition of spat fed with the 3 diets. Carbohydrates, proteins and lipids are expressed as % of dry and ash-free dry weights and as mg ind.⁻¹ *Standardized percentages

	Ash %DW	Carbohydrates			%DW	Proteins		%DW	Lipids	
		%DW	%AFDW	mg ind. ⁻¹		%AFDW	mg ind. ⁻¹		%AFDW	mg ind. ⁻¹
Initial	84.74	1.36	8.91	0.0029	3.54	23.20	0.0074	2.01	13.17	0.0042
		3.00*	19.68*	0.0063*	7.82*	51.24*	0.0164*	4.44*	29.09*	0.0093*
ISO	72.60	2.56	9.32	0.114	11.73	42.89	0.523	4.23	15.65	0.186
		3.83*	13.89*	0.171*	17.47*	63.58*	0.782*	6.11*	22.54*	0.271*
TS	76.87	1.78	7.80	0.044	5.30	22.83	0.131	4.96	21.69	0.121
		3.41*	14.82*	0.084*	10.24*	43.89*	0.254*	9.50*	41.29*	0.234*
MX	75.86	2.05	8.44	0.138	11.23	46.26	0.756	2.92	12.09	0.199
		3.05*	12.63*	0.208*	16.72*	69.16*	1.138*	4.39*	18.23*	0.303*

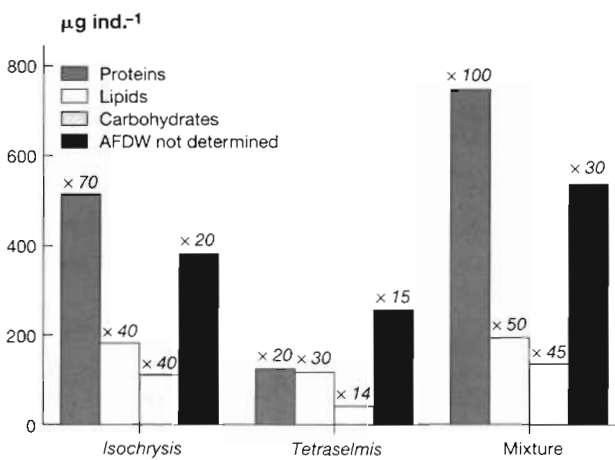


Fig. 7. *Venerupis pullastra*. Increases of the biochemical components of spat fed on 3 diets during the 4 wk of the experiment. AFDW not determined by the analytical methods used is also shown

cells in MX. Although there is a different ingestion depending on the microalgal species, this fact cannot completely explain the differences in growth. The proportion of food ingested is lower for the TS diet than for the other diets. However, the proportion of ingested food used for growth is also lower (Fig. 8a, b, c).

Digestibility of phytoplankton cells can be another factor determining growth. Epifanio (1979) explains the limited nutritive value of *Tetraselmis suecica* to molluscs by the difficulty of digestion of the complex polysaccharides and protein composing the theca of this microalga. In a later study, Romberger & Epifanio (1981) determined assimilation efficiencies of different microalgal species supplied as single and mixed diets for *Crassostrea virginica* juveniles and described an assimilation efficiency of 7 % for *T. suecica* cells and 74 % for *Isochrysis galbana*. However,

we found higher assimilation efficiencies (calculated from growth and estimated respiration costs) for *T. suecica* cells (nearly 60 %) although this value was slightly lower than the efficiency of *I. galbana* cells (70 %). We did not observe an increase in assimilation of cells when they were supplied as a mixed diet.

Another possible explanation for differences in the nutritive value of microalgal species is related to their biochemical composition and their adequacy for the nutritional requirements of bivalves.

Analyses of the phytoplankton cultures used in these experiments (Fig. 5) show that *Tetraselmis suecica* cells have a lower protein content (18 % of organic matter, unstandardized value) than *Isochrysis galbana* (28 %), i.e. almost 40 % less. Flaak & Epifanio (1978) calculated a protein requirement for *Crassostrea virginica* juveniles (>2.5 cm length) of 20 % of diet dry weight, a value slightly higher than the protein content of the *T. suecica* cultures used in our experiment. There are no data in the literature on protein requirements of mollusc spat of the size used in this work (1.4 mm); however, such requirements should be higher than those reported by Flaak & Epifanio since growth rates are higher for this spat size. The considerably higher lipid content of the *I. galbana* cultures (more than 30 % of the organic matter) in relation to *T. suecica* cultures (8 %) could also influence spat growth. Experiments carried out by Holland & Spencer (1973) and Holland & Hannant (1974) demonstrated that

Table 6. *Venerupis pullastra*. Correlation analysis between biochemical composition (standardized percentage of the ash-free dry weight) and growth (dry weight; mg ind.⁻¹) of the spat at the end of the experiment

Growth index	Biochemical component	Correlation coefficient (r)	Significance level (p)	n
Dry weight	Carbohydrates	-0.587	0.220	6
Dry weight	Protein	0.855	0.030	6
Dry weight	Lipids	-0.827	0.042	6

Table 7. *Venerupis pullastra*. Protein conversion indices of spat (mg ind.^{-1}). Δ : protein increases during the experimental period; *Ing*: ingestion of dietary protein during the same time; *CI*: conversion indices

	Δ	<i>Ing</i>	<i>CI</i>
ISO	0.51	0.76	67 %
TS	0.12	0.28	43 %
MX	0.75	0.99	76 %

(1973) and Holland & Hannant (1974) demonstrated that both larvae and early spat make use of lipids as the main energy reserves, whereas adults accumulate energy as glycogen. The carbohydrate contents of the 2 microalgal species are very similar – about 25 % of the organic matter.

That the total organic matter found by biochemical analysis did not correspond with that determined by ashing has been observed by other authors (Heral & Deslous-Paoli 1983). His & Maurer (1988) consider that organic matter not detected by analytical methods consists of proteins not detected by the Lowry method. Present results show that the proportion of organic matter not detected is higher in *Tetraselmis suecica* cultures (45 %), so considering its poor nutritive value it can be supposed that the components undetectable by analytical procedures are refractory to the bivalves' enzymes.

Another difference in the biochemical composition of the 2 algal species is related to fatty acid content. *Isochrysis galbana* shows considerable amounts of 2 polyunsaturated fatty acids regarded as essential for certain species of molluscs, 20:5 ω 3 and 22:6 ω 3, whereas *Tetraselmis suecica* is deficient in the latter (Albentosa et al. unpubl.). Langdon & Waldock (1981) proved that these fatty acids are essential for *Crassostrea gigas* spat by using a combination of algal diets deficient in 20:5 ω 3 and/or 22:6 ω 3 fatty acids, and adding supplements of microencapsulated lipids. Growth of *T. suecica* fed spat was improved by adding supplements of 22:6 ω 3. According to our results (Albentosa et al. unpubl.), the fatty acid composition of *Venerupis pullastra* spat reflected that of the diet. *I. galbana*-fed spat showed a high 22:6 ω 3 content, exactly 8.5 % of the total fatty acids. By contrast, the proportion of this fatty acid was very low (0.34 %) when the diet used was *T. suecica*. Spat fed on the mixture had an intermediate content (3.8 %) of 22:6 ω 3. These results suggest that *V. pullastra* spat is not able to synthesize 22:6 ω 3 from dietary 20:5 ω 3.

Gross biochemical composition of spat was also influenced by diet composition, mainly with regard to protein and lipid content. Increases in the protein content of the spat were double the lipid increases for the

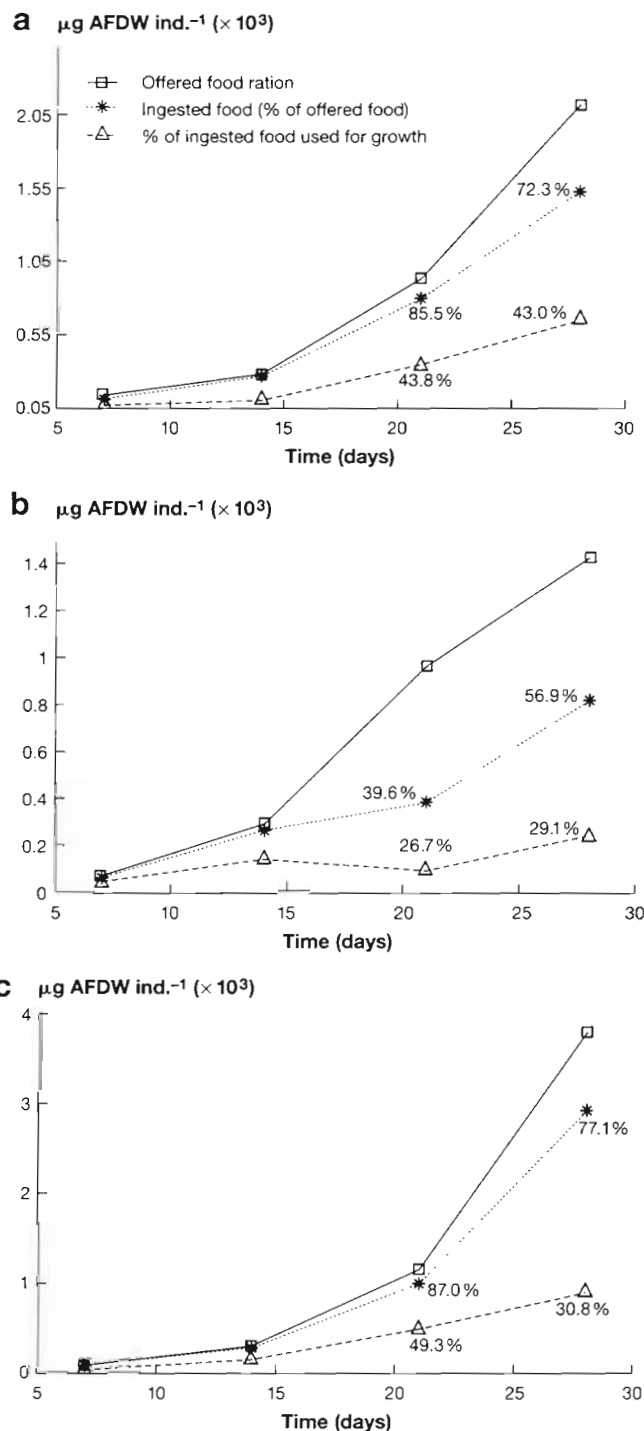


Fig. 8. *Venerupis pullastra*. Percentages of food utilization by spat fed with (a) *Isochrysis galbana*, (b) *Tetraselmis suecica* and (c) the mixed diet

growth-promoting diets (ISO and MX) (Fig. 7), whereas protein increase was lower than lipid increase for TS-fed spat. It seems that TS-fed spat cannot utilize dietary lipids properly. Thus, almost all lipids ingested are immobilized in the body tissues and proteins have

to be used as energetic combustibles. Consequently growth is limited. This situation becomes more critical because the protein content in TS diet is the lowest. With the high-nutritive-value diets (ISO and MX), lipids ingested are optimally used as energetic fuels and protein can be retained for synthesis of new body structures.

The slight increase of spat growth rate with the mixed diet compared to the *Isochrysis galbana* diet cannot be explained by our work. The mixture of the 2 algal species may supply further micronutrients (not evaluated in our experiment) which may be responsible for growth increase.

We consider that the nutritive quality of microalgal species does not depend on any particular factor but rather on the sum of total nutritive properties such as diet acceptability, digestibility and biochemical composition.

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