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Site-specific Growth Rates of Oysters in Chesapeake Bay and Impact of Disease

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ABSTRACT
Extremely rapid growth has been observed in raft cultured oysters in the Chesapeake Bay. In previous studies, animals raised in floating rafts in a shallow tidal creek grew at an average rate of 15 mm/month during their first growing season. Although genetic influences on growth were demonstrated in that study, the very high growth rates in all of the animals suggested that the environment was exceptionally conducive to oyster growth. In an effort to learn more about the relationships between genetics, environment, and growth, we began a series of oyster growth experiments in which animals of the same cohort were raised in different regions of the Chesapeake Bay. Water quality and oyster growth were measured biweekly at these sites. Water qualities measured included salinity, pH, temperature, size-fractionated chlorophyll a levels, chlorophyll b contents, and total suspended solids. Growth was measured as an increase in shell height. Condition indices, disease status, and mortality were also determined. Five sites were chosen based on environmental diversity, security, and availability. Average chlorophyll a levels between sites ranged from 8 ug/l to 25 ug/l and average salinities from 8.0 ppt to 18.0 ppt. Growth rates differed significantly between sites and were positively correlated with both chlorophyll a and salinity. Growth was severely retarded by disease. The potential of raft oyster culture in the Chesapeake Bay will be discussed, and the importance of assessing water qualities when determining the potential of growout sites will be addressed.

INTRODUCTION

The relationship between water quality and bivalve growth has been intensively studied in several species. Many experiments have generated large quantities of data and employed sophisticated statistical treatments to evaluate correlations between specific water qualities and growth or other production traits (e.g., fecundity) (Dame, 1975; Hall, 1984; MacDonald and Thompson, 1985a, b; Brown, 1988; Brown and Hartwick, 1988a,b). These studies were conducted primarily to generate models capable of predicting bivalve production rates based on water qualities for aquacultural considerations. While they have generated statistically significant correlations and are capable of predicting bivalve production rates accurately, these analyses are not particularly useful in making conclusions about the physiological relationships that exist between water qualities and bivalve growth rates.

Recently, we reported very high seasonal growth rates of oysters (*Crassostrea virginica*) raised in floating trays (Paynter and DiMichele, 1990). Oysters examined in that study, raised in a shallow tidal creek environment, grew at rates that averaged 15mm/month over a four month growing season and were higher than those reported in other studies. Furthermore, the growth performance of a population of oysters selectively inbred for fast growth for approximately 10 generations (see Paynter and DiMichele, 1990; Brown and Paynter, 1991) was compared with that in a native Chesapeake population. The selectively inbred population grew approximately 25% faster than the native population in that study. In order to assess the relative contribution of water quality to these high growth rates and to determine whether the selectively inbred population would grow faster in other environments, oysters of both populations were raised in floating trays and concomitant water qualities were measured at several additional sites in the mid-Chesapeake Bay region.

Reported here are observations from two growing seasons (July through November). During the first growing season, growth of selectively inbred and native oysters was compared and correlated with various water qualities. In the second growing season only selectively inbred animals were used. Growth rates and water qualities were related using two different approaches; an instantaneous growth rate analysis (Askew, 1976; Hall, 1984; Brown, 1988) and a time-averaged analysis which related average growth rate at a given site with average water qualities at the same site over an entire growing season. During the growing season, oyster growth rates were insensitive to short-term changes in nearly all water qualities. The results cited in this report support the "time-averaged optimization" theory for feeding and growth in bivalves proposed by Hawkins *et al.* (1985). Incidentally, a significant inhibition of growth was observed in oyster groups infected with the parasitic protozoan, *Perkinsus marinus*.

MATERIALS AND METHODS

In order to assess the effects of water quality on oyster growth, experimental growing sites were established at six locations in the Chesapeake Bay region. Locations were chosen based on 1) preliminary water quality analysis -- sites of differing water quality but similar flow patterns were sought, and 2) accessibility and security. Over a two year study period from 1988 through 1989, 2 groups of oysters representing the selectively inbred and native populations were introduced at each site. Oyster growth, mortality, condition parameters (dry and wet tissue, and shell weights), and disease were monitored along with water quality.

The Maryland (MD) sites comprise two moderate salinity sites (12-15‰) and two low salinity sites (<12‰). The Virginia (VA) sites had mean salinities of approximately 20‰. All of the sites except one VA site had similar ecological characteristics. Typically, a site was a shallow tidal creek, well protected from weather and boat traffic. They were removed from point source pollution such as marinas and sewage outlets. Horizontal water flow was low at all sites but one as judged by casual observation. Many of these factors are known to affect bivalve growth, especially water flow, and their effects have been neutralized in this study by site selection. The one site which differed in several of these charateristics, however, showed no great differences in growth rate compared to a site of similar salinity.

Cultchless oysters were produced from native and/or selectively inbred broodstock using traditional hatchery methods. In general, oysters from 10 to 25 mm in length were introduced within 2 days at all sites in floating rafts. Rafts consisted of wooden frames 3 ft x 2 ft with a polyethylene mesh folded into a rectangular box which hung below the wooden frame and was stapled to the wooden frame along the edges. The resulting mesh box was 3 ft long x 2ft wide x 8 in deep. A 2 ft x 3 ft panel of extruded styrofoam wedged underneath the wooden frame was used to keep the tray afloat. Approximately 1000 oysters were initially placed in a single tray. If a tray became crowded by the oysters' growth the group was split into another

tray. Groups representing different cohorts (animals from the same spawn) were kept in separate trays. Densities were considered non-limiting at all stages in the study.

Growth as shell height was measured every two weeks along with several water qualities. The water qualities shown to affect oyster growth, including temperature, salinity, pH, seston, and chlorophyll, were measured biweekly at each site using standard techniques. Since the purpose of the study was to ascertain which water qualities most closely correlated with growth rate both within a site and between sites, the study was restricted to the seasonal growing periods of the oyster in the Chesapeake Bay region (May through November). Length, total weight, shell weight, wet tissue weight, and dry tissue weight were measured monthly throughout the study period in five animals from each tray at each site. Due to the uniform shape of cultchless oysters raised in floating trays, most allometric parameters were closely related to shell length, including wet and dry tissue weights. Therefore, shell height was a good measure of whole oyster growth. Condition indices were calculated from that data as :

$$CI = \text{dry wt (g)}/(\text{total wt(g)}-\text{shell wt(g)})$$

Bimonthly, 25 animals from each tray at each site were assessed for the presence of disease (*Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo)) using standard histopathological techniques (Burreson, 1991).

The study established a large database of oyster size, condition indices, water qualities, and disease prevalences which could be related using a variety of statistical approaches. It was quickly established that the presence of disease had a significant negative impact on growth. For simplicity, all data associated with infected oysters will be treated in a separate section. A detailed report of all disease effects is currently in preparation (Paynter and Burreson, ms in preparation). Therefore, only the data related to healthy non-infected oysters will be used in the water quality/growth analysis in order to establish a more clear relationship between water quality and oyster growth rates.

Two different analytical approaches were used to determine the relationship between increase in shell height and water qualities. One approach was based on the "instantaneous" growth rate analysis used by Askew (1976), Hall (1984), and Brown (1988), which relates growth (change in shell height) and measured water qualities between consecutive sampling points (in this case two weeks) over an extended sampling time series. Correlations were then determined based on the association of the increase in shell length between two consecutive sampling points and the mean of the water qualities between the same two sampling points. Mean water qualities were calculated as the (intial value + the final value)/2. Correlation coefficients were calculated according to Sokal and Rohlf (1981).

In contrast to the instantaneous growth analysis, average growth rates at a single site over the entire growing season were calculated using simple linear regression. The average growth rate was then correlated to mean water qualities (averaged over the whole season). Differences in average growth rates (regressions) between sites were tested using an F-test (Sokal and Rohlf, 1981).

RESULTS

The results of this study will be reviewed in two parts. First, with respect to the relationship between water qualities and oyster growth rates in healthy oysters, and second, with respect to the impact of disease on oyster growth. The most striking aspect of the data was the lack of variation in the growth rates within a specific site as water qualities changed in a typically seasonal fashion. The increase in shell height between sampling times was constant in all populations within a site throughout the growing season. This occurred at all sites. However, the magnitude of the biweekly increase, and hence the overall growth rate, differed between sites and between populations. Typical increases in shell height over time at low and high salinity

sites are shown in Figure 1. The arrow indicates the first detection of disease. The incremental increases in height between sampling points was essentially constant throughout the study period at all sites until infection occured (the impact of disease will be discussed below). This was also represented in the fit of the regression lines to the data. Regression coefficients were typically above 0.95 for both populations at all sites (Table 1). Differences in average growth rates for a given site were observed between years but the relationships between the sites did not change significantly (i.e., sites supporting better growth the first year with respect to the other sites also supported better growth the second year). The causes of the observed differences between years in absolute growth rates could not be examined since a different group of the selectively inbred animals was used in the second year.

The constant growth rates at all of the sites was further demonstrated by the lack of correlation between any of the variable water qualities and the "instantaneous growth rate" values. The relationships between incremental shell height increase and total chlorophyll a, seston, temperature, and salinity at each site are shown in Figure 2. When oyster growth was related to water quality in this fashion, salinity and chlorophyll *a* levels were found to be significantly correlated with instantaneous growth rates ($p<.05$) although the relationships were weak ($r<.40$). Interestingly, the oysters continued to grow at the same rate while mean temperatures fell from 29°C to 15°C. Temperature did not affect growth rate until it declined to approximately 10°C. Within a site, changes in water quality did not affect growth rates. Yet, growth rates between sites were different.

SITE	SALINITY (o/oo)	GROWTH RATE (mm/month)	S.E.E	r^2
1	11.4	14.8	0.35	.99
2	16.8	8.3	0.33	.96
3	10.1	11.3	0.45	.97
4	14.0	12.5	0.39	.96
5	20.0	15.5	0.40	.98
6	19.7	16.7	0.51	.95

Table 1. *Average salinities and growth rates of oysters associated with experimental grow-out sites in Chesapeake Bay.*

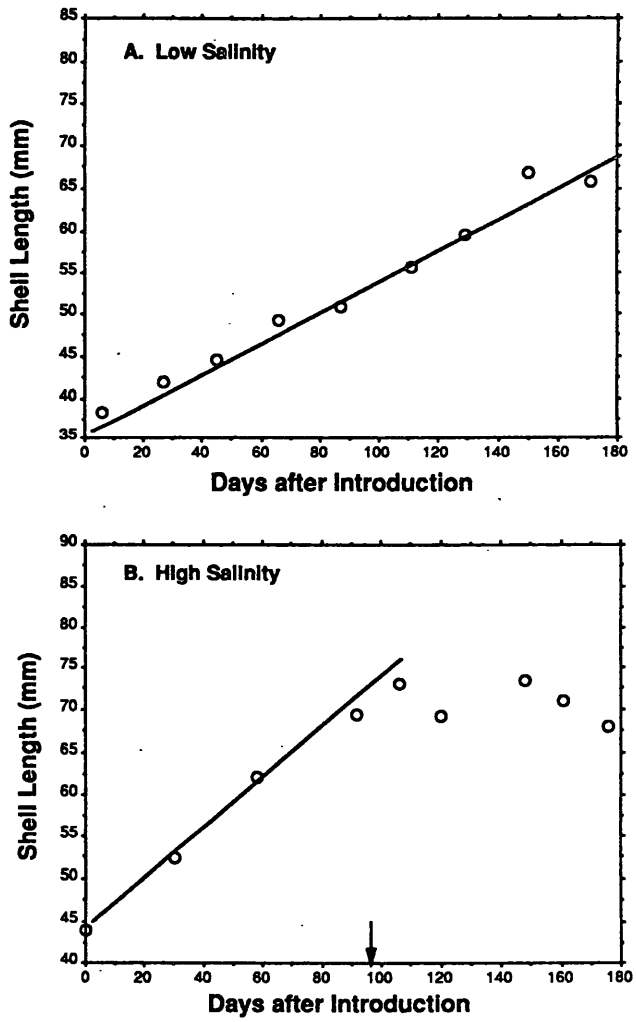

Figure 1. *Typical increases in shell height with time at low (A) and high (B) salinity sites. Time 0 was early May. Lines represent linear regression through shell height measurements in non-infected oysters. Oysters at low salinity were never infected. Infection was first detected in oysters at high salinity approximately 95 days after introduction. Infection prevalence at that time was 20% and all infections were light. Once infected, growth in the group as a whole essentially stopped and shell height remained constant for the remainder of the growing season.*

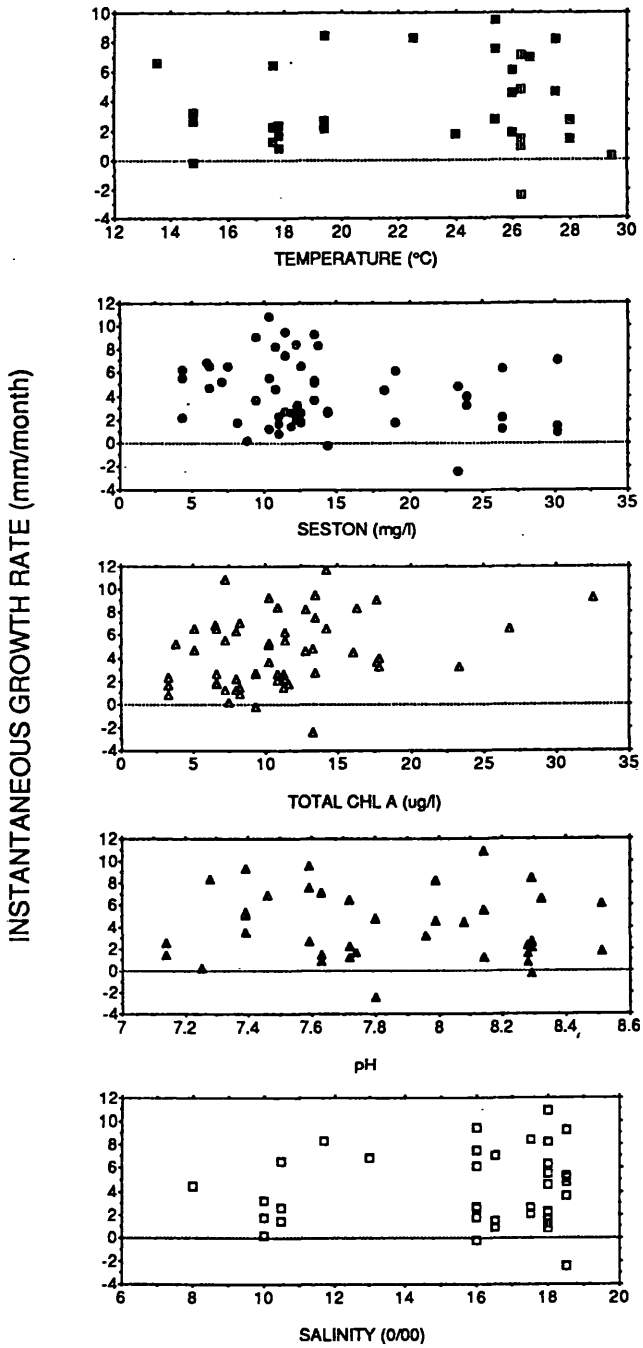

Figure 2. *Instantaneous growth rate analysis of the effects of various water qualities on oyster growth rate. Instantaneous growth (as defined in text) was plotted against temperature, seston, total chlorophyll a, pH, and salinity. Only salinity and chlorophyll a showed any correlations and those were weak but significant. See text for details.*

In order to compare water qualities between sites, and with the knowledge that many water qualities, especially chlorophyll *a* levels, could change dramatically between sampling periods, the relationship between *average* growth rate at a site throughout the growing period and *average* water quality at the same site was examined. Mean growth rate throughout the growing season was calculated by linear regression and was a highly precise estimate of growth rate as evidenced by the high coefficients of determination (r^2) (Table 1). Mean water qualities were calculated as the mean of all measurements taken for a specific water quality at a given site throughout the growing season . When this approach was used, salinity and chlorophyll a could be significantly related to growth rate.

Higher salinities had a significant stimulatory effect on oyster growth rates. The two Virginia sites (≈20‰) supported higher growth than any of the lower salinity Maryland sites (Table 1). Although sufficient data were not available for a rigorous statistical analysis (ANCOVA), the effects appeared to be independent of chlorophyll *a* levels. When the analysis was confined to sites with salinities below 15‰ , chlorophyll levels were highly correlative with growth rate.

Total chlorphyll *a* was related to growth rate in a non-linear, hyperbolic fashion (Figure 3). This behavior was similiar to the relationship between substrate and reaction velocity as catalyzed by an enzyme (see Segel, 1975), and the data were treated in a similar fashion. The hyperbolic relationship was linearized by transformation of the data into a double-reciprocal plot (1/mean growth rate vs. 1/ chlorophyll *a* ; see inset). This allowed linear regression to be used on the transformed data and showed that a relatively robust relationship existed ($P<0.003$; $r^2= .80$) between mean chlorophyll levels and growth rate. When the pooled data were resolved into their component parts of native and selectively inbred populations, the relationships were enhanced.

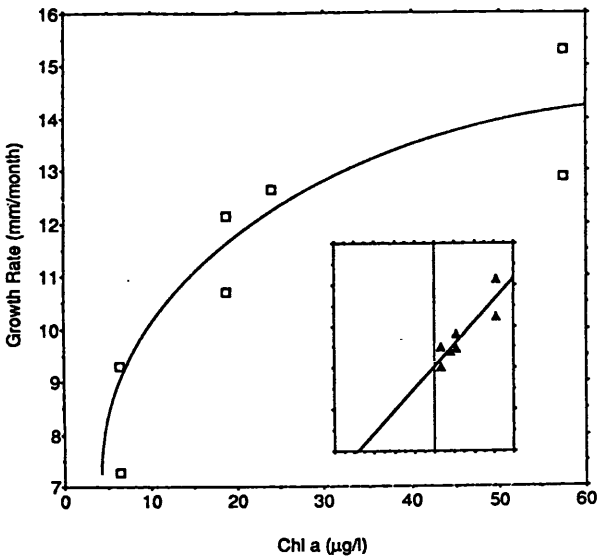

Figure 3. *Site-specific mean chlorophyll a levels associated with site-specific growth rates at low and moderate salinity sites. A double-reciprocal plot analysis (inset) showed a significant relationship existed between growth rates and chlorophyll a.($P<0.0026$; $r^2=.80$).*

The selectively inbred animals grew faster than their native counterparts in 2 of the 4 sites in which a comparison was performed. The double-reciprocal plots of the native and selectively inbred data treated separately demonstrated highly significant correlations ($r^2=.95$ and .97, respectively). The regressions between populations were not statistically different due to the paucity of data for the individual populations. However, some interesting differences appeared to exist between populations and deserve some discussion (see below). Extension of enzyme kinetic theory into this analysis would suggest that the maximally stimulated growth rate at very high levels of chlorophyll *a* would not be different between populations (15 mm/month). However, the amount of chlorophyll *a* required to stimulate half-maximal growth would be lower for the selectively inbred population (4.00 µg) than in the native population (6.5 µg).

Infection by *P. marinus* caused significant inhibition, if not complete cessation, of growth. At higher salinities where healthy growth was greatest, growth halted as soon as any disease was detected, even light infections. Regression analysis of pre- and post-infection growth rates showed that growth was significantly slower in infected animals than in non-infected animals (Fig. 1; F-test, $P<0.001$). Animals growing at moderate salinities were infected but the inhibition of growth was not as severe (data not shown). However, growth in healthy animals at moderate salinity sites was not as great as at higher salinities.

DISCUSSION

An understanding of the environmental parameters that affect oyster growth is important to the management of aquaculture communities. This information can lead to better management strategies and more productive aquacultural ventures. However, the identification of environmental parameters associated with production rates in any bivalve species does not provide any understanding of the physiological traits, characteristics, or qualities associated with growth rate. Genetic differences between oyster species or populations could lead to a variety of physiological differences that might result in differing growth rates. For instance, genetic differences may make one group of oysters more efficient feeders than another, resulting in higher growth rates. Some oyster species may be more tolerant of estuarine conditions, such as *C. virginica,* compared to other species, for example *C. gigas.* These differences are almost certainly based upon the gentically associated physiologies which enable the cells of the animals to regulate their functional integrity over a wide range of environmental conditions. It is therefore important to understand the physiological aspects of bivalve growth in order to understand why different populations behave as they do in response to the environment.

The statistical analysis and correlation of monthly growth rates and water qualities through the year is a productive way to identify water qualities associated with bivalve growth. It is not particularly informative for the study of the *physiology* of bivalve growth. For the most part this problem is due to the generalization that occurs in the statis-

tical modelling, which associates winter periods (low temperature, low seston, and low chlorophyll) with low growth rates. The general observations are, of course, valid but the particular relationship between a given water quality, such as chlorophyll content, and bivalve growth rate during periods of active growth cannot be addressed adequately. We have measured growth rates and accompanying water qualities at six sites during a limited time frame -- the active growing season-- in order to learn more about the specific water qualities associated with very high growth rates. Furthermore, great care was taken to document the presence or absence of disease throughout the study, and healthy *vs.* infected oyster growth rates were treated separately.

Disease (*P. marinus*) inhibited oyster growth significantly. Andrews (1988) noted the same effect over two decades ago, yet the implications of this effect on the oyster populations and the need for subsequent modifications in fishery management have been ignored. Although the mechanism of this effect is unknown, it might be assumed that infection causes a change in the animals' energy budget, which shifts energy away from growth and toward higher maintenance needs required to react to the infection. A more thorough study of disease effects is currently being completed.

Condition index, as calculated in this study, was found to be more of a function of season than site. Condition indices were similar between sites at any given month but differed significantly between months (data not shown). The ratio between shell height and dry tissue weight increased throughout the growing season and showed that growth as dry tissue weight was exponential. Oyster growth at all four sites was essentially constant from July through October even though nearly all water qualities changed significantly. These results suggest that growth was independent of the water qualities measured. Paradoxically, the rates of growth in oysters between some of the sites were different. In light of these preliminary observations, our analysis was conducted in two ways: first, an analysis of instantaneous growth rate correlated with "immediate" water qualities, and second, the correlation of average growth rate at a site and average water qualities at the same site.

While the instantaneous growth rate analysis identified salinity and chlorophyll as determinants of oyster growth, all other water qualities showed no correlation with oyster growth. The instantaneous growth rate analysis failed to identify any relationships because growth at all sites was constant throughout the growing season while all water qualities except salinity varied greatly. This is graphically apparent in Figure 1. It is quite clear that the oysters are growing at a constant rate throughout the growing season even while measures of food levels (chl. *a*) decline by nearly an order of magnitude. The immediate conclusion might be that food levels were at all times saturating, but then how does one account for the differences measured between sites?

The differences in growth rate between sites of low salinity (<15‰) were explained for the most part by variation in mean chlorophyll *a* levels (Fig. 3). These data suggest that oysters regulate their growth rate with respect to the water quality at any given site. That growth rate appears to be one which can be supported throughout the growing season in the face of changing conditions. Essentially, the oysters regulated their growth rates so that conditions were, as the earlier data suggested, saturating throughout the growing season.

These conclusions are compatible with other studies. Hawkins *et al.* (1985) suggested that mussels feed and grow in a time-averaged optimal rate. They argued convincingly that clearance rates, absorbtion and digestion rates, and assimilation rates were actively regulated by the animal so as to maintain some set growth rate in the face of variable environmental parameters -- especially food levels. A recent study completed in our lab suggests that oysters do not grow at rates directly limited by the environment. Paynter and Chen (in review) showed that vertebrate growth hormone stimulated growth in juvenile oysters maintained

in the same upwelling tray as untreated oysters. Since significantly higher growth was supported in identical water qualities, the water qualities could not have been directly limiting growth.

The differences in growth rates between the native and selectively inbred oyster populations are interesting. Although the sparse data set does not allow a statistically robust conclusion, the double-reciprocal analysis suggests several meaningful physiological traits. First, the analysis predicts that the maximum growth rate for both populations would be the same -- about 16 mm/month (this analysis would be applicable only to oysters grown under similar circumstances and at salinities below 15‰). Second, the analysis suggests that the selectively inbred population is more efficient than the native population. The inbred population would require less chlorophyll to support half-maximal growth than would the native population. This is intriguing because much research has been conducted on the feeding and digestive physiology of mussels (see Bayne, 19__ for review) which suggests that feeding and digestion efficiencies and the more general "scope-for-growth" measure are the physiologies closely related to environmental adaptation in bivalves. Furthermore, this conclusion presents a series of questions which can be answered experimentally using the different oyster populations.

Bivalve growth is a complex physiological phenomenon. When studied in relation to the variation typical of an estuary like Chesapeake Bay, it becomes even more complicated. It is clear that the sessile animals, like the oyster which inhabit these estuarine haunts, must possess very sophisticated and powerful biochemical and physiological mechanisms that allow them to constantly acclimate to the changing environment and thrive at the same time.

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