

Growth of mussels (*Mytilus edulis galloprovincialis*) on cultivation rafts: influence of seed source, cultivation site and phytoplankton availability

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Abstract

Mussel seed, obtained from the intertidal zone and permanently immersed collector ropes, was cultivated on ropes suspended from three rafts located at three different sites within the Ria de Arousa (Galicia, NW Spain). Sites were characterized by different levels of phytoplankton availability. The source of seed stock had a marked influence upon subsequent mussel growth; seed originating from collector ropes had higher growth rates than seed collected from intertidal areas and was probably due to the higher condition index and previous adaptation to rope culture conditions (permanent immersion) for these samples. Cultivation site also affected mussel growth; differences in chlorophyll a content and water current speed, which influence phytoplankton availability, were the major factors underlying variation in growth rate and condition index. It is recommended that seed obtained from collector ropes should be used in the commercial exploitation of this species, since it would shorten the total duration of the cultivation process by more than 10%.

1. Introduction

The growth rate, mortality and production traits of mussels vary widely among different stocks of *Mytilus edulis* cultured in Europe and America (Pérez Camacho and Román, 1979; Dickie et al., 1984; Mallet and Carver, 1989; Kautsky et al., 1990). The growth of suspension-feeding bivalves is dependent upon a number of endogenous and environmental factors (Bayne and Newell, 1983), but especially the amount of food ingested, which depends upon the food availability and filtration rate (Wallace, 1980;

Page and Hubbard, 1987). Food availability is linked with phytoplankton dynamics (Tenore and Gonzalez, 1976; Rosenberg and Loo, 1983; Smaal and Stralen, 1990) and varies with the current speed in the cultivation area (Frechette et al., 1989). Variation in the ingestion rate of mussels may result from either adaptations to marked regional differences in quantitative and qualitative seston conditions (Theisen, 1977; Bayne et al., 1984; Navarro et al., 1991), or genetic variation (Koehn and Shumway, 1982; Hawkins et al., 1986). In this sense, the observed variability in mussel production traits under natural conditions may be explained by genetic differences found at both the micro and macrogeographic scales (Levinton and Suchanek, 1978).

The study of intraspecific variation in the physiological adaptations of different populations in response to regional differences in environmental variables is a subject of major interest, in particular in Galicia, where mussel seed originating from various sources is used to support a mussel production of 200 000 tonnes per year. The aim of the present study was to evaluate the quality of mussel seed obtained from different sites, measured as growth, survival and condition index, and to assess the importance of endogenous (either physiological or genetic) and exogenous (phytoplankton availability) factors upon production.

Experiments undertaken in this study were carried out in the major Galician ria, Ria de Arousa, an estuary of 250 km² with an average depth of 19 m and 2.7 m tidal range (Tenore and Gonzalez, 1976), and is among the most important bivalve production zones in the world (Korringa, 1967; Bardach et al., 1972, Perez Camacho et al., 1991). Identifying a seed source showing the most rapid growth and optimal degree of adaptation to culture conditions would be an obvious economic advantage. Furthermore, mussel cultivation of this magnitude relies on obtaining more than 7500 tonnes of seed per year, which is presently collected from the rocky shore. This considerable pressure upon the natural stocks presents an increasingly important problem for mussel cultivators and an alternative source of seed is necessary.

The mussel cultivation process has two stages; pre-fattening and fattening, separated by a thinning out operation. The pre-fattening stage begins with 2 cm long individuals collected from the intertidal area of the rocky shore, preferably from very exposed sites, and is completed after several months when individuals reach a size of ca. 5 cm. During

thinning out, three new ropes are established from each seed rope, and mussels are then cultured until they reach commercial size (7-10 cm) (Perez Camacho et al., 1991). The present study pays particular attention to the first stage, the pre-fattening process. The experiments strictly followed traditional cultivation methods and mussel ropes were prepared and maintained by local mussel cultivators to ensure that conclusions obtained from the present results are applicable to the improvement of rope suspended mussel culture. The study of the second stage, the fattening to commercial size, is currently in preparation.

There is great controversy regarding the taxonomic status of the different European mussels within the *Mytilus* genus. The Galician mussel, traditionally considered as *Mytilus edulis* (Andreu, 1960; Korringa, 1976; Aguirre, 1979; Perez Camacho and Roman, 1979), has recently been classified as *Mytilus galloprovincialis* (Sanjuan et al., 1990; Koehn, 1991; McDonald et al., 1991), and also as the subspecies *Mytilus edulis galloprovincialis* (Gardner, 1992; Gosling, 1992). Since an agreement on the subject has not yet been achieved, we refer to this latter denomination, *Mytilus edulis galloprovincialis*, for the purposes of this study.

2. Materials and methods

2.1. Experimental design

The experiment was conducted on three commercial cultivation rafts (25 m x 20 m), each with 500 ropes, located in the Ria de Arousa (Galicia, NW Spain) at Sites 1, 2 and 3 (Fig. 1), which varied according to production levels. Three sources of seed (stocks) were used; two originated from two of the most productive intertidal regions along the N. Spanish coast, Finisterre (Stock A) and Salvora (Stock B), separated by 100 km, and the third stock from collector ropes placed on a raft anchored at 2 km from B (Stock C; Fig. 1). In order to prevent differences in growth due to seed manipulation, mussels from the three origins were detached from the rocks or collector ropes, handled and attached to 'acclimation' ropes in the same way and for the same time. Acclimation ropes were maintained in a common environment for 6 weeks. In the middle of April the experimental ropes were made up with a density of ca. 2 000 mussels m⁻¹ and they were transferred into the experimental sites.

The initial mean lengths of mussels from stocks A, B and C were 16.0 mm, 18.3 mm and 24.7 mm, and the mean live weights were 0.6 g, 0.8 g and 1.7 g respectively. A total of 18 12 m long seed ropes were prepared (two ropes per stock per site) and placed in the central part of each raft. The experiment ended after 90 days, when mussels had reached the usual size for the thinning out (ca. 5 cm).

2.2. Mussel sampling

Sampling frequency

Duplicate samples of ca. 300 individuals were taken from halfway along the length of each rope at the beginning, after 1.5 months, and again at the end of the experiment.

Length and live weight

Individual mussel length (L) was measured to the nearest 1 mm using callipers and each sample subdivided into 1 mm length classes. Each length class was weighed (live weight, LW) with an accuracy of 0.1 g.

Dry weight and organic matter

Subsamples of five mussels were each taken from five to six length classes covering the entire size range and used to determine dry weight and organic content. With this aim, the abductor muscles were cut and intervalvar water was allowed to drain by placing the mussels with their ventral edge on filter paper. Tissues were then dissected and both shell valves and soft tissues were weighed after drying at 100°C until constant weight, and after ashing at 450°C for 24 h.

Condition index

Condition indices (CI) were calculated from the ratio of tissue dry weight (DW) and the dry weight of the valves (VW), according to the equation (Freeman, 1974):

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

Mortality

Mortality was determined from the number of empty shells in the samples in relation to the total number of individuals sampled.

2.3. Water sampling and current speed

Water samples were taken every 15 days throughout the experimental period in order to measure temperature, salinity and fluorescence at each site by means of a CTD Seabird 25. ENDECO current-meters were placed at a depth of 6 m among the ropes approximately at 2 m from the frontal edge of the rafts to record the water-current speed flowing through the mussel ropes at 1 min intervals during 14 days. Water flow was calculated according to the equation:

$$f = V \times W \times H \times 36$$

where f is water flow ($\text{m}^3 \text{h}^{-1}$), V is current speed (cm s^{-1}), W is the raft width (m, subtracting the width of the ropes) and H is the length of the mussel ropes (m). Water current inside the Ria is due to the tides. Average f values include variation resulting from inlet and outlet currents, high and low tides, and spring and neap tides.

Phytoplankton content of the water at different sampling times was estimated from the chlorophyll a content calculated from the fluorescence data. The amount of phytoplankton available to the mussels on the suspended ropes of each raft can be calculated according to the equation:

$$F = (\text{Ch} \times f) / N$$

where F is grams of chlorophyll a per rope per h, Ch is grams of chlorophyll a per cubic metre, and N is the number of ropes per raft.

2.4. Data analysis

Regression models were calculated for the log LW vs. L relationships from data obtained for 11 length classes 5 mm above and below the mean length. For each stock and site combination, the CI corresponding to the mean length were calculated according to the CI vs. L regression.

The effects of initial size, stock and cultivation site on L and LW growth, CI and mortality of the seed were tested by multifactor analysis of variance (MANOVA), using the least significant difference (LSD) method and the type III estimation of the sums of squares (Lison, 1968; Snedecor and Cochran, 1980; Zar, 1984). The variable initial size did not reach statistical significance ($P > 0.30$) and was therefore excluded from subsequent analyses. A two factor design (stock and site) of three levels per factor and two replicates per level was consequently applied. In view of the direct correlation between means and standard deviations, increases in L and LW were transformed (\log_{10}) in order to achieve homogeneous variances (Lison, 1968). With regard to mortality data, angular transformation was applied to the percentages (Snedecor and Cochran, 1980).

Homogeneity of variances was tested by the Bartlett's test (Snedecor and Cochran, 1980).

Homogeneous groups of stocks and sites were identified by the Tukey's multiple range test (Montgomery, 1991) for both L and LW increase.

Regressions of length and weight increase on chlorophyll a content, current speed and phytoplankton availability for the different stocks and sites were calculated and compared by the analysis of covariance (ANCOVA) (Snedecor and Cochran, 1980).

Regression models, MANOVA, ANCOVA, Tukey's tests and Bartlett's tests were performed by using Statgraphics and Lotus computer software (Manugistics, 1993).

3. Results

3. I. Water flow, phytoplankton content and phytoplankton availability

From Table 1, which shows the average current speeds through the mussel ropes at Sites 1, 2 and 3, the raft from the inner Site 1 experienced a 40% lower water flow compared to the other two sites.

The mean chlorophyll a content of the seawater was similar for Sites 1 and 3, but lower ($3.1 \mu\text{g l}^{-1}$) at Site 2. Therefore, phytoplankton availability varies among sites, with a maximum at Site 3 and markedly lower values for Sites 1 and 2 (Table 1).

3.2. Temperature and salinity

Mean water temperature ranged between 13°C and 17°C to a depth of 10 m. The averages for the entire experimental period were very similar at all three sites studied, ranging between 15°C and 16°C . Salinity varied between 34 and 35 p.p.t. during the experimental period and no differences among sites were detected.

3.3. Seed growth

Fig. 2 and Fig. 3 show the total growth in length and live weight for the three mussel stocks at the three sites of cultivation.

Influence of seed stock

Seed source had a marked effect upon mussel growth rate. Mussel seed from collector ropes (Stock C) had the highest mean length and weight increases of all three sites (27.3 ± 3.5 mm and 8.05 ± 1.78 g for the average of the three rafts). The other two stocks had similar growth increments for L and LW (stock A: 20.7 ± 1.3 mm and 2.82 ± 0.48 g; stock B: 17.5 ± 1.7 mm and 3.35 ± 0.78 g). These differences in both length and weight increase among stocks were highly significant ($P < 0.001$; Table 2), and due to the differences between stock C (collector rope) and the other two stocks (stocks A and B) collected from the rocky shore (Tukey's test $P < 0.01$; Table 3)) which were not significantly different to each other.

Influence of cultivation site

Cultivation site also affected seed growth and a trend towards higher growth at the most oceanic site was demonstrated. The highest L and LW increases corresponded to Site 3, with average values for the three stocks of 24.8 ± 5.6 mm and 5.99 ± 3.58 g. Sites 2 (21.5 ± 2.6 mm, 4.26 ± 1.90 g) and 1 (21.3 ± 2.6 mm, 4.03 ± 1.78 g) showed markedly lower

values. These differences in growth rate due to cultivation sites were highly significant ($P < 0.001$) and resulted from major differences between site 3 (the most oceanic site) and the other two sites (Tukey's test $P < 0.01$; Table 3)) which were not significantly different to each other.

Stock explains 69% and site 15% of the total variation in length growth; stock explains 87% and site 9% of the total variation for increase in live weight.

Interactions

The interaction between the main factors (stock and site) was not statistically significant (Table 2).

3.4. Condition index

At the beginning of the experiment, the mean CI was highest for stock C and lowest for stock A, while there were no significant differences between sites (Fig. 4). Three months after the start of the experiment, the mean CI had increased for all stocks and the differences among them were not significant ($P > 0.05$). With regard to cultivation sites, mean CI increased for Sites 1 and 3 and remained constant at Site 2.

The MANOVA detected highly significant differences among sites ($P < 0.001$) due to differences between Site 2 and the others (Tukey's test $P < 0.05$).

3.5. Mortality

Average mortality was lower than 2% for all stock and site combinations and no significant differences were detected.

3.6. Relationship between growth rate and phytoplankton availability

The following regression equations are obtained if growth rates for each seed source are pooled:

$$\Delta L = 0.142 \pm 0.040 + 0.699 \pm 0.240 F; (r = 0.59, n = 18, P < 0.01) \quad (1)$$

$$\Delta W = e^{(-3.864 \pm 0.498 + 4.968 \pm 2.971F)}; (r = 0.39, n = 18, P = 0.11) \quad (2)$$

where daily length increase (ΔL) and daily live weight increase (ΔW) are expressed as a function of phytoplankton availability (F) .

However, pooling data from groups of mussels showing very different growth rates can hide the relevance of F as the driving factor to explain ΔL and ΔW , also causing low correlation coefficients and lack of statistical significance in (2). Therefore, more meaningful regression models are obtained when mussels from stock C (collector ropes) are considered separately from stocks A and B (rocky shore):

Stock C

$$\Delta L = 0.141 \pm 0.024 + 1.001 \pm 0.144F; (r = 0.97, n = 6, P < 0.01) \quad (3)$$

$$\Delta W = e^{(-3.316 \pm 0.067 + 5.394 \pm 0.450F)}; (r = 0.99, n=6, P < 0.001) \quad (4)$$

Stocks A and B

$$\Delta L = 0.137 \pm 0.0299 + 0.589 \pm 0.171 F; (r = 0.73, n = 12, P < 0.01) \quad (5)$$

$$\Delta W = e^{(-4.144 \pm 0.246 + 4.755 \pm 1.468F)}; (r = 0.72, n = 12, P < 0.01) \quad (6)$$

where F explains 95 and 98% of the variance of the L and W increase respectively for stock C mussels, and 52 and 54% for mussels from stocks A and B.

Further information can be obtained if chlorophyll a content (Ch) and current speed (V) values, rather than phytoplankton availability (F), are introduced in the model:

Stock C

$$\Delta L = - 0.073 \pm 0.002 + 0.057 \pm 0.000 \text{ Ch} + 0.0596 \pm 0.000 \text{ V}; \\ (r = 0.99, n = 6, P < 0.001) \quad (7)$$

$$\Delta W = e^{(-4.510 \pm 0.145 + 0.287 \pm 0.026\text{Ch} + 0.366 \pm 0.027\text{V})}; \\ (r = 0.99, n = 6, P < 0.01) \quad (8)$$

Stocks A and B

$$\Delta L = 0.026 \pm 0.018 \text{ Ch} + 0.042 \pm 0.013 \text{ V}; \\ (r = 0.73, n = 12, P = 0.06) \quad (9)$$

$$\Delta W = e^{(-5.244 \pm 0.600 + 0.274 \pm 0.107\text{Ch} + 0.309 \pm 0.112\text{V})}; \\ (r = 0.73, n = 12, P < 0.05) \quad (10)$$

In these equations V explains 66% of the total variability in (7), 79% in (8), 30% in (9) and 32% in (10), while Ch explains 33%, 19%, 23% and 21% of the variability, respectively.

4. Discussion

The growth rates measured in the present study, ranging from 6.3 to 11.1 mm month⁻¹, were slightly higher than those reported by Perez Camacho and Roman (1979) for mussels from the Ria de Arousa, comparable or occasionally higher than those reported by Page and Hubbard (1987) for Californian *Mytilus edulis*, which were considered by the authors as 'among the highest rates recorded world-wide', and markedly higher than those measured for this species by Rodhouse et al. (1984) in Ireland. These growth rates are also greater than the growth rates of mussels cultured in intertidal areas, for either *M. edulis* (Theisen, 1968; Lube & 1969; Rodhouse et al., 1984; Hager, 1970) or the Mediterranean *Mytilus galloprovincialis* (Ceccherelli and Rossi, 1984).

The present study agrees with Perez Camacho and Roman (1979) regarding the dependence of mussel growth rate on the cultivation site within the Ria de Arousa. Widdows et al. (1984), studying two populations of *M. edulis* in Great Britain, concluded that the physiological differences between the populations, which were the main reasons underlying growth differences, were the result of different environmental conditions rather than genetic factors. Dickie et al. (1984) and Mallet and Carver (1989) also found that site was the main factor affecting growth in Canadian mussels, and Page and Hubbard (1987) detected a close correlation between chlorophyll a and growth in *M. edulis*. In our case, chlorophyll a content of the water is a secondary factor explaining growth variation compared to the major effect of the actual phytoplankton availability, as determined by the current speed, and agrees with the estimations from Frechette et al. (1989). This result is better understood when the high load of mussels suspended from a raft (up to 100 tonnes) and their high filtration activity are considered. As a consequence, seawater passing through the ropes of a cultivation raft loses an average of 60% of its phytoplankton content as measured by chlorophyll a concentration (Perez Camacho et al., 1991). Therefore, the actual amount of phytoplankton available to the mussels depends on the water flow through the mussel ropes, i.e. the current speed. The F parameter, which considers amount of

phytoplankton, current speed and number of ropes, was highly correlated to the growth rate, in particular for mussels from stock C where 3 values exceeded 95%.

However, mussels cultured at Sites 1 and 2 showed similar growth rates despite a 18% higher phytoplankton availability in Site 2. This result may be explained by a higher detritic organic matter content at the inner most site (Site 1) (Oceanology group IIM, unpublished data), not detected by the chlorophyll a analysis, but a potential food source for the mussel (Rodhouse et al., 1984; Perez Camacho et al., 1991). This can also explain the lower condition index for Site 2 mussels, although the occurrence of a spawning event at this site cannot be discarded.

The influence of stock on the growth of mussels has been discussed in previous studies. Mallet et al. (1987) stated that stock differences were important in explaining the variation in shell growth, but site alone accounted for most of the variation in tissue growth. Several authors claimed that stock does not appreciably affect growth rate, while it does affect mortality (Wallace, 1980; Dickie et al., 1984; Page and Hubbard, 1987; Mallet and Carver, 1989). This conclusion was also reached by Fuentes et al. (1992) studying Galician mussels from the Ria de Arousa, although conclusions from this last study should be carefully considered since experimental conditions varied considerably from the traditional rope culture and prevented an appropriate food availability, which lead to growth rates ten times lower than those normally measured in this area. In contrast, the present investigation provides experimental evidence of an important effect of stock on mussel growth rate. This effect may originate from genetic differences among stocks, but a closer view of the present results makes this hypothesis unlikely. First, growth rates of stocks A and B are very similar, despite being located at 100 km from each other. Second, mussels from stock C had much higher growth rates than mussels from stock B, even though stock C was obtained from collector ropes suspended from a raft located only 2 km away from the stock B sampling site. Genetic differences might account for the growth differences obtained only if settlement on either collector ropes or the rocky shore was also affected by genetic factors.

A simpler and more feasible explanation accounting for the differences among stocks might be the physiological adaptations of the mussels to the prevailing environmental conditions at the place of origin, as well as different physiological states at the

beginning of the experiment. Feeding conditions can modify the physiological traits of mussels (Bayne et al., 1984, Bayne et al., 1987) and the differences may persist for several months, as suggested in transplantation experiments (Widdows et al., 1984). Mussels from collector ropes and from the rocky shore experience very different feeding conditions, as the former are permanently immersed and the latter are subject to tidal cycles. Food availability is thus higher for mussels from collector ropes (stock C), and, as a consequence, they show higher condition indices. The condition index of the mussel can be interpreted as an index of growth (Smaal and Stralen, 1990).

The higher growth rate of mussel seed obtained from collector ropes found in the present study, compared to seed obtained from the rocky shore, may dramatically improve current cultivation methods for this species, since it could reduce both the duration of this cultivation stage by more than 30% and the total duration of the mussel culture to commercial size by at least 10%. Besides, the abundance of cultured mussels in the Galician Rias and the natural trend of mussel larvae to settle in large numbers on cultivation ropes (Roman and Perez Camacho, 1982) and experimental rope collectors (Fuentes and Molaes, 1994) makes it feasible to obtain seed from collector ropes suspended from the cultivation rafts themselves. This would enable the cultivator to exert a greater control on the production process and also to avoid the risk of personal injury whilst collecting mussel seed from some of the most exposed shores of the Atlantic coast.

Acknowledgements

The authors are grateful to the Organization de Productores de Mejillon de Galicia (OPMAR), and especially to Olimpio and Jose Castelo, and to Benito, Juan Manuel, Belermo and Victor Dios Otero, for their help in the field work on the rafts. They also thank Guillermo Diaz de l Rio for current speed determinations, Juan Blanco for CTD's data, and Candida Castro, Concepcion Fernandez, Isabel Fernandez and Lourdes Nieto for their technical assistance. The English manuscript was edited by Deborah Morgans. This study was partially funded by the Conselleria de Pesca of the Xunta de Galicia.

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Fig. 1. Map of the Ria de Arousa {Galicia, NW Spain), where the experiment was conducted. Location of the Ria in the Galician coast (top left, above) within the Spanish Atlantic coast (top left, lower) is indicated. See cultivation Sites (1, 2 and 3) and the places of origin of the seed stocks (A, B and C). Polygons indicate areas of mussel cultivation rafts.

Fig. 2. Shell length increase (mm) for mussel seed from the different stocks (A,B,C) cultivated for 3 months at the different Sites (1,2,3).

Fig. 3. Live weight increase (g) for mussel seed from the different stocks (A,B,C) cultivated for 3 months at the different Sites (1,2,3).

Fig. 4. Condition indices at the beginning and at the end of the experiment for mussel seed from the different stocks (A,B,C) cultivated for 3 months at the different Sites (1,2,3).

Table 1

Current speed (V, cm sm⁻¹, range in parentheses), water flow (f m³ per raft h⁻¹), chlorophyll a content (Cl, µg l⁻¹, mean f standard deviation) and phytoplankton availability (F, g chlorophyll a h⁻¹ mussel per rope) at the three experimental sites.

	Site 1	Site 2	Site 3
V	1.81 (0, 12.45)	2.99 (0, 23.50)	3.04 (0, 30.70)
f	14.661	24 219	24 624
Cl	4.2 ± 1.22	3.1 ± 1.88	4.4 ± 1.61
F	0.123	0.150	0.216

Table 2

Multifactor analysis of variance (MANOVA) for shell length and live weight increase of mussels from the three stocks cultivated at the three sites

Source of variation	SS	d.f.	MS	Sig. level
Length increase				
Site	0.0806	2	0.0403	< 0.001
Stock	0.3652	2	0.1826	< 0.001

Interaction	0.0375	4	0.0094	NS
Residual	0.0453	9	0.0050	
Total	0.5286	17		
Live weight increase				
Site	0.4208	2	0.2104	< 0.001
Stock	4.3187	2	2.1593	< 0.001
Interaction	0.0729	4	0.0182	NS
Residual	0.1407	9	0.0156	
Total	4.9530	17		

Table 3

Tukey's multiple range test for shell length and live weight increase of mussels from the three stocks cultivated at the three sites

Stock	Site	LS Mean	Contrast	Sig. Level
Length increase				
A		2.991	A-B	NS
B		3.036	A-C	**
C		3.299	B-C	**
	1	3.017	1-2	NS
	2	3.064	1-3	**
	3	3.245	2-3	**
Live weight increase				
A		1.044	A-B	NS
B		1.184	A-C	**
C		2.146	B-C	**
	1	1.323	1-2	NS
	2	1.380	1-3	**
	3	1.672	2-3	**

* * P < 0.01; NS, not significant.