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Response to artificial selection and realized heritability estimate for shell height in the Chilean oyster *Ostrea chilensis*

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Abstract

Individual (mass) selection for shell height was carried out in the Chilean oyster, *Ostrea chilensis*. The selection was based on the shell height of oysters, 30 months old, which came from the natural population of the Quempillen River Estuary (Southern Chile). Divergent selection was applied, three selected groups were chosen to mass spawn (high, mean selected, and low). Offspring from the mean selected and low groups were obtained. A statistically significant difference ($p < 0.05$) was detected in shell height at 30 months between the offspring of the mean selected and low selected group, suggesting the presence of additive gene effects for growth rate. The realized heritability estimate ($h^2 = 0.34 \pm 0.12$) for a decrease in the shell height, indicates that selective breeding on this trait can be effective in *Ostrea chilensis*.

Keywords : Selection, heritability, *Ostrea chilensis*, growth.

Influence de la sélection artificielle de l'huître du Chili, Ostrea chilensis, *en fonction de la hauteur de la coquille, sur les individus de la première génération.*

Résumé

La sélection individuelle en fonction de la hauteur de la coquille a été étudiée sur l'huître du Chili *Ostrea chilensis*. La sélection a été effectuée sur des huîtres âgées de 30 mois qui provenaient de populations naturelles de l'estuaire du Quempillen (Sud Chili). Une sélection divergente a été appliquée, trois groupes d'huîtres sélectionnées en fonction de la hauteur (hautes, moyennes, petites) ont été choisis pour la reproduction. Des individus de première génération, issus des pontes des groupes d'huîtres moyennes et petites, ont été obtenus. Une différence significative ($p < 0,05$) en ce qui concerne la hauteur a été mise en évidence entre les descendants âgés de 30 mois issus des groupes d'huîtres moyennes et petites, laissant supposer les effets d'un gène supplémentaire sur le taux de croissance. L'estimation du taux d'hérédité potentielle ($h^2 = 0,34 \pm 0,12$) pour une diminution de la hauteur de la coquille indique que l'élevage basé sur cette sélection peut être effectué sur *Ostrea chilensis*.

Mots-clés : Sélection, hérédité, *Ostrea chilensis*, croissance.

INTRODUCTION

The culture of the Chilean oyster, *Ostrea chilensis* Philippi, 1845, has been very important during the last decade; however, it has become restricted by the lack of a reliable source of natural seed (CORFO, 1981; Lepez, 1983). Also, the very slow annual growth of this species due to the short growing season (from November to February) has become an important restriction for the oyster farmers. Oysters are marketable at a shell height of about 50 mm, which is attained after three to four years of growth in culture (Winter *et al.*, 1984).

Oyster culture in Chile, which is 40 years old, has not utilized genetic manipulation. This has been due to the dependence on natural reproduction for seed stock and the consequent lack of control over the complete life cycle, which is a requirement for any type of directed genetic change (Hershberger *et al.*, 1984). The potential role that improved organisms could play in aquaculture development has been addressed in several reports (Wilkins, 1975; Moav, 1976; Newkirk, 1980, 1983; Newkirk and Haley, 1982; Gjedrem, 1983; Gjerde, 1986; Sandifer, 1988). An increase in efficiency of production by a reduction in generation time and/or improvement of survival will contribute to reducing the negative effects of short growing seasons in high latitudes (Newkirk, 1980). One of the exciting and potentially rewarding areas of long-term aquaculture research and development is the replacement of the "wild" animals currently being reared with genetically improved organisms better suited to the culture environment and the consumer's preferences.

Traits of economic importance from a production viewpoint are usually determined by a large number of genes each having a small contribution and under considerable environmental influence (Newkirk, 1980; Lande, 1982). Thus these traits are best studied by means of quantitative genetic techniques, which reduce the most important features of complex genetical systems to relatively few variables that can be estimated from phenotypic measurements (Falconer, 1981; Lande, 1982).

High variation in growth rate of individual Chilean oysters was found within populations (DiSalvo and Martinez, 1985; Toro and Varela, 1988; Toro and Newkirk, 1990). But there have been no studies on genetic parameters or artificial selection for growth in *O. chilensis*. The fact that there is phenotypic variation in growth rate gives hope that there might be significant genetic differences which could be exploited in a selection program to produce faster growing oysters. A selection program requires the estimation of a basic genetic parameter such as heritability for important economic traits. High heritability (h^2) estimates for a particular trait indicate that a large portion of the trait is associated with additive gene action, and that genetic progress can therefore be achieved through mass selection (Newkirk *et al.*, 1977).

The present study was designed to evaluate any difference in the shell height of oysters, *O. chilensis*, that arise as a response to a selection differential applied to the parental population. Also, the realized heritability for shell height was estimated and sources of environmental variation for this trait were analyzed.

MATERIAL AND METHODS

The parental stock was taken from the 1985 natural spatfall from the wild population of *O. chilensis* at the Quempillen River Estuary, located at Chiloé Island, Southern Chile (41°51'S; 73°46'W). The spat collected in 1985 on shells of the mussel *Aulacomya ater* (Molina), were grown out on long lines in the estuary (Toro and Varela, 1988). After a growing period of 14 months a random sample of 4 500 oysters was transferred to subtidal trays and reared at low densities (150 oysters/m^2) in the Quempillén River Estuary.

Parents were selected based on their shell height at 30 months of age (July, 1987). Divergent selection was applied in order to obtain low selected as well as high selected lines. The largest (10 %) was considered "high" selected, the (10%) around the mean was the "mean selected", the smallest (10%) formed the "low" selected group. Individual oysters from the selected groups were labelled by gluing a number on the shell (Newkirk and Haley, 1982, 1983), randomized and grown out on the trays (Toro and Varela, 1988) in low densities at the Quempillén estuary.

On September 22, 1987 each group was placed in a 80 l tank and warmed from ambient water temperature (around 12°C) to 18°C over a 4-week period. The salinity throughout this study was 27 to 28‰; corresponding to the average salinity for the months of September to November in the estuary. The salinity variation in the Quempillén estuary thoughout the year, ranges between 14 and 30‰ with an average of 24‰, while the temperature fluctuates between 8 and 22°C with an annual average of 13°C. The water was filtered through a sand filter and, before heating, passed through a 5 µm filter bag. The water was changed daily and a mixture of *Isochrysis galbana*, clone T-iso, and *Chaetoceros gracilis* Schütt, was added twice a day at an estimated concentration of 500 000 cell/ml.

At the time of water change all groups were checked for larvae. Large quantities of fertilized eggs were aborted after three weeks in the mean selected group. One week later, eggs in the initial stages of divisions were aborted in the High group but no spat were produced from the high selected group. Aborted eggs were not detected from the low group. Clean shells of *A. ater* were placed in the tanks after four weeks of conditioning to collect the spat. Larval settlement from the mean selected and low group occurred within 24 hours. Each collector was labelled to indicate the date and the source of the spat.

The spat obtained were maintained in the laboratory, in a common rearing tank, for 6 days and then transferred, as strings of shells (1.2 m long), to a long line in the Quempillen estuary. Each string was divided into three depths. The spat density was controlled leaving around 20 on every *A. ater* shell. Shell height data were collected monthly during the first 6 months of growth and then every 6 months on

50 juveniles taken at random from each level in every experimental string.

Temperature and salinity measurements in the estuary were taken every 10 days, over the first 6 months (followed by monthly monitoring) during high tide at two different depths in the water column (0.3 m and 2 m from the surface).

In order to find out whether the environmental source of variance followed any pattern, analysis of variance (ANOVA) was carried out with shell height data from offspring of the selected groups as the dependent variable. Two dummy independent variables were established, $string_i$ (where $i = 1, 2, 3, 4$) and $level_j$ (where $j = 1, 2, 3$).

Relative variability analysis was used to test whether there were differences in the coefficients of variation (C.V.) of shell height at two different levels (0.2 and 1.2 m) for the offspring of the mean selected (Y) and low selected (X) group. In this analysis the ratio of variances, $\sigma^2(\ln Y)/\sigma^2(\ln X)$, is tested against the F distribution (Lewontin, 1966; Lande, 1977). This was carried out by transforming the original shell height data to their natural logarithms.

ANOVA was performed with the offspring shell height data to determine whether there was a difference in the size between the offspring of the low selected parents and the offspring of the mean selected parents. Normality of the distributions was tested with a *t*-test (Sokal and Rohlf, 1981; p. 174). The t_s values ranged from 0.57 to 1.93 indicating that the observed skewness (g_1) in all groups did not deviate significantly ($p > 0.05$) from 0 (normally distributed).

In an ANOVA to test the effect of group, shell height was considered as the dependent variable. The dummy variable $level_j$ (where $j = 1, 2$ and 3) was introduced in the model to control for the environmental variance associated with differences between levels in the water column. The independent variable, group, referred to whether the individuals came from the mean selected or the low selected group. Since level is the variable to be controlled for, it is considered first in the ANOVA model.

The realized heritability estimate followed Kirpichnikov (1981). The standar error for the realized heritability was calculated using the formula of Prout (1962).

RESULTS

Offspring (spat) from the mean selected and low selected group were collected on December 21. The mean and standard deviation of shell height for the offspring from the 4 replicate strings and the 3 different depths (0.2, 0.7 and 1.2 m) is summarized in *table* 1 for the mean selected and low selected group.

The temperature and salinity over the first 6 months of growth out, showed a decreasing trend toward the winter months (*fig.* 1) and are in accordance with the results reported by Toro and Winter (1983); however, the decrease is more drastic in the upper layer of the water column, the salinity and temperature decrease from around $30^{o}/_{oo}$ and 16°C to $22^{o}/_{oo}$ and 11°C, respectively, while at the bottom both variables are more constant over the same period.

Results of the string effect and the string depth interaction effect were non-significant ($p > 0.05$) for the 5 ages analyzed (3, 6, 12, 24 and 30 months) in both the mean selected and low selected groups. However, the depth effect was significant ($p < 0.05$) in the offspring of both groups after 3 months.

The relative variability analysis showed no significant differences at $\alpha = 0.05$, indicating that even if significant effect in shell height was found between levels, the coefficient of variation is not affected.

Despite the significant depth effect, the overall means for shell height over the 5 age groups analyzed showed a higher value in the mean selected group. The mean selected group had mean values (in mm) of 10.09 ± 1.06 S.D., 16.52 ± 1.94, 31.40 ± 3.17, 43.90 ± 4.76 and 50.00 ± 5.88 mm for 3, 6, 12, 24 and 30 months of age, respectively; while the mean values for the low group were 9.65 ± 1.07, 15.81 ± 2.92, 27.80 ± 3.38, 38.69 ± 4.41 and 44.79 ± 6.40 for the same ages, respectively.

There was a significant difference in the shell height of the offspring from the mean selected and low selected groups at all analyzed ages ($p < 0.01$); the shell height data for the first two months of age showed no significant difference between the offspring of the two groups ($p > 0.01$). The difference between the two lines was apparent from the 3rd month of age (*table* 2).

In a study aimed at determining realized heritability one has to measure the trait in the individuals of two different generations at one and the same age, and to somehow normalize and standardize the conditions of their cultivation and growth, particularly factors such as the density and culture conditions. The realized heritability estimate for shell height growth rate in *O. chilensis*, based on the selection response was calculated using the selection response (R) over the selection differential (S). The difference of the trait (shell height, in mm) of the mean selected and low lines at 30 months of age, divided by the total selection differential gave an estimated realized heritability of $h^2 = 0.34 \pm 0.12$ (*table* 3).

Table 1. – Mean shell height and standard deviation (SD) of the offspring of the mean selected (M) and low (L) selected group grown at different depths in the water column (0.2, 0.7 and 1.2 m). Four replicate strings (each n = 50).

		STRINGS							
		1		2		3		4	
Age in months	Depth	mean	± SD	mean	± SD	mean	± SD	mean	± SD
(M)	0.2	9.97	0.95	9.70	0.78	10.40	0.97	9.73	0.84
3	0.7	10.20	1.18	10.23	1.10	9.70	1.37	10.13	1.06
	1.2	10.30	1.01	10.43	0.94	10.33	0.88	9.93	1.40
(L)	0.2	9.43	1.15	9.17	1.01	9.70	0.96	9.67	1.23
3	0.7	9.40	0.94	9.67	0.94	9.77	0.80	9.60	1.06
	1.2	9.97	1.16	10.02	0.68	10.13	1.21	9.40	1.42
(M)	0.2	16.27	1.60	16.20	1.82	16.23	2.33	15.93	1.84
6	0.7	16.30	1.53	17.30	1.60	16.40	2.29	16.33	2.32
	1.2	17.57	1.65	16.01	2.09	16.77	2.19	16.90	1.69
(L)	0.2	15.20	2.06	15.80	1.97	15.53	2.02	15.40	1.74
6	0.7	15.30	1.79	15.23	1.47	15.60	2.19	16.47	1.75
	1.2	16.97	1.77	16.03	1.45	16.13	2.19	16.05	2.20
(M)	0.2	31.33	3.00	31.23	3.06	30.73	3.37	31.27	3.51
12	0.7	31.30	2.95	30.87	3.46	31.67	2.83	30.77	2.85
	1.2	32.27	3.27	31.27	3.40	31.90	3.31	32.20	2.96
(L)	0.2	27.40	2.83	26.86	3.31	27.87	3.09	27.96	2.86
12	0.7	27.90	3.57	27.10	2.88	27.53	3.85	27.46	2.99
	1.2	28.53	3.65	28.27	3.25	27.97	2.33	28.80	2.70
(M)	0.2	43.77	4.72	42.77	3.06	44.13	3.55	42.80	3.75
24	0.7	43.07	3.95	43.03	4.42	44.63	2.32	43.67	4.47
	1.2	44.53	4.78	44.20	3.25	44.70	2.94	45.46	4.78
(L)	0.2	37.53	4.32	38.13	4.04	37.83	4.45	39.10	3.85
24	0.7	38.50	3.92	38.23	5.27	38.57	4.12	38.10	3.81
	1.2	39.13	3.91	39.63	3.97	39.76	3.51	39.70	3.90
(M)	0.2	49.77	6.92	48.77	5.64	50.13	4.02	48.80	6.88
30	0.7	49.07	5.36	49.03	6.03	50.63	5.41	49.67	5.17
	1.2	50.53	7.54	50.20	5.32	50.70	3.82	51.46	4.23
(L)	0.2	43.63	7.44	44.23	6.37	43.93	7.63	45.20	4.65
30	0.7	44.60	6.41	44.03	7.62	44.67	4.22	44.20	4.97
	1.2	45.23	5.23	45.73	4.77	45.87	6.34	45.80	5.07

Table 2. – Analysis of variance for shell height. Mean squares (MS) and degrees of freedom (d.f.) of depth (3 different depths), group (mean selected and low) and interaction effects at 3, 6, 12, 24 and 30 months of age.

		Age (months)				
		3	6	12	24	30
Source	d.f.	MS	MS	MS	MS	MS
Depth	2	3.62*	15.94*	26.95**	66.43**	645.03**
Group	1	16.68**	45.51**	1162.80**	2444.01**	2351.11**
Depth Group*	2	0.07	1.28	0.14	0.05	0.10
Error	354	1.12	3.65	5.10	9.34	15.95

* $p < 0.05$, ** $p < 0.01$.

DISCUSSION

A significant response to selection in the offspring of the low selected group was detected from the 3rd month of growth. The difference in the growth between the two lines in *O. chilensis* indicates that there is a significant genetic component which can be used in a selection programme. The significant difference was observed at the 6th and the 12th months of age even though the selection was based on the shell height at 30 months. The response, then, can be measured in a correlated trait (Toro

Table 3. – Determination of realized heritability (h^2) ± SD for shell height at 30 months in *Ostrea chilensis*.

Mean shell height and SD at 30 months (mm)				Heritability
Shell height of parents		Shell height of offspring		$h^2 = \frac{R}{S}$
Low	Control	Low	Control	
34.43 ± 1.80	49.42 ± 1.60	44.79 ± 6.40	49.87 ± 5.88	$\frac{49.87 - 44.79}{49.42 - 34.43} = 0.34 \pm 0.12$
$n = 49$	$n = 48$	$n = 600$	$n = 600$	

and Newkirk, 1990); nevertheless, this indicates that genetic variation for growth rate must be present for the selected trait (shell height at 30 months of age). This result should be taken as an indication of potential genetic gain with some caution because of two factors.

The first is the lack of offspring from the high selected group, thus, only the downwards response has been observed. Experiments in which selection was conducted in both directions, that is, for an increase and for a decrease of the trait, have shown asymmetry; the rate of response in two directions was not equal (Falconer, 1976; Moav and Wohlfarth, 1976; Bondari, 1983, 1986; Campton and Gall, 1988). In general, traits that are related to natural fitness give little response to selection for an increase and more response for a decrease of the trait (Falconer, 1981; Kirpichnikov, 1981). Growth rate has been found to be highly correlated with fertility in oysters (Walne, 1964, 1979) and this is a component of fitness (Falconer, 1981). Thus, the selection toward decreasing the growth rate should give higher response than the selection to increase it. However, Bondari (1983, 1986) reports that divergent selection yields a better response for increasing than for decreasing the body weight in Channel catfish (*Ictalurus punctatus*). Furthermore, much progress has been made in livestock to increase traits such as growth rate.

The second factor is the presence of maternal effects that are a source of environmental variance, and might be of some importance in *O. chilensis* because this species broods the larvae until they are ready to set. Maternal effects can cause initial asymmetry in the response to selection. Bigger oysters might produce larger eggs which result in better growth and survival. However, because fecundity has been found to be correlated with size, this might counter-balance the effect of maternal size on the individual eggs. Ryman (1972) reported that large females of the guppy-fish (*Lebistes reticulatus*) had large broods which was correlated with a decrease in the mean growth rate of individual progeny. A similar effect is reported by Campton and Gall (1988) with the mosquitofish (*Gambusia affinis*). Fortunately, maternal effects generally decrease with ageing of offspring (Monteiro and Falconer, 1966; Falconer, 1981;

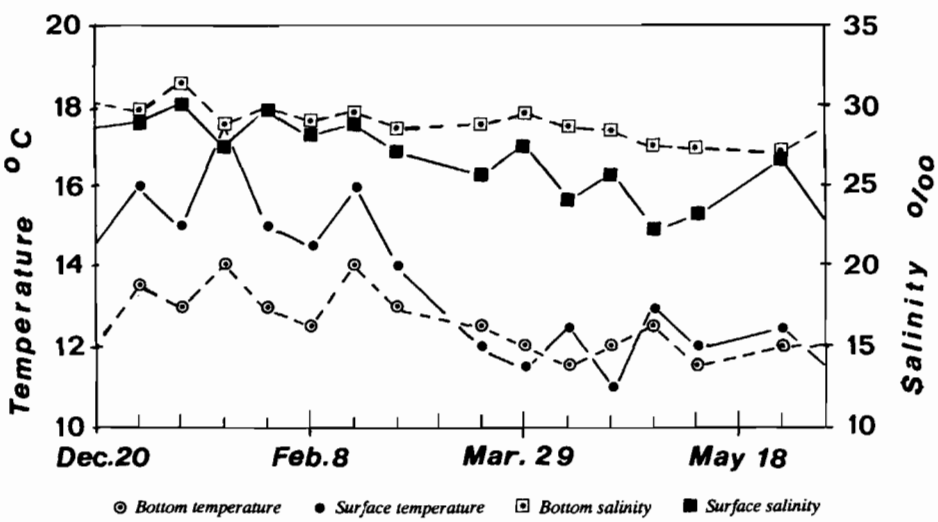

Figure 1. – Temperature (°C) and salinity (°/$_{oo}$) measurements taken every 10 days from December 1987 to June 1988, during high tide for the surface (0.3 m) and the bottom (2 m) in the Quempillén River Estuary.

Kirpichnikov, 1981; Campton and Gall, 1988) and therefore are usually only important in the earlier stages of development (Arthur, 1984). In the present study the difference between the two groups became apparent only after the third month of age suggesting that maternal effects were of little importance.

There are many causes of asymmetrical response to selection: random genetic drift, selection differential, maternal effects, inbreeding depression, genetic asymmetry, major gene effects, scalar asymmetry (Falconer, 1981). There is no way of predicting if these were likely to have been important in this case. Nevertheless, the results of this selection experiment suggest that growth rate in *O. chilensis*, as predicted by the response obtained in this study, can be increased by artificial selection.

With the improved technology at present available in oyster hatcheries, the potential for producing genetically improved seed has become very important. One of the reasons that artificial selection and genetic parameter estimates have not been carried out in *O. chilensis*, is that the control of the whole life cycle in this species has been accomplished only recently with the implementation of hatcheries (DiSalvo *et al.*, 1983; 1984). Another difficulty in working with this species is that the female broods the larvae until they are ready for set, making certain types of controlled crosses difficult. However, since the larval stage of other species of oysters (*Crassostrea virginica*, *Ostrea edulis*, *Crassostrea gigas*) constitutes the most delicate and the most expensive phase of production for the hatcheries, *O. chilensis* has a potential advantage over those species. The advantages and disadvantages of long larval periods have been discussed from the ecological point of view by Vance (1973), Strathmann (1974), Crisp (1978) and Grassle and Grassle (1978).

Mass spawning was used with all the parental selected groups. The most difficult problem with the mass spawning methods used in these experiments is the lack of control on the number of parents contributing to a group. Although about 40 to 50 oysters were in each spawning tank, there was certainly variability in the spawning success. Thus, the sampling of parental genes is unknown, especially the male contribution. This was discussed in Newkirk (1986) where success in controlled mating was reported with *O. edulis*.

The mass spawning design was utilized in order to ensure the success in obtaining offspring from the selected groups. Some constraints with the limited hatchery facilities also indicated that mass spawning should be used. Newkirk (1986) reported the success of the pair mating design. However, this approach needs more hatchery facilities and had not yet been experienced with *O. chilensis*. Nonetheless, no spat were obtained from the high selected groups, although large amounts of recently fertilized eggs were aborted (around 8.10^5 eggs in this experiment and more than 11.10^5 in a separate experiment without controls) between the third and fourth week of conditioning. Several attempts to rear the eggs were made using the technique described for *O. edulis* (Jayarahband *et al.*, 1988), but without success. The cultures became contaminated with protozoa and after a few days the embryos dissolved. Early release of eggs in *O. edulis* has been reported by Loosanoff and Davis (1963) and Walne (1979) when the animals were under stress. This may also be the case in the present experiment; these oysters were disturbed daily to clean them and refill the containers with new water and algal food. No other indication of the cause of this early release of eggs can be suggested.

Although when using mass spawning one does not know how many individuals contribute genes to the offspring obtained, some estimation of the female contribution can be made by keeping track of the number in each brood of eyed larvae. Fecundity in *O. chilensis* ranges between 10 000 to 115 00 larvae per season with an average of 60 000 (Solis, 1967; Padilla *et al.*, 1969; Gleisner, 1981; Lepez, 1983; Winter *et al.*, 1983). Thus, by the amount of larvae released, more than 950 000 in the mean selected group, one could assume that at least 12 females had contributed larvae. In the low selected group, more than 700 000 larvae were released, so at least 8 different females released larvae. These estimations of the female contribution in the mean selected (12 females) and low (8 females) group may be an underestimation because in this species some of the eyed larvae released will be set as soon as 5 minutes (DiSalvo *et al.*, 1983).

The present experimental design allows us to collect quantitative information on the growth pattern and growth rate. Such information can be utilized to improve the accuracy of selection techniques and to maximize genetic gain. The depth in the water column in which the offspring were grown in the estuary was found to have a small but significant effect ($p < 0.05$) on their growth rate, thus the depth effect makes a significant contribution to the total environmental variation affecting the growth rate of the juveniles in the Quempillen Estuary. This is of considerable importance for the control of breeding programmes and genetical studies of *O. chilensis* at this site.

Although several factors could have contributed to the reduced growth (varying degrees of competition from different communities of fouling organisms at each level, temperature, salinity, oxygen saturation, seston content, etc.), and no cause-and-effect relationship can be identified, it may be worth noting that salinity gradient towards the fall months was increasing (*fig.* 1). Several authors have shown the negative effect of low salinity on the growth rate of bivalves (Davis, 1958; Davis and Ansell, 1962; Castagna and Chanley, 1973, Innes and Haley, 1977; Bøhle, 1972; Navarro, 1988). Newkirk *et al.* (1977) report that

there are genetic differences between oyster populations (*C. virginica*), for tolerance to low salinity. The selected parents in the present study came from a spatfall of a natural population at Quempillen estuary, where the salinities on the bottom fluctuate between 25 to 31°/$_{oo}$ (Toro and Winter, 1983). Thus the offspring came from a population apparently adapted to high salinities.

The present study is the first report on artificial selection for growth rate in *O. chilensis*. The significant response to selection and the relatively high realized heritability, indicates the presence of an additive genetic component in the trait, shell height at 30 months of age, suggesting that effective progress could be made through selection for faster growing oysters.

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