

Growth of Tray-Cultured Oysters (*Crassostrea virginica* Gmelin) in Chesapeake Bay

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ABSTRACT

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Growth rates of cultchless oysters raised in floating trays in a shallow tidal creek of Chesapeake Bay were examined. Growth of individuals from a population of *Crassostrea virginica* selectively inbred for fast growth was compared to growth of spat produced from the native parent stock. The oysters grew only during part of the year (July through October) and growth did not occur during the spring season even though the environmental conditions (temperature, salinity) were similar to those during the active growing season. All animals grew quickly in floating raft culture (10-15 mm/month) during the growing periods and at a relatively constant rate with respect to length. The selected animals exhibited significantly faster growth during both growth seasons examined in the study. The rates measured during the growing season suggest that oysters could be grown to market size in 6 months of continuous active growth.

INTRODUCTION

Growth rates of commercially important bivalve species such as *Crassostrea virginica* (Gmelin) have been studied for many years (Korringa, 1976; King, 1977; Brown, 1988; Brown and Hartwick, 1988a,b). Unfortunately, direct comparison of these databases is difficult due to the differences in cultivation and measurement methods. It has been clearly established, however, that oysters have optimal temperature, salinity and food requirements for a high growth rate. In addition, the basic findings of many studies suggest that growth rate may have a significant genetic component. Several of these studies have shown that bivalve growth rate is positively correlated with multilocus heterozygosity (Singh and Zouros, 1978, 1981; Zouros et al., 1980; Koehn and Shumway, 1982;

Gaffney and Scott, 1984; Zouros and Foltz, 1984; Foltz and Chatry, 1986), while others have identified specific genetic loci which are associated with variation in size or growth rate (Singh and Zouros, 1978; Zouros et al., 1980; Adamkewicz et al., 1984a,b; Gaffney and Scott, 1984; Zouros and Foltz, 1984; Foltz and Chatry, 1986).

Intrinsic physiological factors also affect growth. A molluscan insulin-like peptide (MIP) has been isolated from *Lymnea stagnalis* which demonstrates significant growth-promoting properties (Ebberink and Joosse, 1985; Smit et al., 1988). Morse (1984) showed that bovine growth hormone or insulin increased the average growth rate of juvenile abalone (*Haliotis rufescens*) and eliminated much of the size variation exhibited by untreated groups. This suggests that physiological variation in molluscan growth rate (i.e. the variation *not* due to environmental factors) may be due as much to variation in growth stimulating peptides as to genetic variation at metabolically important loci or multi-locus heterozygosity.

In order to address this issue, the growth of native Chesapeake Bay oysters was compared to a population of oysters which had been selectively bred for fast growth over eighteen generations by a local oyster grower. The selected population exhibited different allele frequencies when compared to native Chesapeake Bay animals. This finding prompted an experimental growout which compared the growth rates of animals derived from the selected population with those of animals produced from native Chesapeake Bay stock and showed that the selected population grew significantly faster than the native population.

MATERIALS AND METHODS

Native animals (*Crassostrea virginica*) were collected from a natural oyster bar (Tolley Point Bar, Chesapeake Bay, Maryland) in the vicinity of the bar from which the selected population originated. Gametes were stripped from individuals of each population and two crosses were made between a limited number males (2) and females (3) of each population. This produced two groups of animals, native and selected, representing those populations. The animals were stripped of their gametes in mid-June and combined with eggs or sperm from other broodstock animals of the same population and sex. Sperm and eggs were then mixed to produce larvae. These two groups of larvae were raised in 500-l tanks to the eyed stage. The larvae were maintained in filtered seawater (50 μm clearance; 12–14 ‰ salinity) which was changed twice daily. They were induced to undergo metamorphosis on 29 June (native) and 1 July 1987 (selected) by the addition of epinephrine (10^{-4} M, final concentration) to the seawater resulting in metamorphosis without attachment to a substrate (Coon et al., 1985). During the first 4 weeks after metamorphosis, the spat were maintained in floating trays on water tables in a flow-through filtered downwelling seawater system at the hatchery. The trays consisted of wooden

frames (approximately 20 cm high) with nitex screen (E.A. Case, Andover, NJ) bottoms. The size of the nitex screen was changed as the animals grew larger. In general, mesh sizes were kept to about one-half of the average animal length (e.g. 4 mm animals were maintained on 2 mm mesh).

When the animals reached 8–10 mm in length, they were moved to a local tidal creek and maintained there in floating rafts. The rafts consisted of plastic trays (approximately 60×75×15 cm) with polypropylene mesh liners to contain the oysters within the tray, and a styrofoam float that covered the entire tray. The oysters and trays were rinsed once a week and the mesh was changed as the animals grew larger. Initial mesh size was 3 mm (1/8 in) and final mesh size was 19 mm (3/4 in). Subsequently, the mesh was changed whenever it became fouled.

For growth studies, 100 animals from each population were placed in a single tray which was divided into quadrants using 6 mm (1/4 in) galvanized wire mesh. Each quadrant held 50 of the selected or native animals. This assured that experimental animals were grown in equivalent environments and were kept separate throughout the duration of the growout. Length was measured from the umbo to the ventral shell margin (shell height; Galtsoff, 1964) in 20 of the 50 animals in each quadrant biweekly during the growing periods and total individual weight was measured at 45 and 111 days of age. The animals were cleaned of fouling organisms and blotted dry before weighting. In order to minimize non-random sampling, the animals in a single quadrant were pushed together, then approximately 20 were picked up en masse using a scoop. All the animals removed were measured and returned to their quadrant.

Starch gel electrophoresis was performed on 20 adult animals from native populations taken from Chesapeake Bay, and on 20 broodstock animals of the selected population (not on the animals produced from the crosses). The parents of the populations under study were not examined. Standard electrophoretic techniques were used (Schaal and Anderson, 1974). The animals were scored for malate dehydrogenase (E.C. 1.1.1.37; MDH), glucose phosphate isomerase (E.C. 5.3.1.9; GPI), leucine aminopeptidase (E.C. 3.4.11; LAP), and 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44; GPD). Allele frequencies of *Lap-1*, *Lap-2*, *Mdh-1*, *Mdh-2*, *Gpi*, and *Gpd* and heterozygosity levels were calculated and reported as described by Buroker (1983).

In January of 1988, 250 animals were measured for length, total weight and wet tissue weight in order to examine the relationship between length and biomass in these animals. The animals were collected from the trays, cleaned of fouling organisms and mud, measured for length and weighed whole. The oysters were then removed from the shells, blotted, and weighed. Care was taken to collect all of the animal tissue including any parts of the adductor muscle that remained attached to the shell.

All chemical reagents were purchased from Sigma (St. Louis, MO). All statistical treatments were performed according to Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

The selected animals showed less variation at three loci (Table 1). Of the five loci tested, only *Lap-2* was monomorphic in the native population but the remaining loci showed significant polymorphism. In the selected group, however, *Lap-2* was monomorphic and *Lap-1*, *Gpi*, and *Gpd* were nearly monomorphic. *Mdh* allele frequencies were similar in both groups. The selected population appears to be less genetically variable, possibly due to inbreeding. The 20 native animals were the offspring of natural spawning in Chesapeake Bay and presumably of mixed parentage, but since the relationship of the animals in neither population can be firmly established, the differences in the observed genetic heterogeneity cannot be addressed definitively.

Buroker has surveyed genetic variation in *Crassostrea virginica* in Chesapeake Bay (1984) and along the Atlantic coast (1983). He identified several polymorphic loci in the Chesapeake populations: aminopeptidase-1, adenylate kinase, aspartate aminotransferase-2, esterase-1 and -3, leucine aminopeptidase-1 and -2, mannose phosphate isomerase, 6-phosphogluconate dehydrogenase, phosphoglucose isomerase, and phosphoglucomutase-1 and -2. Four of the five loci tested in the present study were polymorphic in both sets of animals of this study, but alleles of three of those four loci were nearly fixed in the selected animals. The allele frequencies in the native animals were similar to those found by Buroker (1984).

Larval sizes at metamorphosis were not significantly different between populations. Fig. 1 shows the increase in shell length of juvenile oysters during the

TABLE 1

Allele frequencies for native and selected oyster populations

Locus		Native	Selected
<i>Lap-1</i>	100	0.57	0.97
	98	0.43	0.03
	H	0.40	0.05
<i>Gpi</i>	100	0.58	0.97
	94	0.42	0.03
	H	0.70	0.05
<i>Gpd</i>	106	0.01	0
	100	0.76	0.97
	97	0.23	0.03
	H	0.31	0.05

Tissues (gill) from 20 animals were subjected to starch gel electrophoresis. Relative mobilities were scored by assigning 100 to the most common allele and the other alleles were scored with respect to that position. H = pooled heterozygote frequency.

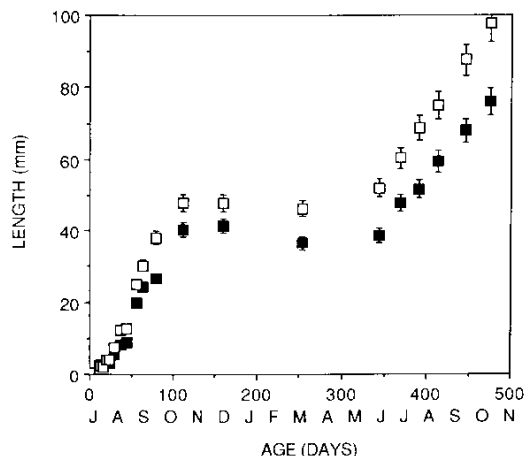


Fig. 1. Increase in shell length with age. Shell length was measured beginning with newly metamorphosed spat (1 July 1987) and followed through 480 days of age (October 1988) for native (closed squares) and selected (open squares) oysters. There were distinct growing seasons (July–October) in which growth rates were high (see Table 2) and a long non-growing season (November–May). Mean lengths of groups of the same population were not different and were pooled. Error bars represent the standard errors of the mean.

study period (July 1987–November 1988). Growth occurred in two distinct time periods during the study: a first growing season (days 0–120) and a second growing season (days 350–480). The season coincided with July through October of 1987 and 1988, respectively. During August, September and October, the animals exhibited the highest growth rates (Fig. 1). The mean water temperature in the tidal creek ranged from 35°C in July and August to 3°C in the winter and salinity ranged from 10–6‰ throughout the year. The animals stopped growing in early November when the water temperature declined to an average of 10°C. Interestingly, the animals did not begin to increase shell length again until late June the following year even though the environmental conditions (temperature, salinity) in early April appeared to be equivalent to those of the first growing season. Mortality was <2% in both populations throughout the growout period.

Throughout both growing seasons, the increase in animal length was constant for both groups and significantly different between populations. A least-squares regression analysis was used to estimate growth rates during the active growing seasons. Increase in shell length/unit time was linear with a high coefficient of determination ($r^2=0.95$). During the first growing season, the selected group exhibited an average growth rate of 15.15 mm/month while the native group grew at a significantly slower rate of 11.85 mm/month (Table 2). For the second growing season, the growth rate of the selected population (10.17

TABLE 2

Regression analysis for the first and second year growth in native and selected oysters

Growing season	Population	Growth rate (mm/month)	s.e.	r^2
First (July–October 1987)	Selected	15.15	0.63	0.965
	Native	11.85*	0.54	0.958
Second (June–October 1988)	Selected	10.17	0.21	0.997
	Native	8.22*	0.57	0.963

*The selected animals grew faster than their native counterparts (*t*-test, $P < 0.05$).

Seasonal growth rates with the standard error of the estimate (s.e.) were calculated from linear regression of shell length on time for the first growing season (days 0–120) and second growing season (days 340–480). The slope of the calculated linear regression represents the mean growth rate over each season.

mm/month) was significantly greater than for that of the native group (8.22 mm/month).

The cultchless induction of the animals using epinephrine and subsequent rearing in floating trays resulted in a nearly uniform teardrop shape of both groups of animals, and length was a good index of growth. Due to this uniform shape, a statistically precise correlation could be calculated between length and whole individual weight (shell + animal), wet weight (animal tissue alone) or dry weight (dried animal tissue). The relationship between length (L) and wet weight (W) of the animals in this study was described by the following equation: $W = (6.79 \times 10^{-5}) L^{2.49}$; with a high coefficient of determination ($r^2 = 0.94$). Of course, the relationship between wet weight and length may change during the year as the animals undergo changes in tissue weight due to seasonal physiological processes such as spawning. Nevertheless, the relationship indicates that the linear increase throughout the growing season was correlated with an expected logarithmic increase in tissue weight. Furthermore, it shows that length is a good index of growth in these animals. This is most likely a result of the relatively uniform shape of the animals grown under the conditions described above. It is not surprising either that the second year growth (based on shell-length measurements) was slower since 1 mm of length increase in those larger animals represented significantly more growth in total biomass. The seasonal and interpopulation variation in the length/biomass relationship is currently being studied. Although faster growing animals are thin-shelled, it was noted that the shell of the slower-growing native oyster was also thin. Shell thickness may not be determined as much genetically as environmentally by frequent disturbance by predators and other secondary factors.

The mean water temperature declined from 35°C in late July and early Au-

gust to approximately 10°C in early November when the animals stopped growing. Since oysters are poikilotherms, one would have expected to see a significant alteration in growth with a temperature decline, yet, the animals continued to exhibit a constant growth throughout this temperature range. Malouf and Breese (1977) reported that growth in the Pacific oyster, *Crassostrea gigas*, was related more to suspended particulate organic material (food) than to water temperature. In the present study, water quality at the study site was measured during the second growing season and although total seston load and total chlorophyll *a* declined with water temperature, the 3–10- μ m-size fractionated chlorophyll *a* content, a measure of food available to the oysters, remained constant (Paynter, unpublished data).

The rates reported here reflect extremely fast growth in oysters. On the average, in 4 months the selected animals grew 48 mm during the first growing season. The native animals also grew well to an average length of 40 mm in the same time period. Beaven (1950) noted growth rates of animals set on oyster shell in floating rafts in various regions of Chesapeake Bay. In that study the greatest growth occurred in animals raised in Pocomoke Sound (a few grew to 76 mm [3 in.; market size] in a single growing season). The animals raised in the present study reached a maximum of 63.5 mm. According to Beaven, Chesapeake Bay animals growing in the natural benthic habitat will take three growing seasons to reach 76 mm. Beaven also pointed out the large differences in average growth rates of different oyster bars in Chesapeake Bay. These differences could be the result of differences in habitat or genetic differences or, most likely, both. Mallet and Haley (1983) produced spat by making between and within crosses of various populations of *Crassostrea virginica* in New Brunswick, Canada. On the average, all of the animals in that study, whether produced from a pure population cross or a between population cross, grew approximately 5 to 10 times more slowly than the animals reported in this study. Ogle et al. (1978) reported maximal growth rates of 4.2 mm/month in oysters (*Crassostrea virginica*) raised in suspended culture off the coast of the Gulf of Mexico. Nell and Holliday (1988) reported growth rates in *Saccostrea commercialis* and *Crassostrea gigas* with respect to salinity. They suggested that optimum salinities for growth of those two species were above 20‰. The animals in the present study were raised in a mean salinity of 14‰, well below those levels. Brown (1988) and Brown and Hartwick (1988a,b) examined growth rates of *C. gigas* in floating rafts or suspended bags in the Pacific Northwest region. During the periods of highest growth in their studies, those oysters grew approximately 10 mm/month, equal to the average of the slowest growing group in this study, and those animals were growing in salinities twice that of the oysters reported on here.

Although the selected animals grew faster than the native animals during both growing seasons, we cannot definitively conclude that the selected animals represent a faster growing "strain" of oysters because the parentage of

these populations was limited. A larger "mass cross" might allow a better estimation of the genetic contribution to growth differences between selected and native animals. We have identified several genetic loci which are associated with growth rate in these animals (Paynter and Chapman, in prep.) and plan to test the heritability of these associations by producing genotype-specific crosses. In conclusion, oysters grown in floating trays in a tidal creek of Chesapeake Bay exhibited extremely high rates of growth (10-15 mm/month) during discreet growing periods during the year (late June through October). Animals grew to market size (76 mm; 3 in) in 15 months although they reached 48 mm in 4 months during the first growing season. If spat could be produced earlier in the year, it may be possible to grow large numbers of oysters from spat to market size in 6 months.

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