Journal of Shellfish Research, Vol. 13, No. 1, 67–75, 1994.

GROWTH AND BIOCHEMICAL PROFILE OF JUVENILE MUSSELS (*MYTILUS GALLOPROVINCIALIS* Lmk) FED ON DIFFERENT ALGAL DIETS

J. P. FIDALGO, A. CID, I. LÓPEZ-MUÑOZ, J. ABALDE, AND C. HERRERO
Laboratorio de Microbiología
Dpto. de Biología Celular y Molecular
Facultad de Ciencias
Universidad de La Coruña
Campus de A Zapateira, s/n
15071 La Coruña. Spain

ABSTRACT The food value of different microalgal diets to juvenile *Mytilus galloprovincialis* Lmk was assessed. Three different marine microalgae species (*Tetraselmis suecica, Dunaliella tertiolecta* and *Phaeodactylum tricornutum*) were fed singly and in various mixtures. Composition of the mixtures was based on the dry weight of the cells. Significantly higher growth rates in length and volume were obtained with diets containing *T. suecica*. Differences in food value between diets containing *T. suecica* and the remainder also were observed in terms of growth in dry and organic weights, gross growth efficiencies and condition indexes. All these parameters were correlated significantly with each other. The biochemical profile of the mussels also was modified by the diet. Mussels fed on diets containing *D. tertiolecta* showed increased levels of carbohydrates. Growth rates, gross growth efficiencies and condition indexes were correlated significantly with the protein and lipid deposited as body constituents, and lipid content (%DW) of the mussels. In the diets of higher growth, 44–47% of algal protein supplied was deposited as body protein, against 4.2–4.5% of lipids and 33–64% of carbohydrates.

KEY WORDS: mussel, *Mytilus galloprovincialis*, microalgal diets, growth, biochemical profile

INTRODUCTION

Extensive cultivation of mussels is a world-wide activity of growing economic importance. Mussel spat traditionally has been obtained from collector ropes on rafts, or natural banks on the coast (Mariño et al. 1983, Dardignac-Corbeil 1990). The first stages of culture (spat and young mussels) are critical in rearing bivalves, and improvements in the production require improvements in the quality of the spat.

The nutritional requirements of juvenile mussels are poorly understood (Hawkins and Bayne 1991). The nutritional value of different microalgal species as food for juvenile bivalves has been studied widely in clams and oysters, but only a few studies have been focussed on juvenile mussels (e.g. Walne 1970, Strömgren and Cary 1984).

Mussels are also used as test organisms or bioindicators in marine environmental research, and an important number of studies on their biology has been carried out. Convenient diets and optimum culture conditions improve such laboratory research. The optimization of the culture conditions in our department has produced an important increase in mussels survival and in the number of metaphases obtained for cytogenetic studies. In this way, differences in the physiological state of the mussels were reflected by the mitotic indices. Freshly collected mussels show variable, but rather low, mitotic indices. As the feeding time in the laboratory became longer, mitotic indices increase and deviations from the mean shorten (Martínez-Espósito et al. in press). Microalgal species used may also have an important influence on the results obtained from physiological experiments.

The algal diet must supply the nutrients required by juvenile bivalves, providing energy for metabolic demands and growth. The food value of a microalgal species depends on cell size, digestibility, toxicity and biochemical composition (Webb and Chu 1982). Mixed diets, with more than one algal species, have generally promoted better growth than monoalgal diets (Epifanio et al. 1976, Epifanio 1979, Strömgren and Cary 1984).

One useful way to evaluate differences in food value is estimate the relative growth efficiency of the spat fed with different diets. The importance of providing bivalve spat with a suitable algal diet is very clear: they will grow faster and more efficiently, reach higher quality and perform better when transferred to the natural environment (Laing and Millican 1986).

In the present work, the food value of different algal diets to juvenile *Mytilus galloprovincialis* Lmk, measured in terms of growth rate, biochemical composition of mussels and efficiency of food conversion, has been analysed.

MATERIALS AND METHODS

Microalgal Cultures

Three marine microalgal species were used as potential diets: *Dunaliella tertiolecta* Butcher (Chlorophyceae), *Tetraselmis suecica* (Kylin) Butch (Prasynophyceae) and *Phaeodactylum tricornutum* var. *bicornutum* Bohlin (Bacillariophyceae). *D. tertiolecta* was obtained from the Culture Center of Algae and Protozoa, Cambridge, U.K., and *T. suecica* and *P. tricornutum* were obtained from Dr. Fábregas, University of Santiago. They were cultured in seawater filtered through a 0.45 μm filter, autoclaved at 120°C for 45 min, and enriched with commercial nutrients for marine microalgae (Algal-1; Nutrición Avanzada S. A., Santiago de Compostela, Spain) (Herrero et al. 1991). Salinity of seawater was 35‰. Unialgal cultures were carried out in 6 l PYREX vessels, and maintained in exponential growth. All cultures were incubated in a controlled environmental chamber at 18 ± 1°C and illuminated with 3 fluorescent lamps placed under the flasks and 3 above them (about 110 $\mu E\ m^{-2}\ s^{-1}$) on a 12:12 light:dark cycle.

Cellular density of the algal cultures was determined daily by cell counting with a Neubauer haemocytometer.

Culture of Mussels and Algal Diets

Juvenile mussels were collected from Ría de Arousa, Galicia, NW of Spain. On arriving at the laboratory, mussels between 6 and 13 mm were selected and randomly distributed in groups of 20 individuals. A group of mussels was used to measure length and volume; then the flesh was separated, pooled and stored at −20°C until biochemical analysis. This group represents the "wild mussels". The remaining groups were fed with *T. suecica* during an acclimation period of 7 days; after that mussels were pooled, and redistributed randomly in 20-spat groups. They were not fed during the 24 h before the feeding experiments. From these mussels, a sample was withdrawn for the initial biochemical composition.

Mussels were cultured in 1-liter polyethylene cylindric tanks filled with 0.45 μm-filtered ultraviolet light-treated seawater, in a culture room at 18 ± 1°C and 12:12 h light:dark. Tanks were aerated to maintain an adequate O_2 tension and to avoid food sedimentation. The pH of the seawater was 7.9. The tanks were emptied, cleaned and refilled with freshly filtered ultraviolet light-treated seawater twice each week. Each day, the seawater was filtered (5 μm) to remove faeces and pseudofaeces, pH was recorded and dead mussels were removed and measured.

Experiments were carried out for 60 days. The following algal diets, both mono-species and mixed-species, were assessed: *T. suecica* (Ts diet); *D. tertiolecta* (Dt); *P. tricornutum* (Pt); *T. suecica* + *D. tertiolecta* (TsDt); *T. suecica* + *P. tricornutum* (TsPt); and *D. tertiolecta* + *P. tricornutum* (DtPt). A control no fed was also established.

During the acclimation period, the food supplied was gradually increased and established in a dietary level of 1.20 mg algal DW per mussel per day. This food ration was supplied in several doses throughout the light period. The ration per doses was established as the ration at which pseudofaeces production was minimum. Before adding the algal cell suspensions, a volume of seawater equivalent to the volume of algal suspension was removed from experimental tanks to maintain a constant volume.

Equivalent cell densities were established, based on the dry weight of the algal cells of the different species used. For the mixed diets, rations were adjusted so that each microalga contributed an equal dry weight to the ration.

The food ration was increased up to 1.81 mg dry algae matter per spat per day throughout the experimental period.

Initial conditions for the different experimental diets are presented in Table 1.

Growth and Biochemical Analysis

The shell-length and volume were monitored at periodic intervals. Shell-length was measured by a calliper (±0.1 mm). Whole mussel volume was determined from weight of the displaced distilled-water volume, by a BOSCH balance (±0.1 mg).

At the end of the trials, flesh from the mussels fed on each diet was pooled and freeze-dried. Biochemical contents of the algae and mussels were measured using the phenol-sulphuric technique for carbohydrate (Dubois et al. 1956), and a charring method for lipid (Marsh and Weinstein 1966). Protein content of mussels was determined in a Tekator autoanalyser by the micro-KJELDAHL method (A.O.A.C. 1965). Protein of microalgae was measured by the dye-binding method (Bradford 1976), and RNA was determined as described by Kochert (1978). The ash weight was determined by ashing at 540°C (A.O.A.C., 1965), and the ash free dry weight (AFDW) was calculated by subtraction.

TABLE 1.

Initial length and volume of juvenile mussels (*M. galloprovincialis*) for experiments with different algal diets.

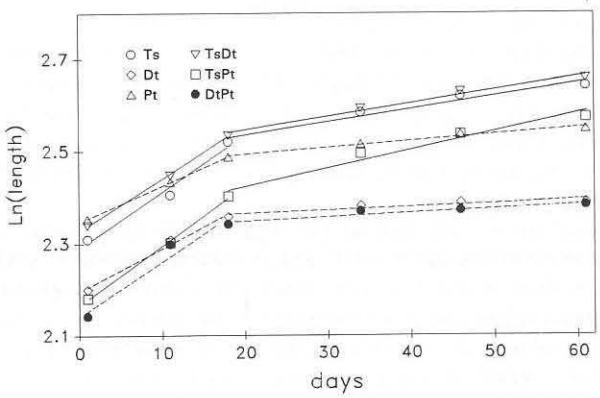
	Length (mm)		Volume (mm^3)	
Diet	**Mean**	**95% C.I.**	**Mean**	**95% C.I.**
Ts	10.07	9.78–10.38	93.06	84.55–102.42
Dt	9.03	8.12–10.03	70.00	50.00–97.00
Pt	10.52	9.83–11.27	116.32	96.23–140.62
TsDt	10.38	10.00–10.70	100.01	85.66–116.77
TsPt	8.86	8.16–9.61	70.96	57.30–87.87
DtPt	8.52	8.06–8.99	62.03	51.44–75.66

Diets: *T. suecica* (Ts), *D. tertiolecta* (Dt), *P. tricornutum* (Pt) and mixed diets (1:1 dry weight).

RESULTS

Growth in Shell-Length

Figure 1 represents the time course of shell-length (as the mean of natural logarithms of length) for each diet. Growth tended to fit curves with inflexion points on day 18 of culture. Because of this, the experimental time was divided in two periods: the first one, until day 18, and the second one after day 18. Straight lines were drawn using linear regression in each one of these periods; the regression coefficients were compared using the analysis of covariance described by Snedecor and Cochran (1989). No differences were observed among the slopes during the first experimental period ($P > 0.05$). Differences appeared in the second period.



	Days 1 - 18			Days 18 - 61		
	b_0	b_1 (x10^{-3})	r	b_0	b_1 (x10^{-3})	r
Ts	2.29±0.03	11.41±2.07	0.988	2.48±0.02	2.81±0.44	0.978
Dt	2.20±0.01	8.86±0.81	0.993	2.35±0.01	0.69±0.26	0.891
Pt	2.35±0.00	7.37±0.16	0.999	2.46±0.01	1.43±0.16	0.983
TsDt	2.33±0.01	10.69±0.71	0.999	2.49±0.01	2.86±0.26	0.990
TsPt	2.17±0.01	12.14±0.89	0.999	2.34±0.02	3.96±0.50	0.978
DtPt	2.14±0.03	11.41±2.11	0.978	2.33±0.01	0.86±0.20	0.937

Figure 1. Growth in length of juvenile mussels fed on different algal diets: *T. suecica* (Ts), *D. tertiolecta* (Dt), *P. tricornutum* (Pt), and mixed diets. Equations, for two growth periods, were adjusted by linear regression: $Y = b_0 + b_1X$; where Y is the length as Ln(length), and X is the time in days. The coefficients (mean ± SE) are shown below the figure.

Results of ANCOVA for the data of the second experimental period are presented in Table 2. Since significant differences were observed, comparisons of slopes were carried out between *T. suecica* (Ts) diet and each one of the remaining diets (analysis not shown). No differences were observed among the regression coefficients obtained in the diets Ts, TsDt and TsPt ($P > 0.05$), coefficient of Ts diet being different from slopes in Dt, Pt, and DtPt diets ($0.05 > P > 0.01$). The slope for Dt diet was not different from 0 ($P > 0.05$).

From these results, the diets were divided in two groups. Higher growth rates were observed with diets containing *T. suecica* (Ts, TsDt, and TsPt); TsPt being the diet which supported the highest growth throughout the experimental period. Lower growth rates were observed with the remaining diets.

A relative length growth rate (*Gr*, Table 3) was calculated, with *T. suecica* (Ts) diet as control, as:

$$Gr = (G \text{ diet}/G \text{ control}) \times 100$$

where G is the length growth rate, calculated using the equation:

$$G = \text{Ln}(L_t) - \text{Ln}(L_o)/t$$

being L_t the length at time t, L_o the initial length at day 1, and t the time in days.

Relative length growth rate obtained in the TsPt diet increased with respect to *T. suecica* diet throughout the experimental period, while replacing 50% *T. suecica* with *D. tertiolecta* (TsDt diet) resulted in a relative growth rate similar to Ts diet. In Pt diet, relative length growth rate remained stable, with values about 60%, after 18 days of culture. In Dt and DtPt diets, relative length growth rates declined continuously throughout the experimental period, because growth was almost zero from day 35.

A synergistic effect was observed in the mixed diets regarding their respective monoalgal diets (Table 3). *Gr* values for mixed diets were higher than the average of *Gr* values of monoalgal diets.

Length of unfed mussels did not vary throughout the experimental time.

Growth in Volume

Figure 2 represents the time course of volume (as the mean of natural logarithms of volume) for each diet. The coefficients obtained by linear regression are presented below the graph. The slope for mussels fed on *D. tertiolecta* was not different from 0 ($P > 0.05$). Data were treated as data of length, and results of the comparison of slopes from day 18 are given in Table 4. Two groups of diets were again observed: growths observed for mussels fed on diets containing *T. suecica* were no different among them ($P > 0.05$), but faster than in the remaining diets: Dt and DtPt ($P < 0.01$) and Pt ($0.05 > P > 0.01$).

Volume growth rates (*Gv*) and relative volume growth rates (*Gvr*) were calculated as for length, but replacing L_t and L_o for V_t and V_o, expressed in mm^3. Relative volume growth rates are represented in Table 5. In Pt diet relative volume growth rate was stable (50–60%) throughout the experimental time, while relative volume growth rates of Dt and DtPt diets declined up to 40% lower values at the end of trials. After day 18, *P. tricornutum* supported faster growth than DtPt and Dt diets (Fig. 2), although not significantly different ($P > 0.05$).

Volume of unfed group did not change throughout the experimental period.

Length and volume growth rates for the whole experimental period ($t = 60$; Table 6) were closely correlated ($r = 0.95$, $P < 0.01$) (Table 7).

Growth in Weight

Growth rates in dry body weight (G_{DW}) and organic weight (G_{OW}) (Table 6) were calculated as $\text{Ln}(W_t/W_o)/t$, where W_t and

TABLE 3.

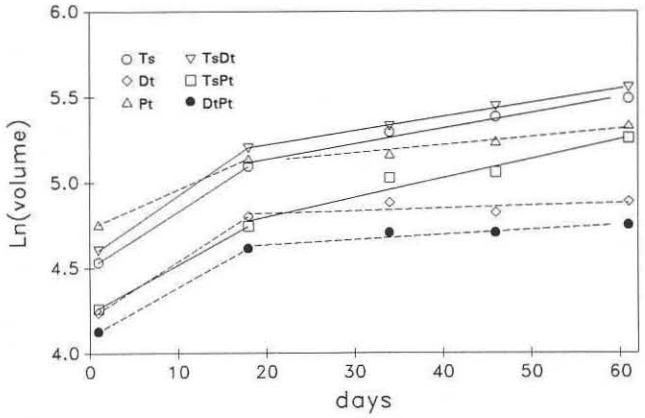
Relative length growth rate (*Gr*) at days 11, 18, 34, 46, and 60, of mussels fed on different algal diets.

Diet	Gr_{11}	Gr_{18}	Gr_{34}	Gr_{46}	Gr_{60}
Unfed	29	16	15	12	8
Ts	100	100	100	100	100
Dt	97	69	61	56	52
Pt	82	64	59	59	59
TsDt	110	94	95	94	97
TsPt	126	107	114	115	120
DtPt	163	97	83	75	72

TABLE 2.

Comparison of regression lines obtained for the growth in shell-length of juvenile mussels fed on different algal diets. *x:* time (days); *y:* mean Ln(length).

					Regression Coefficient	Deviations from Regression		
	d.f.	Σx^2	Σxy	Σy^2		d.f.	SS	MS
Within								
Ts	3	996.75	2.792	0.00820	0.00280	2	0.00038	0.00019
Dt	3	996.75	0.688	0.00061	0.00069	2	0.00014	0.00007
Pt	3	1010.00	1.443	0.00211	0.00143	2	0.00005	0.00002
TsDt	3	1010.00	2.885	0.00838	0.00286	2	0.00014	0.00007
TsPt	3	998.75	3.954	0.01616	0.00396	2	0.00051	0.00025
DtPt	3	998.75	0.862	0.00082	0.00086	2	0.00008	0.00004
						12	0.00128	0.00011
Pooled, *W*	18	6011.00	12.624	0.03628	0.00210	17	0.00977	0.00057
		Difference between slopes:				5	0.00848	0.00170
Comparison of residual variances: (Test de Barlett)				F = 2.00		$P > 0.05$		
Comparison of slopes:				F = 15.85(5,12)		$P < 0.01$		



	Days 1 - 18		Days 18 - 61		
	b_0	b_1 ($\times 10^{-3}$)	b_0	b_1 ($\times 10^{-3}$)	r
Ts	4.500	33.01	4.94±0.04	9.11±0.99	0.988
Dt	4.207	33.17	4.79±0.05	1.48±1.24	0.644
Pt	4.734	22.52	5.03±0.04	4.62±0.90	0.967
TsDt	4.570	35.22	5.05±0.07	8.21±0.15	0.999
TsPt	4.234	28.42	4.56±0.08	11.32±1.17	0.974
DtPt	4.099	28.67	4.57±0.03	2.98±0.71	0.942

Figure 2. Growth in volume of juvenile mussels fed on different algal diets: *T. suecica* (Ts), *D. tertiolecta* (Dt), *P. tricornutum* (Pt), and mixed diets. Linear regressions were calculated for two growth periods, and the coefficients are shown below the Figure ($Y = b_0 + b_1X$; where Y is the Ln(volume) and X is the time in days).

W_o are final and initial dry weight (DW), or ash free dry weight (AFDW). Higher rates in weights were obtained in diets that promoved faster growth in length and volume: *T. suecica* + *P. tricornutum*, *T. suecica* and *T. suecica* + *D. tertiolecta* ($20.63 - 24.78 \times 10^{-3}$ d^{-1}).

G_{DW} and G_{OW} were closely correlated with length growth rate, G_{60} (r = 0.99 and 0.97, respectively; $P < 0.001$), and with volume growth rate, Gv_{60} (r = 0.93 and 0.89, respectively, $P < 0.001$) (Table 7).

Condition Index, Gross Growth Efficiency and Mortality

The selected condition index (Ci) relates dry flesh weight (mg) with total volume (mm^3):

$$\text{Ci} = \text{dry flesh weight/volume}$$

The traditionally used Ci in studies with bivalves relates flesh DW with internal volume. However, juvenile mussels present thin shells, and the calculated Ci was considered useful.

Ci values were between 90 and 100 in mussels fed with diets containing *T. suecica* (Table 6). In Pt and DtPt diets, Ci declined to similar values of "wild" mussels (69.13), and Ci was lower than "wild" mussels for those fed on *D. tertiolecta* (51.07). Ci fell to 25.80 in the unfed mussels. Condition index for freshly collecty mussels of equivalent volume to mussels fed on *Tetraselmis* diets was 59.88.

Gross Growth Efficiency (K_1) is the proportion of the organic weight of the algal cells cleared from suspension that is incorporated into organic growth of animals (Laing and Millican 1986).

$$K_1 = I/C$$

where I is the increase in total organic weight of the mussel in a certain time (in these trials, 60 days), and C is the organic matter of the algal cells cleared per mussel during the same period. Since cell deposition on culture vessels was minimal, an assumption was made that all supplied algal biomass was cleared by mussels. The organic matter contents of the microalgae used were 63.46 mg 10^6 cells^{-1} for *T. suecica*, 61.32 mg 10^6 cells^{-1} for *D. tertiolecta*, and 20.66 mg 10^6 cells^{-1} for *P. tricornutum*.

Higher K_1 values (Table 6) were observed in faster growth diets, those with *T. suecica* in their formulation: Ts, TsDt and TsPt. K_1 was correlated with G_{60} (r = 0.90, $P < 0.01$), Gv_{60} (r = 0.93, $P < 0.01$), G_{DW} (r = 0.94, $P < 0.01$), G_{OW} (r = 0.91, $P < 0.01$) and condition index (r = 0.98, $P < 0.001$) (Table 7).

Instantaneous mortality rate (Ricker 1973) was calculated using the equation:

$$Z = \text{Ln}(N_o - N_t)$$

where N_t is the number of living mussels at time t, and N_o is the initial number of mussels. The Z_{60} values (t = 60 days) are represented in Table 6. Unfed mussels maintained a high survival until day 40. From this moment, effect of starvation resulted in a Z_{60} of 0.43. Mortality rate was not correlated with any other parameter (Table 7), but Dt diets all had higher mortality rates.

Biochemical Profile and Food Conversion

Biochemical composition, as mg per mussel, and ratios between components, is shown in Table 8. The highest absolute contents of all components were obtained in mussels that grew faster (diets containing *T. suecica*).

Expressing data of Table 8 as % of dry weight, protein was the component more abundant, between 54.60–69.02% DW. Carbohydrates accounted for 9.04–25.14% DW, and diets containing *D. tertiolecta* showed the highest values (18.05–25.14%). Lipids were between 3.27 and 4.99% DW for cultured mussels, and 9.02 for "wild" mussels.

The lipid:protein ratio (Table 8) reached a maximal value in "wild" mussels (0.15); all the cultured mussels showed values between 0.059–0.083, being maximum in diets containing *T. suecica*. Carbohydrate:protein ratios were minimum in mussels fed on the diet of *Phaeodactylum*, and maximum in mussels fed on the three *Dunaliella* diets and in "wild" mussels. Lipid:carbohydrate ratio was minimum in *Dunaliella* diets (0.15–0.24) and increased up to values >0.30 in the remaining diets and in "wild" mussels.

Taking into account the gross biochemical composition of microalgal cells (Table 9), efficiencies of conversion of protein, carbohydrates and lipids from the diets into protein, carbohydrates and lipids deposited as body constituents were calculated. The components deposited as body constituents were obtained subtracting the initial values from final values. In the diets of higher food value (*T. suecica* diets) values between 44 and 47% of protein offered to mussels was deposited as body protein, compared with 33–64% of carbohydrates and 4.2–4.5% of lipids (Table 10).

Relationships Among Parameters

Correlation analysis among growth, physiological, and biochemical parameters was carried out using the statistical software SPSS/PC+ (*v.* 4.01). Parameters considered were growth rates in length (G_{60}), volume (Gv_{60}), dry body weight (G_{DW}) and ash free body weight or organic weight (G_{OW}); gross growth efficiency (K_1); condition index (Ci); mortality rate (Z_{60}); depositions of protein (D_P), carbohydrates (D_C) and lipids (D_L) (mg per mussel);

TABLE 4.

Comparison of regression lines obtained for the growth in volume of juvenile mussels fed on different algal diets. *x*: time (days); *y*: mean Ln(Volume).

	d.f.	Σx^2	Σxy	Σy^2	Regression Coefficient	Deviations from Regression d.f.	SS	MS
Within								
Ts	3	996.75	9.077	0.08463	0.00911	2	0.00197	0.00098
Dt	3	996.75	1.478	0.00528	0.00148	2	0.00309	0.00154
Pt	3	1010.00	4.667	0.02319	0.00462	2	0.00162	0.00081
TsDt	3	1010.00	8.293	0.06813	0.00821	2	0.00004	0.00002
TsPt	3	998.75	11.318	0.13452	0.01133	2	0.00626	0.00313
DtPt	3	998.75	2.973	0.00984	0.00298	2	0.00099	0.00050
						12	0.01397	0.00116
Pooled, *W*	18	6011.00	37.806	0.32559	0.00629	17	0.08781	0.00517
		Difference among slopes:				5	0.07384	0.01477
Comparison of residual variances: (Test de Barlett)				F = 1.77		$P > 0.05$		
Comparison of slopes:				F = 12.68(5,12)		$P < 0.01$		

final composition as percentages of DW, and ratios among chemical components. Matrix of correlation coefficients is presented in Table 7. Analysis were carried out without data of unfed mussels.

Depositions of protein and lipids were significantly correlated between them and with all growth rates, gross growth efficiency and condition index; carbohydrate depositions were correlated with none of these parameters.

Significative and positive relationships were also detected among lipids (as percentage of DW) with all growth rates, K_1, depositions of protein and lipids per mussel, and lipid:protein ratio. Ash contents (as % DW) were negative correlated with growth rates, K_1, and depositions of protein and lipids. Protein and carbohydrate levels were correlated with none of the parameters.

Lipid:protein ratio was correlated with volume growth rate, and lipids as %DW.

DISCUSSION

During the acclimation period, food ratio was gradually increased from low levels to a dietary level of 1.20 mg algal DW per mussel per day, 21% of dry body weight per day, or 10–15% of organic matter weight depending on the diet. Food rations of 10–20% of dry weight per day have been reported for 0.2 g mussels (Bayne et al. 1976). Food ration was increased throughout the experimental period up to 1.81 mg algal DW per mussel per day in all the diets. However, feeding rates were different throughout the experimental period because of the different increases in body weight for each diet. Thus final food ration varied between 22–27% of dry body weight (*Dunaliella* and *Dunaliella* + *Phaeodactylum* diets) and 7.5–13% (remaining diets).

The unfed control group was included to show that growth was not produced by any factor other than microalgal diets. Data obtained show that growths in length or volume did not occur in the unfed mussels. Therefore, growths observed in the other groups of mussels were indeed due to the microalgal diets used.

Length and volume growth rates were not constant throughout the experimental period. There were two periods clearly different in the growths in length and volume (Figs. 1, 2). In all diets, growth rates were higher during the first 18 days. Nielsen (1988) had observed this same behaviour in juvenile *M. edulis*, as the acute response (initial increase) and acclimation to increasing temperature. Environmental temperature in Arousa waters is 12–13°C in April, and the experiments were carried out at 18°C. Therefore this increase may provoke a response in the growth rate. However, mussels were acclimated to laboratory conditions during 8 days before beginning the experiments. In these experiments a new steady-state in growth rate was reached after day 18 (Figs. 1 and 2). A strong decrease in growth rate was also reported by Nielsen (1988) at 18°C. Other physiological parameters, as filtration rate, respiration rate or scope for activity show acclimation to temper-

TABLE 5.

Relative volume growth rate (*Gvr*) at days 18, 34, 46, and 60 of mussels fed on different algal diets.

Diet	Gvr_{18}	Gvr_{34}	Gvr_{46}	Gvr_{60}
Unfed	1	0	0	0
Ts	100	100	100	100
Dt	100	80	65	65
Pt	68	53	56	60
TsDt	107	95	99	99
TsPt	86	101	94	105
DtPt	87	76	69	64

TABLE 6.

Length (G_{60}, $\times 10^{-3}$), volume (Gv_{60}, $\times 10^{-3}$), dry flesh weight (G_{DW}, $\times 10^{-3}$) and organic weight (G_{OW} $\times 10^{-3}$) growth rates; mortality rate (Z_{60}), condition index (Ci), and gross growth efficiency (K_1) for mussels fed on different algal diets.

	G_{60}	Gv_{60}	G_{DW}	G_{OW}	Z_{60}	Ci	K_1
Ts	5.41	15.70	21.12	20.94	0.05	93.91	32.11
Dt	3.07	10.63	7.48	5.19	0.11	51.07	2.81
Pt	3.17	9.50	10.51	8.85	0.05	67.96	10.73
TsDt	5.19	15.56	20.71	20.63	0.11	95.93	31.59
TsPt	6.41	16.35	24.78	24.75	0.00	93.02	27.85
DtPt	3.93	10.23	13.78	14.85	0.36	69.42	9.47
Wild	–	–	–	–	–	69.13	–

TABLE 7.

Matrix of correlations among growth and biochemical parameters for mussels fed on different algal diets.

	G_{60}	Gv_{60}	G_{DW}	G_{OW}	Z_{60}	K_1	Ci	D_P	D_C	D_L	C/P	L/P	L/C
G_{60}	1.00												
Gv_{60}	.945*	1.00											
G_{DW}	.987**	.930**	1.00										
G_{OW}	.972**	.892**	.992**	1.00									
Z_{60}	−.340	−.431	−.293	−.178	1.00								
K_1	.899*	.932*	.937*	.910*	−.410	1.00							
Ci	.903*	.893*	.956*	.947*	−.277	.980**	1.00						
D_P	.895*	.935*	.932*	.899*	−.444	.998**	.977**	1.00					
D_C	.643	.744	.698	.711	.126	.747	.786	.745	1.00				
D_L	.946*	.978**	.961*	.940*	−.306	.964**	.959*	.964**	.824	1.00			
C/P	−.225	−.197	−.237	−.116	.848	−.345	−.309	−.369	.289	−.151	1.00		
L/P	.804	.917*	.815	.782	−.262	.850	−.216	.865	.905*	.932*	.135	1.00	
C/L	.350	.299	.375	.303	−.812	.457	−.020	.483	−.154	.279	−.544	.747	1.00
LIP (%)	.946*	.933*	.976**	.953*	.376	.972**	−.118	.977**	.744	.972**	.046	.990**	.801*
ASH (%)	−.945*	−.945*	−.982**	−.987**	−.164	−.986*	−.613	.991**	−.789	−.847	−.053	−.575	−.502

Minimum pairwise N of cases: 6; 1-tailed signif.: * .01 **.001.

ature between 10 and 20°C (reviewed by Bayne et al. 1976) and a new steady-state is established in two weeks after perturbance. However, growth is a more complex physiological process.

The effects of diets on growth were more evident during the second period. Two types of diets can be established in relation to their food value (Figs. 1, 2): a) diets that include *T. suecica* in their formulation, and b) *D. tertiolecta* diet, *P. tricornutum* diet and the mixed diet of them. Different food values were also observed in growths in weight, gross growth efficiencies, and condition indexes (Table 6), all these parameters being significantly correlated among them (Table 7). Mussels fed on *T. suecica* diets utilized more efficiently the ingested ration for growth (K_1 about 30%), and the condition indexes were almost 100, against 59.88 for freshly collected mussels of equivalent volume.

Shell-length growths of mussels fed with *T. suecica* diets were 0.10–0.14 mm day^{-1} during the first 20 days, decreasing to 0.04–0.05 mm d^{-1} from this day. These values are lower than other reported for mussels in nature: 0.10–0.34 mm d^{-1} for 26 mm mussels (Aguirre 1979), 0.24 mm d^{-1} for 18.2 mm mussels (Pérez and Roman, 1979), for mussels cultured on rafts in Arousa waters; 7–11 mm mo^{-1} from other studies in nature (reviewed by Jorgensen 1990). However, growths obtained in our experiments were similar to others obtained in laboratory (Jorgensen 1990). According to Jorgensen (1990) mussels growing at 0.34 mm d^{-1} exploit the whole potential for growth; thus, in our experiments mussels exploited ½ of the potential for growth during the first experimental period, but only ⅕ after day 18 of culture.

A synergistic effect was observed in mixed diets, mainly in those including *Tetraselmis*. Based on a linear relationship between cell concentration of suspension and growth rate (Strömgren and Cary 1984), if only additive effects among diets were true, relative growth rates of mixed diets would be similar to means of relative rates of monoalgal diets. Relative growth rates were higher than means of relative rates of monoalgal diets (Tables 3, 5). *Tetraselmis* + *Phaeodactylum* diet provided the highest growth rates, while *T. suecica* and *Tetraselmis* + *Dunaliella* supported similar growths. *P. tricornutum* had a food value higher than *Dunaliella,* and kept a constant relative growth rate in relation to *T. suecica* throughout the experimental period. Diets of *Dunaliella* and *Dunaliella* + *Phaeodactylum* supported a relative good growth only during the first experimental period, their relative growth rates declining throughout the experimental period.

Certain factors associated with algal cells have been suggested for explaining why some algal species are better than others as food source of molluscs, for example, cellular size, wall composition, digestibility, toxic metabolites and gross biochemical composition (Webb and Chu 1982). None of these characteristics alone has offered an entirely satisfactory explanation. Under the present conditions of culture, gross biochemical composition of the three used algae was not very different (Table 9), except for a higher

TABLE 8.

Biochemical composition, as mg per mussel, and relationships between components of mussels fed on different algal diets and wild mussels.

	DW	PRO	CHO	LIP	Ash	Cho/Pro	Lip/Pro	Lip/Cho
Ts	22.77	14.63	3.24	1.07	3.83	0.22	0.073	0.33
Dt	6.70	3.70	1.22	0.22	1.82	0.35	0.063	0.18
Pt	14.11	8.57	1.28	0.53	3.38	0.14	0.059	0.41
TsDt	24.78	14.53	4.99	1.21	4.06	0.34	0.083	0.24
TsPt	17.90	12.35	2.31	0.89	2.88	0.20	0.075	0.39
DtPt	8.04	4.88	2.02	0.30	1.83	0.41	0.061	0.15
Wild	3.50	1.85	0.41	0.29	0.51	0.30	0.154	0.54

DW, dry meat weight; PRO, protein; CHO, carbohydrates, and LIP, lipids.

TABLE 9.

Gross biochemical composition (% of dry weight) of *T. suecica*, *D. tertiolecta* and *P. tricornutum* in exponential growth phase.

	Protein	Carbohydrates	Lipids	RNA	Ash
T. suecica	21.20	4.69	9.95	5.80	26.49
D. tertiolecta	24.74	8.34	15.44	6.13	15.64
P. tricornutum	20.34	5.66	12.83	4.59	40.70

carbohydrate content for *D. tertiolecta* and a higher ash content for *P. tricornutum*.

The low nutritive value of *D. tertiolecta* for juvenile bivalves has already been reported (Walne 1970, Wikfors et al. 1984, Enright et al. 1986a). *T. suecica* and *D. tertiolecta* are of similar shape and size; the main difference is the presence of a medium rigid cell wall in *T. suecica* and the absence of a cell wall in *D. tertiolecta*, which presents a glycocalix-type envelope (Oliveira et al. 1980). The *Dunaliella* cell is more flexible than other microalgal cells; flexibility probably causing a higher resistance to breakage by the crystalline style. Moreover, it has been reported that the filtrates of *D. tertiolecta* cultures contain ectocrines that inhibit the filtration activity of *M. edulis* (inhibition dependent of concentration), but no *T. suecica* (Ward and Targett 1989).

Composition and balance of fatty acids of the diet has frequently been related with food value of microalgae (Langdon and Waldock 1981, Webb and Chu 1982, Enright et al. 1986b), mainly regarding their contents of polyunsaturated fatty acids. The low nutritive value of *D. tertiolecta* has been related with the absence of C20 and C22 polyunsaturated fatty acids, while *T. suecica* contains 20:5*w*3 (Langdon and Waldock 1981; Volkman et al. 1989) and *P. tricornutum* 20:5*w*3 and 22:6*w*3 (Siron et al. 1989).

However, analytical data obtained in our laboratory for these microalgae in the present culture conditions showed the presence of C20 and C22 polyunsaturated fatty acids in *D. tertiolecta* (Herrero et al. 1992). Thompson et al. (1990) studied the influence of irradiance on the fatty acid composition of different microalgae and they also found C20 and C22 polyunsaturated fatty acids in *D. tertiolecta* in similar conditions of irradiance that those used in our experiments.

Nevertheless, it must be taken into account the marked differences in fatty acid content and composition found for some species grown in different laboratories, that raises the question whether consistent results can be obtained for the same species cultured under similar conditions (Volkman et al. 1989).

Molluscs require a dietary source of arginine, histidine, methionine, cysteine, leucine, isoleucine, valine, lysine, tryptophan, threonine and proline (Boudreau 1985). All these aminoacids are supplied for the microalgae used in these experiments (Fábregas and Herrero 1985, Herrero et al. 1985). The amount of aminoacids are very similar in all of them, although *P. tricornutum* and *T. suecica* have significantly higher amounts of methionine (2.28 and 1.45 gr per 16 gr of N, respectively) and threonine (5.19 and 5.27 gr per 16 gr of N, respectively) than *D. tertiolecta* (0.80 gr per 16 gr of N of methionine and 2.58 gr per 16 gr of N of threonine). Vitamin content (Fábregas and Herrero 1990) and mineral composition (Fábregas and Herrero 1986) are very similar in *T. suecica* and *D. tertiolecta*, except for a higher content of tocopherol, Zn and Cu in *T. suecica*, and for β-carotene in *D. tertiolecta*.

The mixed diet of *T. suecica* and *P. tricornutum* is probably the best balanced diet regarding their biochemical composition, promoting the fastest growth of all tested diets.

T. suecica has the biggest cell size among the microalgae used in this work with the highest biomass per cell. For instance, four *P. tricornutum* cells yield the same organic matter as one *T. suecica* cell. These different organic matter:volume ratios act on the energetic cost of feeding. This fact may be particularly important when the food value of ingested organic matter is low. Laing and Millican (1986) reported that in the lowest food value diets for *O. edulis*, clearance rates and size-specific metabolic rates were higher, associated with lower organic growth of the animals. They suggested that it may represent an attempt by the spat to obtain the required amounts of essential nutrients from the low food value diets by increasing the filtration rate, leading to a greater metabolic demands on the assimilated ration, with less energy available for organic growth.

The biochemical composition of the mussels was also modified by the diet (Table 8). *D. tertiolecta* has the highest carbohydrate content of the three algae used (Table 9), and the inclusion of *D. tertiolecta* in the diet produced increasing carbohydrate levels.

From biochemical data, an alternate hypothesis explaining growth dynamics shown in Figs. 1 and 2 emerges. "Wild" mussels at the beginning of the experiment contain considerably more lipid (9%DW) than experimentally-fed mussels (3–5%DW) at the end of the experiment. This decrease in lipids as %DW was lower in diets supporting faster growth. Moreover, in diets of *Dunaliella* and *Dunaliella* + *Phaeodactylum* lipid contents as mg per mussels also decreased. The period of rapid initial growth may be supported by some stored lipid component, exhausted from day 18 in diets of *Dunaliella* and *Dunaliella* + *Phaeodactylum*, and that diets containing *T. suecica* must include in higher amounts.

Growth rates and gross growth efficiencies were significantly correlated with body lipids as percentages of dry flesh weight

TABLE 10.

Efficiencies of conversion of protein, carbohydrates and lipids offered in the diets by mussels fed on diets of *T. suecica*. Data correspond to the whole experimental time (60 days).

	Protein			Carbohydrates			Lipids		
Diet	Offer.	D_P	%	Offer.	D_C	%	Offer.	D_L	%
Ts	24.9	10.9	43.8	5.5	2.4	44	11.7	0.49	4.2
TsDt	23.1	10.4	44.9	6.4	4.1	61	12.5	0.56	4.5
TsPt	21.3	10.0	46.9	5.5	1.8	33	12.1	0.53	4.4

Offer., mg offered in diets; D_P, D_C, D_L, depositions of protein, carbohydrates, and lipids (mg per mussel), respectively.

(Table 7). Lipid:protein ratio was also higher in diets promoting faster growth. Therefore, mussels fed with diets that supported faster growth reached higher lipidic contents. This was also found by Laing and Millican (1986) with cultured *O. edulis* spat, and spat with higher lipid levels grew faster and with higher survival when planted out in the sea.

In the diets of higher food value, between 44 and 47% of the protein offered to mussels was deposited as body protein (Table 10), at a deposition rate of 0.17–0.18 mg d^{-1}, against a 33–64% of carbohydrates (0.03–0.07 mg d^{-1}) and 4.2–4.5% of lipids (0.009 mg d^{-1}).

Starved mussels (unfed group) had a high survival rate ($Z = 0$) during the first 20 days. Mortality increased to Z values of 0.43 at the end of the experiments (Table 6). During the starvation, the loss of dry flesh was 0.32 mg per day, or 64% of initial dry flesh. Riisgard and Randlöw (1981) and Strömgen and Cary (1984) found that *M. edulis* may have some shell growth when not fed or fed below maintenance levels. In our experiments losses per day were 0.17 mg d^{-1} of protein, 0.09 mg d^{-1} of carbohydrates and 0.03 mg d^{-1} of lipids. A preferred utilization of proteins as metabolic substrate has been observed for sexually mature mussels during winter (reviewed by Gabbot 1976), and an increasing catabolism of endogenous protein to support metabolic requirements at lower energy intakes for 10 mg dry weight mussels (Hawkins and Bayne 1991).

ACKNOWLEDGMENTS

This work was supported by a grant of Plan Nacional I + D, CICYT, Spain, reference AGF92-0736. A. Cid, J. P. Fidalgo, and I. López-Muñoz hold Fellowships from the Consellería de Educación, Xunta de Galicia.

REFERENCES CITED

Aguirre, M. P. 1979. Biología el mejillón (*Mytilus edulis*) de cultivo de la Ría de Vigo. Ph.D. thesis. Universidad Complutense, Madrid.

A.O.A.C. 1965. Official methods of analysis of the Association of Official Agricultural Chemist. USA.

Bayne, B. L., R. J. Thompson & J. Widdows. 1976. Physiology: I. *In* Marine mussels. Their Ecology and Physiology. ed. B. L. Bayne. Cambridge University Press, Cambridge, pp. 121–206.

Boudreau, K. N. 1985. Aquaculture nutrition: protein. *In* Biological Aspects of Aquaculture Nutrition. eds. J. D. Castell, et al. World Conference on Aquaculture and International Aquacultural Trade Show.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein, utilizing the principle of dye-binding. *Anal. Biochem.* 72:248–254.

Dardignac-Corbeil, M. J. 1989. Traditional mussel culture. *In* Aquaculture. ed. G. Barnabé. Vol. 1. Ellis Horwood, Chichester. pp. 285–341.

Dubois, M., K. A. Gille, J. K. Hamilton, P. A. Rebers & F. Smith. 1956. Colorometric method for determination of sugars and related substances. *Anal. Chem.* 28:350–356.

Enright, C. T., G. F. Newkirk, J. S. Craigie & J. D. Castell. 1986a. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. *J. Exp. Mar. Biol. Ecol.* 96:1–13.

Enright, C. T., G. F. Newkirk, J. S. Craigie & J. D. Castell. 1986b. Growth of juvenile *Ostrea edulus* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition. *J. Exp. Mar. Biol. Ecol.* 96:15–26.

Epifanio, C. E., C. M. Logan & C. Turk. 1976. The culture of six species of bivalves in a recirculating seawater system. *In* 10th Eur. Symp. on Marine Biology, Vol. 1. eds. Persoone, G. & E. Jaspers. Universa Press, Wetterem, Belgium. pp. 97–108.

Epifanio, C. E. 1979. Growth in bivalve molluscs: nutritional effect of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria mercenaria* L. *Aquaculture* 18:1–12.

Fábregas, J. & C. Herrero. 1985. Marine and microalgae as a potential source of single cell protein (SCP). *Appl. Microbiol. Biotechnol.* 23: 110–113.

Fábregas, J. & C. Herrero. 1986. Marine microalgae as a potential source of minerals in fish diets. *Aquaculture* 51:237–243.

Fábregas, J. & C. Herrero. 1990. Vitamin content of four marine microalgae. Potential use as source of vitamins in nutrition. *J. Appl. Microbiol.* 5:259–264.

Gabbot, P. A. 1976. Energy metabolism. *In* Marine Mussels. Their Ecology and Physiology. ed. B. L. Bayne. Cambridge University Press, Cambridge, pp. 293–355.

Hawkins, A. J. S. & B. L. Bayne. 1991. Nutrition of marine mussels: factors influencing the relative utilizations of protein and energy. *Aquaculture* 94:177–196.

Herrero, C., J. Abalde, B. Cabezas, J. Fábregas & B. Regueiro. 1985. Isolation of the diatom *Phaedoctylum tricornutum* var. *bicornutum* from Galician waters. Amino acid pattern and biological value. *Bol. Acad. Gal. Ciencias* 4:35–39.

Herrero, C., A. Cid, J. Fábregas & J. Abalde. 1991. Yields in biomass and chemical constituents of four commercially important marine microalgae with different culture media. *Aquacul. Engin.* 10:99–110.

Herrero, C., E. Vecino & J. Abalde. 1992. The marine microalga *Dunaliella tertiolecta* (Butcher): nutritional properties and hypocholesterolemic effects. *In* Profiles in Biotechnology. eds. T. G. Villa & J. Abalde. Santiago University Press, pp. 271–288.

Jorgensen, C. B. 1990. Bivalve Filter feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology. eds. Olsen and Olsen. Fredensborg, Denmark. 140 pp.

Kochert, G. 1978. Quantitation of the macromolecular components of microalgae. *In* Handbook of Phycological Methods. Physiological and Biochemical Methods. eds. J. A. Hellebust & J. S. Craigie. Cambridge University Press, London. pp. 189–195.

Laing, I. & P. F. Millican. 1986. Relative growth and growth efficiency of *Ostrea edulis* L. spat fed various algal diets. *Aquaculture* 54:245–262.

Langdon, C. J. & M. J. Waldock. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. *J. Mar. Biol. Ass. U.K.* 61:431–448.

Marsh, J. B. & D. B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipids Res.* 7:574–576.

Mariño, J., A. Pérez & G. Roman. 1983. El cultivo del mejillón (*Mytilus edulis* L.) en la Ría de Arousa. *Bol. Inst. Esp. Oceanogr.* No. 350.

Martínez-Espósito, M. J., J. J. Pasantes & J. Méndez. Proliferation kinetics of mussels (*Mytillus galloprovinvialis*) gill cells. *Mar. Biol.* in press.

Nielsen, M. V. 1988. The effect of temperature on the shell-length growth of juvenile *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* 123:227–234.

Oliveira, L., T. Bisalputra & N. J. Antia. 1980. Ultraestructural observation of the surface coat of *Dunaliella tertiolecta* from staining with cationic dyes and enzyme treatments. *New Phytologist.* 85:385–392.

Pérez, A. & G. Roman. 1979. Estudio del mejillón y su epifauna en los cultivos flotantes de la Ría de Arousa. II. Crecimiento, mortalidad y producción del mejillón. *Bol. Inst. Esp. Oceanogr.* 5(267):23–41.

Ricker, W. E. 1973. Linear regression in fisheries research. *J. Fish. Res. Board Can.* 30:409–434.

Riisgard, H. V. & A. Randlöw. 1981. Energy budgets, growth and filtra-

tion rates in *Mytilus edulis* at different algal concentrations. *Mar. Biol.* 61:227–234.

Siron, R., G. Giusti & B. Berland. 1989. Changes in the fatty acid composition of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* during growth and under phosphorus deficiency. *Mar. Ecol. Prog. Ser.* 55:95–100.

Strömgrem, T. & C. Cary. 1984. Growth in length of *Mytilus edulis* L. fed on different algal diets. *J. Exp. Mar. Biol. Ecol.* 76:23–34.

Thompson, P. A., P. J. Harrison & J. N. Whyte. 1990. Influence of irradiance on the fatty acid composition of phytoplankton. *J. Phycol.* 26:278–288.

Volkman, J. K., S. W. Jeffrey, P. D. Nichols, G. I. Rogers & C. D. Garland. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* 128:219–240.

Walne, P. R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mecenaria* and *Mytilus. Fish. Invest.,* London. Ser. 2, 26(5):62 pp.

Ward, J. E. & N. M. Targett. 1989. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. *Mar. Biol.* 101:313–321.

Webb, K. L. & F. L. Chu. 1982. Phytoplankton as a food source for bivalve larvae. *In* Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. eds. G. D. Pruder, C. Langdon & D. Conklin. Lousiana State University, Baton Rouge, LA. pp. 272–291.

Wikfors, G. H., J. W. Twarog, Jr. & R. Ukeles. 1984. Influence of chemical composition of algal food sources on growth of juvenile oysters, *Crassostrea virginica*. *Biol. Bull.* 167:251–263.