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Energy balance of mussels *Mytilus galloprovincialis*: the effect of length and age

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ABSTRACT: Clearance and ingestion rates, absorption efficiencies and respiration rates were measured in mussels *Mytilus galloprovincialis* Lmk of different lengths (53 to 89 mm) and age (10 to 24 mo) from cultivation rafts in the Ría de Arosa (Galicia, Spain). The experiments were carried out either in the laboratory, using monoalgal food (*Isochrysis galbana*) with an organic content of 91%, or under natural conditions of food availability in cultivation rafts with seston, the organic content of which ranged from 33 to 69%. Food concentrations ranged from 0.57 to 1.00 mg l^{-1} of total particulate matter (TPM), a load which is below the threshold for the production of pseudofaeces in *Mytilus*. These experiments proved that the ingestion rate (IR = mg TPM h^{-1}) of food increases with the size of the mussel (measured as g of soft-tissue dry weight [DW]) according to the power equation $IR = 12.661DW^{0.619}$, this model accounting for over 90% of the variance of the IR. Behavioural patterns that tended to maintain constant IR regardless of the density of the food were observed. Absorption efficiency (AE) is positively related to the organic content (OC) of the food according to the following hyperbolic equation: $AE = 1.015 - 0.163(1/OC)$ ($r = 0.940$). AE is independent of mussel size for most of the size range used in this study, but there is a critical length around 85 mm, above which there is a noticeable decrease of AE. Metabolic expenditure, measured in terms of oxygen consumption standarized per unit of dry weight of flesh, tends to increase with the age of the mussel. The results obtained led to the conclusion that physiological traits such as the regulation of ingestion or differences in AE between groups do not explain the differences in growth between mussels of the same age. These differences must therefore be due to the limited food and space available as a result of the large numbers of mussels on the cultivation rafts and the agglomeration of mussels on the cultivation ropes.

KEY WORDS: *Mytilus* · Ingestion rate · Respiration · Absorption efficiency · Food quality

INTRODUCTION

Unlike mussels from natural populations on intertidal rocks, which exhibit a certain regularity of length for each age group, mussels cultivated on rafts are strikingly different in length, although at the start of the cultivation, all the mussels on a rope are normally of the same age and origin and of very similar length.

The cultivation rafts, upon which over 10 000 million mussels can be found on the NW Spanish coast alone, are responsible for the role of the mussel as a key species in the ecosystem of the 'rías' (Tenore & González 1976).

The growth of mussels depends on environmental factors, particularly the amount of food ingested and its quality, which strongly determine the efficiency of food assimilation. The filtering activity of bivalves tends to decrease as the food concentration in the water increases, thus regulating the amount of food ingested and maintaining the number of particles filtered in a given period of time relatively constant (Winter 1973, 1978, Foster-Smith 1975, Griffiths & King 1979, Widdows et al. 1979, Riisgård & Randløv 1981).

On the other hand, filtering activity, and hence the amount of food ingested, increases as the mussel grows according to an allometric function with $b < 1$. In consequence, the daily ration ingested, expressed as % of dry weight of flesh, decreases with increasing size of the mussels (Winter 1978, Navarro & Winter 1982).

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Absorption efficiency (AE) varies inversely with the density of suspended food and hence the ingestion rate (Widdows & Bayne 1971, Thompson & Bayne 1972, 1974, Griffiths & King 1979, Griffiths 1980, Navarro & Winter 1982, Bayne et al. 1989). Although some authors have reported that AE is also influenced by body size (Bayne et al. 1976), most consider AE to be independent of this variable (Vahl 1973, Thompson & Bayne 1974, Widdows 1978, Navarro & Winter 1982).

Given these considerations, how can we explain differences in size between mussels of the same age or differences in age between mussels of the same size? It is clear that for mussels maintained under identical conditions in which AE is size independent, differences in length between specimens of the same age should be expected to be due to differences in the rate of food uptake. This could be the consequence of limitations of food and space (see Fréchette & Lefaivre 1990) that arise from the high density of mussels in the culture system. However, differences in growth rate could also be attributed to differences in feeding rate and/or AE that would have enabled some mussels to grow bigger in the same time span. The existence of such fast- and slow-growing groups of mussels could then be tested by comparing feeding behaviour of specimens of the same size and different age under common feeding conditions.

While size effects on several aspects of physiology of mussels have been extensively reported, we are not aware of any other study in which age effects have also been considered. In the present study we aimed to establish the influence of age and length on physiological variables of the mussels, and to determine to what extent differences in growth can be attributed to variations in feeding rate or AE, or whether they can be explained by variations in the amount of food available due to the agglomeration of mussels on the cultivation ropes (comparable to the aggregation of mussels in natural populations on intertidal rocks), which cannot be compensated for by changes in the clearance rate.

MATERIAL AND METHODS

Measurements. ***Total dry matter and organic matter:*** Samples of water and faeces from each mussel were filtered on pre-ashed GF/C filters and rinsed with isotonic ammonium formate. Total dry particulate matter (TPM) was computed from the increase in weight after the filters were dried to a constant weight at 110°C. Particulate organic matter (POM) corresponded to the weight lost after ignition to constant weight at 450°C in a muffle furnace. The weights of TPM and POM were measured in µg. Food quality was expressed in terms of its organic content (OC) by weight (OC = POM/TPM). Once these measurements were made, soft tissues from each mussel were excised, dried at 100°C and ashed at 450°C to determine dry weight (DW) and the weight of organic matter (OW) of the soft tissues. The weight of the valves (VW) was also determined. The weights of DW, OW and VW were measured in mg. A condition index (CI) was calculated as follows (Pérez Camacho et al. 1997):

$$CI = 100(DW/VW)$$

Clearance and ingestion rates: In Expt 1, which was performed in the laboratory, mussels were acclimatized for 1 wk under experimental conditions before measurements began. Clearance rate (CR) was determined in triplicate for each of the 15 mussels used in the experiment (5 of each length), using individual 300 ml metacrylate containers with running seawater (5 l h^{-1}) and a concentration of 50000 cells ml^{-1} of *Isochrysis galbana* equivalent to 1.1 mg l^{-1} of TPM with an organic content of 91.31%. Containers without mussels were used to calculate any possible sedimentation of phytoplankton, which proved to be negligible. Flow was controlled with 12-channel variable-flow ISMATEC peristaltic pumps. The following equation was used to calculate the CR:

$$CR = f[(C_i - C_e)/C_e]$$

where C_i and C_e represent the concentration of *I. galbana* (cells l^{-1}) in the inflow and outflow, respectively, and f is the flow measured in l h^{-1}.

The ingestion rate (IR) (mg TPM h^{-1}) was determined using the following equation:

$$IR = CR \times TPM$$

where TPM corresponds to the concentration of *Isochrysis galbana* (in mg l^{-1}). The IR per gram of soft-tissue DW of the mussels, or mass-specific IR (IR_s), was also determined.

In the experiments performed *in situ* on the cultivation rafts (Expts 2 and 3), the measurements were performed on mussels of the same origin cultivated on ropes at a depth of 6 m. The mussels were sorted according to their lengths, their byssus carefully cut and epibionts removed, after which they were placed in mesh bags which were suspended from the raft at a depth of 6 m for 24 h. Subsequently 18 mussels of each size-group were transferred to individual compartments in feeding tanks, which received a flow of 90 l of seawater per hour from a submersible pump situated at a depth of 6 m in the same place where the mesh bags had been suspended. Samples of seawater for analysis were taken at half-hourly intervals, and the faeces every 2 h, using the methods described by Navarro et al. (1991) and Iglesias et al. (1996). In this

case the IR was calculated indirectly from the egestion and the inorganic content of the seston (Navarro et al. 1991, Iglesias et al. 1996) according to the following equation:

$$IR = ER(FIC/SIC)$$

where ER is the egestion rate, measured in mg DW h^{-1} of faeces, FIC is the fecal inorganic matter content (%) and SIC is the inorganic matter content of the seston. CR was then computed by the following expression:

$$CR = IR/TPM$$

Absorption efficiency: AE was calculated according to Conover (1966). Absorption rates (A) were estimated as the difference between organic ingestion rates (OIR) and organic egestion rates. Absorption efficiencies (AE) corresponded to the ratio A/OIR.

Oxygen consumption rate: Oxygen consumption rate (VO_2, mg O_2 h^{-1}) and mass-specific VO_2 (VO_s) were estimated only for Expts 2 and 3 in the same mussels used to estimate the CR. Measurements were taken at half-hourly intervals, using YSI electrodes in 400 ml glass chambers maintained at the environmental temperature with flowing water pumped from the sea. Measurements were concluded before concentration of oxygen in the chambers attained 50% of the initial concentration.

Experimental design. The experiments were carried out with mussels from the experimental cultures in the Ría de Arosa (Galicia, Spain). All the mussels shared the same origin (spat gathered from rocks on the Island of Sálvora), and were of similar age and size at the start of cultivation. These mussels remained attached to ropes suspended from a raft for between 6 and 18 mo before the experiments began. Three different experiments were performed, 2 of which were intended to determine the influence of size on physiological variables, comparing results obtained in the laboratory with monoalgal food to those obtained on the raft with natural food, whilst the third was designed to study the independent effects of size and age of mussels on the same physiological variables.

Expt 1: This experiment was performed in the laboratory, using *Isochrysis galbana* as food, with an organic content of 91%, dosed at a TPM concentration of 1.010 mg l^{-1}. Three groups of 5 mussels each were used, measuring between 60 and 63 mm (average length: 61.6 ± 0.9 mm), 72 and 75 mm (average length: 73 ± 1.2 mm), and 87 and 90 mm (average length: 88.9 ± 1.2 mm), respectively (means ± standard error). At the time of the experiment the approximate age of these mussels was 24 mo.

Expt 2: This experiment took place on the cultivation raft, using natural food with an organic content of 69%, at a TPM concentration of 0.568 mg l^{-1}. Mussels used in this experiment were of the same origin as those used in Expt 1, were of the same age (24 mo), and were divided into 3 groups of 18 mussels each, with lengths ranging from 56 to 66, 71 to 77 and 85 to 92 mm (average lengths: 60.1 ± 3.4, 73.9 ± 2.8 and 88.5 ± 2.6 mm, respectively).

Expt 3: This experiment was carried out under the same natural conditions as Expt 2, but the organic content of seston was 33% and TPM concentration was 1.007 mg l^{-1}. Mussels belonging to 2 age groups of 10 (juvenile) and 22 (old) mo were subsequently divided each into 2 size-groups. Groups of 'old mussels' had average lengths of 55.7 ± 1.0 and 77.9 ± 0.8 mm, and groups of 'juvenile mussels' had average lengths of 53.85 ± 0.83 and 79.42 ± 0.56 mm. Thus, measurements of Expt 3 were performed on 4 groups of mussels in which age and size varied independently.

Statistical methods. All statistical procedures were performed with STATGRAPHICS software. The differences between means for the variables studied were compared by means of analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The Bartlett test was used to check the homogeneity of the variances, with logarithmic transformations being performed when necessary. Multiple comparisons were carried out with the least significant differences (LSD) multiple range test (Snedecor & Cochran 1980, Zar 1984).

RESULTS

Expt 1

Table 1 shows the mean values for size parameters (L, DW, OW and VW) of the mussels used in this experiment, and Table 2 those for physiological variables (CR, IR, OIR, IRs and AE). These data showed a clear increment of the feeding variables with the size of mussels (except AE and IRs), which were found to be

Table 1. Expt 1. Mean values for length (L), dry weight (DW) and organic weight (OW) of the soft tissues, and valve weight (VW) in mussels of varying lengths (S, small; M, medium-sized; LA, large) of the different parameters analysed. The data correspond to the mean values ± SE (n = 5)

	L (mm)	DW (g)	OW (g)	VW (g)
S	61.60 ± 0.96	1.026 ± 0.344	0.312 ± 0.049	7.811 ± 1.029
M	73.00 ± 1.12	1.834 ± 0.399	0.458 ± 0.084	11.309 ± 0.800
LA	88.90 ± 1.24	3.762 ± 1.016	1.058 ± 0.441	19.644 ± 2.542

Table 2. Expt 1. Mean values of the clearance rate (CR), seston ingestion rate (IR), organic matter ingestion rate (OIR), ingestion rate of total particulate matter per g of mussel soft tissue dry weight (IR_s) and absorption efficiency (AE) in mussels of varying lengths (S, small; M, medium-sized; LA, large), and significance of the ANOVAs of the different parameters analysed. The data correspond to the mean values ± SE (n = 5)

	CR ($l\ h^{-1}$)	IR ($mg\ h^{-1}$)	OIR ($mg\ h^{-1}$)	IR_s ($mg\ h^{-1}\ g^{-1}$)	AE (%)
S	2.051±0.250	2.256 ± 0.275	2.060 ± 0.250	2.199 ± 0.268	88.34 ± 0.58
M	4.159 ± 0.321	4.575 ± 0.353	4.177 ± 0.320	2.495 ± 0.193	88.21 ± 0.38
LA	5.634 ± 0.390	6.197 ± 0.429	5.658 ± 0.392	1.647 ± 0.114	75.49 ± 4.53
F-ratio	9.327	9.327	9.327	1.526	7.667
Significance level	<0.01	<0.01	<0.001	>0.05	<0.01
df	2,12	2,12	2,12	2,12	2,12

statistically significant when testing for differences between mean values of CR, IR and OIR for the 3 groups of mussels (ANOVA, $p < 0.05$). Differences were significant between all groups (LSD test, $p < 0.05$).

When ANOVA included the size of mussels (given as the soft-body DW) as a covariable, non-size-related differences were found to be non-significant for any of the above variables ($p > 0.05$). The same results were obtained by comparing IRs computed for different groups of mussels by ANOVA ($p > 0.05$).

The AE of the small- and medium-sized mussels were similar, whilst that of the large ones shows a noticeable decrease, its difference from the 2 former groups being statistically significant (ANOVA, $p < 0.01$; LSD test, $p < 0.05$). This observation points to the existence of a critical length (≅88 mm) above which AE declines drastically.

Expt 2

Table 3 shows the mean values for size and Table 4 those for physiological variables measured in mussels during this experiment. CR, IR and OIR increased with the size of the mussels. However, the only statistically significant differences in this case were those between the groups of small mussels and the medium-sized plus large ones (ANOVA, $p < 0.01$; LSD test, $p < 0.05$), differences between the latter 2 groups being not statistically significant. This was probably connected with a lower range of weight in the mussels used in this experiment, coupled to the greater variability of the physiological determinations. The ANOVA for these parameters when the DW of the mussels is used as a covariable did not reveal significant differences ($p > 0.05$), and neither are the differences in IR_s significant (ANOVA, $p > 0.05$).

As in Expt 1, the AE of the large mussels was significantly lower than that of the small- and medium-sized ones (ANOVA, $p < 0.001$; LSD test, $p < 0.05$), for which there were no significant differences. This consistency confirms the existence of a critical size (88 mm) above which AE shows a noticeable decrease.

Table 3. Expt 2. Mean values for length (L), soft-tissue dry weight (DW) and organic weight (OW), and valve weight (VW) in mussels of varying lengths (S, small; M, medium-sized; LA, large) of the different parameters analysed. The data correspond to the mean values ± SE (n = 16)

	L (mm)	DW (g)	OW (g)	VW (g)
S	60.06 ± 0.84	0.848 ± 0.059	0.653 ± 0.05	15.684 ± 0.187
M	73.50 ± 0.77	1.698 ± 0.089	1.211 ± 0.064	10.350 ± 0.319
LA	88.38 ± 0.66	2.677 ± 0.173	2.130 ± 0.146	15.946 ± 0.418

Expt 3

In accordance with the design of this experiment, the mean values for size parameters (L, DW, OW and VW) were significantly different between size groups (small- vs medium-sized; ANOVA, $p < 0.001$; LSD test, $p < 0.05$), whilst there were no significant differences between age groups (juvenile vs old; LSD test, $p > 0.05$) (Table 5).

As in the previous experiments, there were significant differences in CR, IR and OIR of different sized mussels (ANOVA, $p < 0.001$; LSD test, $p < 0.05$). However, neither the ANOVA of these variables when DW was used as a covariable ($p > 0.05$) nor that of the IR_s (ANOVA, $p > 0.05$) showed any significant differences (Table 6).

As for the AE, the small-old mussels was the only group that showed any statistically significant difference (ANOVA, $p < 0.001$; LSD test, $p < 0.05$). The mean AE for this group is

Table 4. Expt 2. Mean values of the clearance rate (CR), seston ingestion rate (IR), organic matter ingestion rate (OIR), ingestion rate of seston per g of mussel soft-tissue dry weight (IR_s) and absorption efficiency (AE) in mussels of varying lengths (S, small; M, medium-sized; LA, large), and significance of the ANOVAs of the different parameters analysed. The data correspond to the mean values ± SE (n = 16)

	CR ($l\ h^{-1}$)	IR ($mg\ h^{-1}$)	OIR ($mg\ h^{-1}$)	IR_s ($mg\ h^{-1}\ g^{-1}$)	AE (%)
S	3.611±0.594	2.051 ± 0.327	1.417 ± 0.232	2.545 ± 0.438	80.21 ± 0.53
M	6.931 ± 0.841	3.936 ± 0.479	2.720 ± 331	2.281 ± 0.268	81.59 ± 0.74
LA	9.406 ± 1.750	5.343 ± 0.994	3.691 ± 0.687	1.910 ± 0.298	70.80 ± 2.34
F-ratio	6.437	6.437	6.437	0.839	8.511
Significance level	<0.01	<0.01	<0.01	>0.05	<0.001
df	2,45	2,45	2,45	2,45	2,45

Table 5. Expt 3. Mean values of length (L), soft-tissue dry weight (DW) and organic weight (OW), and valve weight (VW) of mussels of different sizes and ages (Sj, small juvenile; So, small old; Mj, medium juvenile; Mo, medium old). Significance of the ANOVAs of the different parameters analyzed and LSD multiple range test. The data correspond to the mean values ± SE (n = 14)

	L (mm)	DW (g)	OW (g)	VW (g)	Homogeneous groups
Sj	52.9 ± 0.9	0.562 ± 0.029	0.428 ± 0.024	3.679 ± 0.218	×
So	55.7 ± 0.9	0.618 ± 0.035	0.470 ± 0.029	4.496 ± 0.218	×
Mj	80.6 ± 0.8	1.327 ± 0.047	1.075 ± 0.038	10.217 ± 0.328	×
Mo	78.8 ± 0.8	1.314 ± 0.068	1.009 ± 0.052	10.970 ± 0.461	×
F-ratio	76.07	78.85	83.07	124.31	
Significance level	<0.001	<0.001	<0.001	<0.001	
df	3,52	3,52	3,52	3,52	

Table 6. Expt 3. Mean values of the clearance rate (CR), seston ingestion rate (IR), organic matter ingestion rate (OIR), total particulate matter ingestion rate per g of mussel soft-tissue dry weight (IR_s) and absorption efficiency (AE) of mussels of different sizes and ages (Sj, small juvenile; So, small old; Mj, medium juvenile; Mo, medium old). Significance of the ANOVAs of the different parameters analysed and LSD multiple range test. The data correspond to mean values ± SE (n = 14)

	CR ($l\ h^{-1}$)	IR ($mg\ h^{-1}$)	OIR ($mg\ h^{-1}$)	IR_s ($mg\ h^{-1}\ g^{-1}$)	AE (%)
Sj	2.283 ± 0.224	2.299 ± 0.225	0.759 ± 0.074	4.171 ± 0.407	55.950 ± 2.478
So	2.248 ± 0.286	2.264 ± 0.288	0.747 ± 0.095	3.816 ± 0.620	45.533 ± 2.127
Mj	4.032 ± 0.533	4.061 ± 0.537	1.334 ± 0.177	3.051 ± 0.409	51.941 ± 2.238
Mo	4.154 ± 0.577	4.183 ± 0.581	1.380 ± 0.192	3.363 ± 0.521	54.152 ± 2.061
F-ratio	5.960	5.960	5.960	0.984	5.684
Significance level	<0.001	<0.001	<0.001	>0.05	<0.001
df	3,52	3,52	3,52	3,52	3,52

lower than for the other groups measured in this experiment (Table 6), which to some extent would provide a reasonable explanation for their small size in relation to age.

Ingestion versus size

As previously described, ingestion by mussels has a direct bearing on their size, and therefore the heaviest mussels (which are also the longest) are those that ingest a greater amount of food. Thus, the ingestion rate of mussels can be related to size through regression lines. Table 7 shows these regression equations computed from ingestion data obtained with natural food only (Expts 2 and 3).

If the mean values of AE for the small- and medium-sized mussels in all 3 experiments are compared to the organic content of the food ingested (OC), we can see that AE increases in direct proportion to OC, following an asymptotic tendency (Fig. 1). On fitting this to the model put forward by Navarro et al. (1991), the following equation is obtained (values ± SE; r = 0.873):

Table 7. Parameters of the regression lines of the total particulate matter ingestion rate (IR, mg h^{-1}) and mass-specific ingestion rate (IR_s, mg h^{-1} g^{-1}) on the body size of *Mytilus edulis* (DW, g of dry soft tissue; L, mm). *a* and *b* are fitted parameters in the allometric equation $y = aX^b$; r is the correlation coefficient; $IR_s = IR \times DW^{-1}$; n = 10 (the values are the mean of between 5 and 16 measurements)

	Parameter	Estimate	SE	*T* value	p	r
IR vs L	*a*	0.001	0.000	−7.435	0.0001	0.952
	b	1.970	0.225	8.751	0.0000	
IR vs DW	*a*	2.960	0.520	20.851	0.0000	0.934
	b	0.599	0.081	7.385	0.0001	
IRs vs DW	*a*	2.960	0.520	20.851	0.0000	0.868
	b	−0.401	0.081	−4.949	0.0011	

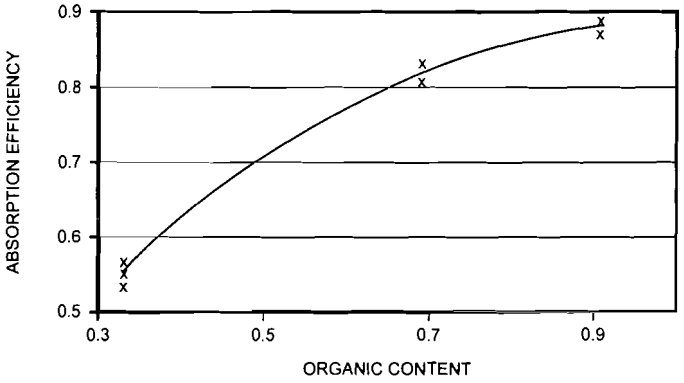

Fig. 1. Absorption efficiency as a function of the proportional organic content of the ration. The line was fitted by eye

$$AE = 0.919 \pm 0.188\ (1 - e^{-2.784 \pm 2.814\ (OC - 0.030 \pm 0.214)})$$

where the exponential coefficients are not statistically significant. An improved fit is obtained if the AE is related to 1/OC along a linear regression (values ± SE; r = −0.940):

$$AE = 1.015 \pm 0.045 - 0.163 \pm 0.021\ (1/OC)$$

In addition to a better description of data in statistical terms, the hyperbolic function also corresponds to theoretical expectations (see Navarro et. al. 1991).

Respiration

The oxygen consumption rates (VO_2) of the mussels from Expts 2 and 3 are shown in Table 8. In both these experiments, which were performed at temperatures of 17 to 19°C, the VO_2 increases with the weight (and length) of the mussels, with differences between the values of VO_2 for the different mussel groups being highly significant ($p > 0.001$) when compared by ANOVA. The LSD test also showed significant differences ($p > 0.05$) among all the size groups in both Expts 2 and 3.

When the regression of VO_2 over the DW of the soft tissues of the mussels ($VO_2 = aDW^b$) is estimated for data obtained in these 2 experiments, it can be seen that, in both cases, DW accounts for over 84% of the variation in VO_2 (Table 9). The values of the slopes and the intercepts of the 2 equations are very much alike, and no significant differences appeared when they were compared by means of an ANCOVA.

DISCUSSION

Clearance and ingestion rates

The relation between ingestion rates and body size in mussels (and other filter-feeders) has been broadly discussed in previous publications (e.g. Walne 1972, Winter 1978, Bayne & Hawkins 1990). In general terms it can be said that the amount of organic material ingested increases allometrically with the size of mussels (dry-tissue weight), with a value of $b < 1$ (Winter 1973, 1978, Navarro & Winter 1982). Our results on the whole agree with those of the above-mentioned studies (see Table 7), and the slopes of the regression lines of ingestion (expressed as IR or as mass-specific IR) on dry-tissue weight are very similar to those reported by Navarro & Winter (1982) for *Mytilus chilensis* (−0.43 and between 0.38 and 0.42 respectively in the first case, and 0.62 and between 0.58 and 0.62 in the second).

As a general rule, clearance and ingestion rates will increase rapidly as the concentration of particles increases until the ingestion rate reaches a maximum. After this point, the clearance rate declines whilst the ingestion rate remains constant until the whole digestive apparatus collapses at a very high concentration of particles, and then the ingestion rate drops considerably (Winter 1978, Riisgård & Randløv 1981, Bayne & Hawkins 1990). Our experiments provide clear evidence of this regulation since the ingestion rates of mussels of similar size are also very similar (Tables 1

Table 8. Oxygen consumption rates per individual (VO_2) and per mg soft-tissue dry weight (VO_s) of the mussels in Expts 2 (L, large; M, medium; S, small) and 3 (Sj, small juvenile; So, small old; Mj, medium juvenile; Mo, medium old). Means (n = 16) ± SE. Relationship of homogeneous groups within each experiment according to the LSD multiple range test. Temperature: 17 to 19°C

Expt:	2	2	2	3	3	3	3
Mussel:	S	M	L	Sj	So	Mj	Mo
Dry flesh weight (g)	0.727 ± 0.105	1.524 ± 0.727	2.546 ± 0.106	1.261 ± 0.073	0.895 ± 0.048	2.601 ± 0.137	2.492 ± 0.247
VO_2 (ml h^{-1})	0.482 ± 0.015	0.832 ± 0.056	1.168 ± 0.092	0.696 ± 0.042	0.581 ± 0.040	1.059 ± 0.053	1.320 ± 0.093
VO_s (ml h^{-1} g^{-1})	0.680 ± 0.045	0.561 ± 0.060	0.454 ± 0.023	0.564 ± 0.044	0.647 ± 0.017	0.409 ± 0.013	0.547 ± 0.030
Homogeneous groups (VO_2)	×	×	×	×	×	×	×

Table 9. Parameters of the regression line of the oxygen consumption rate (ml h^{-1}) versus soft-tissue dry weight (g dry flesh weight) of the mussels in Expts 2 and 3 (jm = 10 mo old mussels, om = 20 mo old mussels). *a* and *b* are fitted parameters in the allometric equation $y = aX^b$; r is the correlation coefficient

	Range of dry flesh weight	Temp. (°C)	Number of observations	*a*	*b*	r	p
Expt 2	0.80–2.68	18–19	18	0.329 ± 0.045	0.647 ± 0.070	0.917	<0.001
Expt 3	0.90–2.60	18–19	32	0.325 ± 0.037	0.690 ± 0.054	0.918	<0.001
Expts 2 and 3	0.56–2.68	18–19	50	0.328 ± 0.028	0.674 ± 0.042	0.918	<0.001
Expt 3 (jm)	1.26–2.60	18–19	16	0.319 ± 0.049	0.569 ± 0.085	0.874	<0.001
Expt 3 (om)	0.90–2.49	18–19	16	0.347 ± 0.028	0.803 ± 0.047	0.977	<0.001

to 6). These results are even more significant if one takes into account the differences between the mussels used in the experiments (mussels of varying length and age), the different composition of food used (cultured phytoplankton and natural seston), and the different experimental conditions (laboratory and raft).

The concentration of particulate material in our experiments (from 0.5 to 1 mg l^{-1}) falls within the normal range for the Galician rías (Navarro et al. 1991) but is below values reported for other estuaries sustaining actively growing populations of mussels (Smaal et al. 1986, Bayne et al. 1993), and well below the threshold for the production of pseudo-faeces (3 mg l^{-1}, Bayne et al. 1993). Under these conditions, and in the absence of pre-ingestive selection that could increase the organic fraction of ingested food (Bayne et al. 1993), the regulation of the ingestion rate is based on TPM rather than organic material. A comparison of the results of Expts 1, 2 and 3 (Tables 2, 4 & 6) shows this to be the case, with similar IR_s for mussels of similar length, whilst the ingestion rates of organic material vary widely and are directly proportional to the organic content of the material.

Absorption efficiency

Not much information is available regarding the influence of the size of mussels on AE, although, as a general rule, AE appears to be size independent in *Mytilus edulis* (Vahl 1973), *Mytilus chilensis* (Navarro & Winter 1982) and *Modiolus modiolus* (Winter 1978). The results of Expts 1 and 2 in our study, performed with different food concentrations (1.01 mg and 0.57 mg TPM l^{-1}) and organic contents (91 and 67%), clearly show a noticeable decrease of AE in mussels reaching a length of 85 mm. This does not necessarily mean that there is a contradiction with the above mentioned findings, since the specimens used in their experiments were well below the critical length of 85 mm, above which the 'ageing' of the mussel becomes evident. Below this length the AE remains constant, regardless of the age and length of the mussels, with the exception of the small old mussels in Expt 3, whose reduced AE might well account for their low growth rates.

In *Mytilus* fed on concentrations of seston below the threshold level for the production of pseudo-faeces, the AE rises asymptotically with the increase in food qual-

ity (Navarro et al. 1991) according to a function which in theory would be hyperbolic, and which can be transformed into a linear regression by means of the inverse transformation of the independent variable (Navarro et al. 1996). Our experimental data fit these theoretical expectations, as the hyperbolic model accounts for 97% of the variance in AE as being dependent on the quality of ingested food (r = 0.940). The equations in Table 8 allow us to estimate the theoretical maximum value for AE, which would be 0.889 for 100% OC. Similarly, the OC below which AE would be negative is 0.160, which in terms of quality expressed as POM per unit of particulate volume would be 0.22. These values resemble those previously estimated by Navarro et al. (1991) for mussels from the Ría de Arousa.

Oxygen consumption rate

Oxygen consumption is a good measure of metabolic demands for activity in bivalves (Winter 1978). However, differences in VO_2 associated with variable feeding conditions are not expected to be important since the metabolic cost of feeding and digestion represents a minor component of the overall metabolic rate (Bayne et al. 1989, Widdows & Hawkins 1989, Navarro et al. 1991). The main variation in oxygen consumption rates is accounted for by an allometric dependence on body size given by the equation $y = ax^b$. Although b values reported in the literature are very variable, in our experiments the mean slope of this equation is 0.67, which is close to the arithmetic mean calculated by Bayne et al. (1976) using values of the slope estimated by several different authors ($b = 0.71$).

In addition to this, a detailed examination of values from Expt 3 (Table 8) would suggest that the mass-specific VO_2 of young mussels is lower than that of old mussels of the same length. In fact, if the mussels used in this experiment are divided into 2 age groups ('young' and 'old' mussels), the ANCOVA applied to VO_2 data using DW as a covariable results in significant differences between both groups (Table 10). This demonstrates that there is a clear ageing effect which is evident irrespective of the size of the mussel, causing the mass-specific VO_2 to increase with age. The consistency of these data is supported by the similarity between the VO_2 of the 'old' mussels in Expt 3 and that of the mussels of the same length in Expt 2, which were also of approximately the same age (Table 8). On the other hand, if the groups are established according to the length of the mussels, without taking age into account, then the ANCOVA with DW as a covariable does not show any significant differences (Table 10). The increase in oxygen consumption with age is clearly illustrated in Table 9, which shows that the slope for the regressions between oxygen consumption and soft-tissue weight changes from 0.57 in 10 mo old mussels to 0.80 in 22 mo old mussels and which results in greater respiration rates predicted for old mussels of the same size. This difference in oxygen consumption associated with age may well provide an explanation, if only partial, for the noticeable variation in the coefficients of equations calculated by various authors to relate oxygen consumption and body size in mussels (see Bayne et al. 1976), particularly when it is taken into account that the wide range of body sizes used necessarily implies age differences.

Growth rate

It is important here to point out that even if an ageing effect has been described which may account for the decline of the growth rate in mussels of greater body size or age neither the variations between the different body sizes and efficienciess nor the regulatory processes discussed herein are sufficient (except for small-old mussels, whose AE was noticeably lower than for large mussels) to account for the differences in growth between cultivated mussels of the same age, at least under the same environmental conditions. An alternative explanation for this phenomenon may be found in the limitations of food and space (see Fréchette & Lefaivre 1990) that arise from the high density of mussels in the culture system, resulting in a limitation of ingestion rates, for both the whole raft and the individual cultivation ropes.

In the case of Galicia, the rafts are located in areas that are characterised by a low concentration of particulate matter, which nevertheless has a high organic content (between 0.5 and 1 mg POM l^{-1} and 50% OC: Pérez Camacho et al. 1991, Navarro et al. 1991, this study). The reduction in particle concentration in the water flowing past the raft (as high as 50%, Pérez Camacho et al. 1991) and the decrease in ingestion

Table 10. ANCOVA of the oxygen consumption rate (ml h^{-1}) of mussels of different sizes and ages in the Expt 3 (covariate = DW, g dry flesh weight)

Factor	F-ratio	Significance level	df
Length			
Covariate: DW	21.560	0.0001	1,1,29
Principal effect	1.045	0.3151	
Age			
Covariate: DW	183.341	0.0000	1,1,29
Principal effect	12.097	0.0016	

rate in mussels when the concentration of TPM drops below 0.4 mg l^{-1} (Riisgård & Randløv 1981) may mean that, at least at those times of the year when the proportion of seston in the water is at its lowest and in spite of the regulation mechanism, the ingestion rates of the mussels would be noticeably lower at the back of a raft than at the front, leading to a clearly inferior growth rate in the former (a fact widely borne out through the experience of the mussel growers).

In very much the same way the high density of mussels on the cultivation ropes (between 400 and 500 mussels m^{-1}) means that they are superimposed on each other in several layers, with the subsequent competition for food between mussels on the same string, and a reduced availability of seston for those mussels that form the inner layers. If, in addition, we also consider the probable decrease in filtration and ingestion rates due to the reduced opening of the valves caused by the pressure that the mussels exert on each other (Jørgensen et al. 1986), the differences in growth rates between mussels with a common origin and of the same age, cultivated on the same rope, would easily be explained.

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