

# Growth of the pearl oyster *Pteria sterna* under different thermic and feeding conditions

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**ABSTRACT:** The combined influence of temperature and food concentration on *Pteria sterna* growth was determined, using 3 temperatures and 3 food concentrations. During 15 wk, the shell was measured weekly along the axis of maximum growth. The 2 higher growth rates (4.8 and 4.2 mm mo<sup>-1</sup>) were obtained with the highest ration, at 30 and 25 °C, while with the lowest food concentration growth was not temperature dependent. By the response surface analysis, a synergistic effect of temperature and food concentration on growth was found. Food concentration, but not temperature, had an important influence on condition index (ash free meat dry weight/shell dry weight × 100).

## INTRODUCTION

Restocking natural beds with natural or laboratory-produced juveniles has been suggested by various authors if a natural resource is exploited above the level of natural recruitment (e.g. Espina 1989 for *Tivela stultorum* and Hahn 1989 and literature therein for *Haliotis* spp., *Trochus niloticus* and *Strombus gigas*). This is the case for the pearl oyster *Pteria sterna* Gould, which is considered an endangered species in Mexico (Baqueiro 1987), in spite of a permanent ban on harvesting enacted by the Mexican government in 1939. Studies in proximity of some of the known natural beds of this mollusk have revealed that spat settlement is scarce (Bückle-Ramírez et al. 1992) due to the low numbers of larvae competent for settling in the plankton of those areas (Serrano-Guzmán 1992). For this reason, it has been proposed that laboratory-produced juveniles be used for restocking purposes as well as for aquacultural activities (Baqueiro 1987, Araya-Nuñez 1988, Bückle-Ramírez et al. 1992).

The juveniles used for these purposes should be in good physiological condition, in order to withstand the stress of the change from the laboratory to the natural environment. Further, acclimation should be done when water conditions are close to optimum for body growth, in order to ameliorate the possibility of survival to subsequent adverse conditions. The literature

available on *Pteria sterna* growth is based on field observations (Aguirre-Hinojosa 1987, Singh-Cabanillas 1990, Bückle-Ramírez et al. 1992) showing high growth rates during summer months, with notably lower values in winter. However, since food availability is at its lowest during colder months (Serrano-Guzmán 1992), these observations are not sufficient to determine the relative importance of temperature and food availability for *P. sterna* growth.

The aim of the present work was to study in the laboratory how these factors influence both shell and body growth. It also considers the limits within which their interactions have the best effects in laboratory conditions and conversely if, outside these limits, either factor might cease to be effective, or even have a negative effect on body growth, as evaluated from the organisms' condition index.

## MATERIALS AND METHODS

Specimens of between 5 and 10 mm shell height were collected on July 4–5, 1990, from spat collectors after 2 mo of immersion in 'La Gringa' cove located in Bahía de Los Angeles (113° W, 28° N). They were separated from the substrate with a scalpel, held for 24 to 48 h in an ice box at ca 27 °C, and subsequently transported to the laboratory where they were maintained in

an aquarium of capacity 45 l and fed ad libitum twice daily for 8 wk with *Chaetoceros* sp. (clone CH-X-1 from the collection of Centro de Investigación Científica y de Educación Superior de Ensenada; Voltolina-Lobina et al. 1991). This was chosen in preference to other microalgae used in aquaculture for the following reasons: (1) Good results have been obtained with this strain for various filter-feeders such as the mussels *Mytilus edulis*, *M. galloprovincialis* and *Modiolus capax*, the oyster *Crassostrea gigas* and several penaeid and brine shrimps (Voltolina-Lobina et al. 1991). (2) Unicellular *Chaetoceros* spp., such as the one used, are common and abundant worldwide in coastal areas, and several strains have been isolated in Baja California coastal waters (López-Elías pers. comm.). (3) The genus *Chaetoceros* has been mentioned as one of the common constituents of Bahía de Los Angeles phytoplankton communities (Ayala-Sánchez & Michel 1980).

*Chaetoceros* sp. was also used for the experimental phase, using different semi-continuous cultures with f/2 medium (Guillard & Ryther 1962) at daily dilution rates of between 50 and 33 % for the 18 and 400 l cultures respectively.

The 9 experimental aquaria were divided into groups of 3, each kept in a constant temperature water bath at either 20, 25 or 30 °C. Each aquarium was subdivided with acrylic walls into 3 sections, to allow the experiment to be run in triplicate. Each aquarium received all 3 diets, 1 per section predetermined at random.

The size ration and the feeding frequency were based on observations made during the acclimation period. It was decided that the maximum ration allowable was, for a twice daily feeding routine, ca 8 % of the meat dry weight, after which pseudofaeces began to be produced. The other rations were 50 and 25 % of the highest allowable value, resulting in 8, 4 and 2 % of meat dry weight.

After verifying the normality of the size distribution by chi-square goodness of fit test ( $\alpha = 0.05$ ), 9 juveniles were stocked into each section of the experimental aquaria. By 2-way ANOVA, it was also shown that size distributions were homogeneous ( $\alpha = 0.05$ ).

Temperature was measured twice daily and dissolved oxygen, pH and salinity every 24 h. These were kept approximately constant by frequent water changes. The aquaria were cleaned every second day with UV-sterilized filtered sea water.

The food rations were calculated on the basis of the equation  $DW = 0.00869 \text{ AMG}$ , where  $DW$  = oyster dry weight (mg); and  $\text{AMG}$  = radius along the axis of maximum growth (cm) (Bückle-Ramírez et al. 1992). The volume of culture necessary was determined by the microalgal density, their average dry weight (30  $\mu\text{g}$  per  $10^6$  cells; López-Elías 1990) and the calculated dry

weight of *Pteris sterna*. Care was taken to start each feeding with the same microalgal density (20, 40 and  $80 \times 10^3$  cells  $\text{ml}^{-1}$ ) by modifying the volume of water added to the aquarium.

The experiment lasted 15 wk (31 August to 14 December). The initial dry weight and condition index were obtained using 20 organisms from the original population. The experimental design consisted of 3 temperatures (20, 25 and 30 °C) simulating the thermal range of the original environment between May and November (Aguirre-Hinojosa 1987) and 3 food rations (2, 4 and 8 % of meat dry weight). AMG was measured once a week (mm). Growth with different treatments was compared with analysis of covariance and Student-Newman-Keuls (SNK) tests (Zar 1974, Steel & Torrie 1988).

After 15 wk, the organisms were starved for 24 h, measured and weighed (total wet weight; wet and dry weights of shell; wet, dry and ash weights of the meat) by drying the shell and the meat at 60 °C for 2 and 24 h respectively, and ashing the meat at 590 °C for 12 h. The condition index was estimated using the equation  $\text{DWM}/\text{WDS} \times 100$  where  $\text{DWM}$  = ash-free meat dry weight and  $\text{WDS}$  = shell dry weight (Lucas & Beninger 1985).

Response surface analysis is a technique used to describe in a bi-dimensional form the relative importance of the experimental factors and their interactions on a given biological process (Alderdice 1972, Lough & Gonor 1973a, b). It consists of generating a function, usually quadratic since this generally allows an optimum interpretive response (Schnute & McKinnell 1984), which relates experimental conditions (factors) to biological responses. The technique involves the use of stepwise multiple regression (Zar 1974) to obtain the best model and the percentage of the total variance explained by the model itself.

In order to compare growth in the laboratory and in the field, 110 organisms of the original batch were kept in 2 Nestier cages at the collection site.

## RESULTS

Temperature, salinity, oxygen saturation and pH did not vary widely (Table 1), and it was therefore concluded that their effects on growth were similar and that they did not affect survival, which was high in all cases (Table 2).

Since no differences were found among the 3 replicates of each treatments (1-way ANOVA;  $\alpha = 0.05$ ) the data were pooled for statistical analysis. The initial mean size ranged between 11.3 and 12.3 mm (overall mean 11.7 mm), and the final mean size of each group depended on the treatments. The growth curves of

Table 1. Physical and chemical variables (mean and SD) for the 9 treatments. Temperature was measured twice a day, other variables once a day

Temperature: Food:	20 °C			25 °C			30 °C		
	2 %	4 %	8 %	2 %	4 %	8 %	2 %	4 %	8 %
Temperature (°C)	19.84 (0.38)	19.86 (0.36)	19.86 (0.36)	25.00 (0.50)	25.01 (0.51)	24.96 (0.46)	29.85 (0.82)	29.78 (0.87)	29.81 (0.83)
Salinity (‰)	32.45 (1.93)	32.45 (1.99)	32.60 (1.85)	33.06 (2.23)	32.94 (2.18)	33.31 (2.03)	32.15 (2.78)	32.15 (2.73)	32.33 (2.69)
Oxygen (%)	95.02 (3.20)	95.04 (2.63)	94.77 (2.96)	95.42 (2.56)	95.12 (2.52)	94.55 (3.07)	93.46 (3.57)	92.81 (4.13)	91.64 (6.49)
pH	8.03 (0.12)	8.00 (0.13)	7.97 (0.12)	8.07 (0.19)	8.02 (0.20)	8.02 (0.20)	8.06 (0.14)	8.02 (0.15)	7.95 (0.18)

Table 2. *Pteria sterna*. General data (mean and SD) from the growth experiment. Superscript letters indicate growth rates not significantly different from one another (obtained by covariance analysis and Student-Newman-Keuls test,  $\alpha = 0.05$ ). AMG: radius along axis of maximum growth. Total no. of oysters was 28

Temperature: Food:	20 °C			25 °C			30 °C		
	2 %	4 %	8 %	2 %	4 %	8 %	2 %	4 %	8 %
Mortality (%)	0.0	3.7	0.0	3.7	3.7	0.0	7.4	3.7	0.0
Initial AMG (mm)	11.7 (1.9)	11.9 (2.0)	11.8 (1.6)	11.3 (1.6)	11.4 (2.1)	11.4 (2.4)	11.7 (2.0)	11.9 (1.9)	12.3 (2.1)
Final AMG (mm)	17.4 (2.8)	20.2 (3.6)	21.0 (3.8)	17.6 (2.2)	22.9 (3.8)	26.2 (4.9)	17.8 (2.7)	23.9 (2.7)	29.2 (3.9)
Growth (mm wk <sup>-1</sup> )	0.4 <sup>a</sup>	0.6 <sup>ab</sup>	0.6 <sup>bc</sup>	0.4 <sup>ab</sup>	0.8 <sup>c</sup>	1.0 <sup>d</sup>	0.4 <sup>ab</sup>	0.8 <sup>c</sup>	1.1 <sup>d</sup>
Weight									
Meat (%)	21.8 (1.8)	26.0 (4.2)	28.6 (3.1)	20.6 (2.1)	26.3 (1.4)	33.8 (2.1)	24.0 (3.7)	25.2 (2.0)	31.5 (1.4)
Dry meat (%)	20.1 (1.8)	21.2 (4.2)	23.4 (3.1)	18.0 (2.1)	16.6 (1.4)	21.1 (2.1)	15.6 (3.7)	17.5 (2.0)	19.9 (1.4)
Organic weight (%)	81.5 (3.4)	82.6 (6.5)	86.1 (5.0)	77.8 (1.6)	78.4 (2.8)	84.8 (4.0)	73.7 (5.7)	79.8 (2.2)	85.1 (1.7)
Shell (%)	73.5 (0.1)	69.6 (5.2)	66.7 (4.0)	72.1 (5.0)	68.0 (3.5)	60.9 (5.6)	71.6 (4.1)	70.0 (6.7)	63.5 (3.5)
Dry shell (%)	78.2 (1.9)	74.0 (2.6)	71.4 (3.2)	79.5 (3.9)	73.7 (4.6)	66.2 (3.7)	76.0 (3.8)	74.8 (12.7)	68.5 (2.9)
Conversion rate (%)	13.5	23.5	19.7	5.0	22.0	27.8	0.7	15.3	21.0
Condition Index	52.5 (12.3)	70.9 (21.1)	100.4 (32.0)	43.3 (9.6)	60.4 (10.3)	118.2 (35.2)	39.6 (9.7)	58.0 (13.7)	97.2 (18.8)

juveniles throughout the experiment were nearly linear (Fig. 1). Covariance analysis showed a slope difference among treatments related to both variables and to their interaction, with significantly higher growth for the treatments at 30 °C, 8 % and 25 °C, 8 % and lower values for all other treatments of high temperature and high or intermediate food concentrations (Table 2). The lowest growth rates were those of 2 % ration, irrespective of temperature. The growth for the 30 °C and 8 % treatment was 2.75 times higher than the 2 % treatments.

In order to determine the linear and quadratic effects of temperature ( $T$ ,  $T^2$ ), and food ration ( $A$ ,  $A^2$ ) and of

their interaction ( $T \times A$ ), multiple regression analysis was applied to growth rates ( $C$ , mm wk<sup>-1</sup>). Statistical analysis (Table 3) shows that all variables were significantly important ( $p < 0.05$ ) with the exception of the linear effect of food, and that the most important effects were those of the interaction and of the square power of food. The polynomial obtained:

$$C = -1.4736 + 0.1401 T - 0.0031 T^2 - 0.0142 A^2 + 0.0090 T \times A$$

explained 93 % of the observed variance. The response surface clearly shows the interaction between food concentration and temperature (Fig. 2).

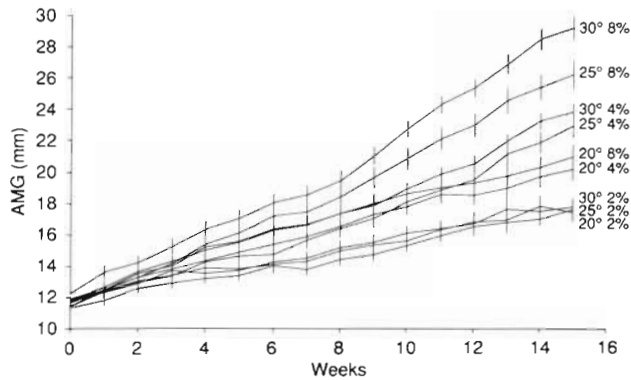


Fig. 1. *Pteria sterna*. Growth under different temperatures (20, 25, 30 °C) and feeding regimes (2, 4 and 8 % of meat dry weight). AMG: radius (mm, mean  $\pm$  1 SE) along axis of maximum growth

Meat content varied between 20.6 to 31.5 % of the total wet weight and the organic content of meat from 73.7 to 86.1 %; both showed a tendency to increase in a direct relation to food concentration and an inverse relation to temperature (Table 2). At low food concentrations, the daily rate of food conversion was inversely related to temperature, and for the highest concentration, the maximum was at 25 °C.

The model for the Condition Index (CI), that included the effects of all variables (linear, quadratic and interaction), explained only ca 60 % of the total vari-

ance observed, with low probabilities (Table 4). The model was simplified by stepwise multiple regression to include only  $A + T^2$ ; it explained the same percentage of the total variance but with high probabilities. The resulting polynomial was:

$$CI = 36.862 - 0.020 T^2 + 10.089 A$$

The response surface shows the strong effect of food concentration (Fig. 3).

*Pteria sterna* juveniles kept in Bahia de Los Angeles grew better than the lab-cultured organisms. From an initial mean ( $\pm$  SD) AMG in July of  $7.35 \pm 1.78$  mm, the specimens grew to  $30.7 \pm 4.60$  mm by November and  $38.7 \pm 8.25$  mm by January 1991. Condition indexes were similar, but more variable under natural conditions than in the laboratory:  $81.09 \pm 17.47$  and  $106.95 \pm 41.96$  mm, for November and January respectively.

## DISCUSSION

The response surfaces (Figs. 2 & 3) indicate that our experimental conditions did not reach an optimum for growth and condition index. Temperature had a direct influence on growth with high food concentrations, but its increase caused a lower condition index, probably because of the higher energy requirement of the organisms at high temperatures (Bayne 1973, Widdows 1978), and not through its effect on filtration rate. This

Table 3. *Pteria sterna*. Square model evaluation of growth ( $\text{mm wk}^{-1}$ ). T: temperature, A: food ration

Independent variable	Coefficient	SE	R <sup>2</sup> (%)	t	p
$T \times A$	0.008	0.001	79.40	6.215	0.000
$A^2$	-0.017	0.004	88.96	-4.709	0.000
$T^2$	-0.003	0.001	91.48	-2.766	0.012
T	0.144	0.057	93.02	2.531	0.019
A	0.050	0.050	93.03	1.016	0.321
Constant	-1.633	0.714		-2.288	0.033

Table 4. *Pteria sterna*. Square and simplified model evaluation of condition index (CI). Variables as in Table 3

Independent variable	Coefficient	SE	R <sup>2</sup> (%)	t	p
Square model					
A	3.107	5.024	58.45	0.619	0.537
$T^2$	-0.178	0.115	59.81	-1.545	0.127
$T \times A$	0.174	0.132	59.97	1.321	0.188
T	7.099	5.790	60.08	1.226	0.221
$A^2$	0.255	0.368	59.99	0.692	0.490
Constant	-34.199	72.395		-0.472	0.637
Simplified model					
A	10.089	0.541	58.45	18.643	0.000
$T^2$	-0.020	0.007	59.81	-2.998	0.003
Constant	36.862	5.106		7.220	0.000

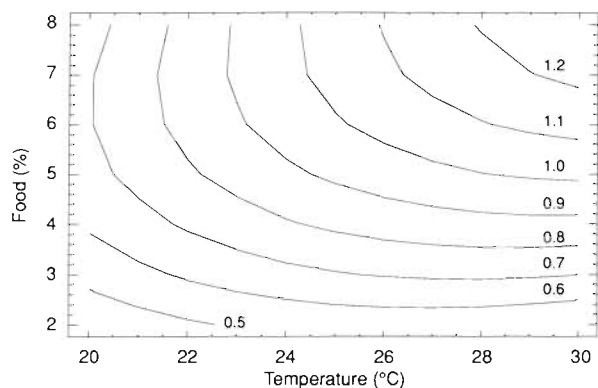


Fig. 2. *Pteria sterna*. Growth response ( $\text{mm wk}^{-1}$ , measured along axis of maximum growth) for temperatures 20 to 30 °C and food ratios 2 to 8 % of meat dry weight

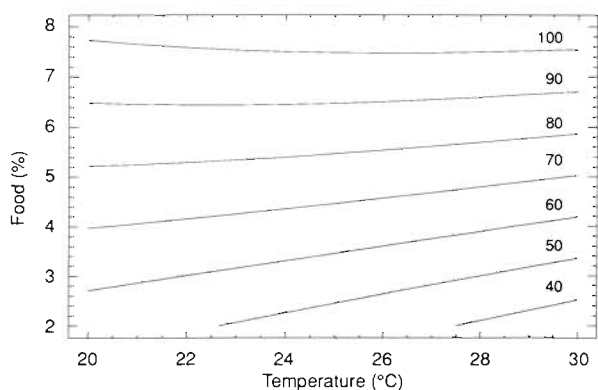


Fig. 3. *Pteria sterna*. Response under simplified model of condition index (ash free meat dry weight/shell dry weight  $\times 100$ ) for temperatures 20 to 30 °C and food ratios 2 to 8 % of meat dry weight

was found in preliminary experiments to be in the range  $4.9$  to  $12.4 \text{ l d}^{-1} \text{ ind.}^{-1}$ . Furthermore, for the temperatures and food concentrations used in the present work, the filtration rate was unaffected by both variables. This agrees only in part with many reports in the literature that the feeding activity of filter-feeders is not affected significantly by temperature changes, at least within a specific thermal range (e.g. Wilbur & Owen 1964, Widdows 1978, Bayne 1985) but is directly related to particle concentrations.

In *Mytilus edulis* changes in meat and shell increments may be produced by seasonal variations (Hilbish 1986). These changes consist of an uncoupling between tissue and shell growth, which results in a staged growth: tissue increases first and shell later. In *Crassostrea virginica* such cycles were detected with a duration of between 5 and 7 wk (Ukeles et al. 1984). These examples indicate that the growth of bivalve molluscs may be heterogeneous even under laboratory conditions. In our case, even if meat increase was not

measured at regular intervals, such cycles do not appear to exist since shell growth was practically linear in all treatments (Fig. 1).

The growth rates of many bivalves are optimal at temperatures below the highest used in this study. For instance, Pandya (1976) reported that the pearl oyster *Pinctada fucata* has a higher growth rate at temperatures between 19 and 28 °C than at 28 to 32 °C, and Chellam (1978) reports similar results for that species. In another example, the growth rates of cultured *Tapes semidecussata*, *T. decussata* and *Mercenaria mercenaria* increased between 10 and 25 °C and decreased at higher temperatures (Laing et al. 1987). *Pteria sterna* showed a somewhat similar trend as the former examples with respect to meat dry weight, CI and food conversion rate, which were all higher for the intermediate temperature treatment.

The differences in growth between juveniles kept in the laboratory and those reared in Bahía de Los Angeles could be due to different factors. First the diet used in the laboratory was much more abundant than that available under natural conditions, since each individual was fed 1200, 2400 and 4800  $\text{mg d}^{-1}$ , equivalent to 840, 1680 and 3660  $\text{mg}$  of organic matter respectively (López-Eliás 1990). The few data available on the concentration of total seston and of its organic content in the area, for the period July to November 1986, indicate that the above variables range from maxima of 16.0 and 12.3 to minimum values of 1.4 and 0.3  $\text{mg m}^{-3}$ , respectively (Serrano-Guzmán 1992). Taking into consideration that the highest filtration rate we measured for our organisms was only  $12.4 \text{ l d}^{-1}$  and that this was not affected by temperature and food concentration, the quantity of seston each individual might have retained in Bahía de Los Angeles was no higher than  $2 \text{ mg d}^{-1}$ , of which only  $1.75 \text{ mg}$  was organic matter.

Second, laboratory rations were consumed in a very short time (each half ration usually lasted about 3 h, after which the number of cells in suspension was negligible) and we never noticed the presence of pseudofaeces. Such a high concentration might have affected the efficiency of food assimilation, which is usually low when food is available in excess. In nature, food is not only available (though in lower quantities) on a continuous basis; it is also much more varied, which might also account for the high growth rates of those oysters.

The growth rates observed in the laboratory and in the field are comparable to those of other field studies on *Pteria sterna*, which range from  $2.8$  to  $7.5 \text{ mm mo}^{-1}$  (Aguirre-Hinojosa 1987, Araya-Nuñez 1988, Singh-Cabanillas 1990) compared to 1.6 (lowest) and 4.8 (highest)  $\text{mm mo}^{-1}$  in the laboratory, and 5.8 and 5.2  $\text{mm mo}^{-1}$  in the field, the latter considering 2 extra winter months (December 1990 and January 1991) when temperatures and food availability are lowest

(Serrano-Guzmán 1992). A review of the few data available in the literature seems to point to the fact that the wide range of the growth rates reported is due to seasonal differences as well as to the initial size of the organisms. Since growth is strongly influenced by the interaction of food and temperature, it would seem logical to expect that field experiments initiated during the period of spring-summer would result in better growth rates than those carried out in late summer, since more food was available and because temperature was close to the optimum for this species.

### CONCLUSIONS

The analysis of the response surfaces indicates that optimum conditions were not reached in the ranges of our experimental conditions and that growth is mainly under the influence of the interaction between food availability and temperature. The condition index depends principally on food, with a slight negative quadratic effect of the other variable which may explain the higher condition index noted for the lowest temperature.

The fact that optimum conditions were not reached is probably due to food quality or variety, given the tendency of the growth response surface to increase towards temperature and food concentration values which are outside the normal ranges found in the natural environment, in which growth rates are generally higher than those noted in this work.

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