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## THE INFLUENCE OF INTERTIDAL HEIGHT ON GROWTH, MORTALITY AND HAPLOSPORIDIUM NELSONI INFECTION IN MSX MORTALITY RESISTANT EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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**ABSTRACT** Oysters, *Crassostrea virginica* (Gmelin), selectively bred for resistance to mortality associated with *Haplosporidium nelsoni* (Haskin, Stauber and Mackin 1966), were held at five different intertidal levels in the Delaware Bay during their first growing season. Survival was directly proportional to aerial exposure (AE) with the most exposed oysters (28.2% AE) suffering the highest mortality (30%). Growth, condition, fouling, *Polydora* infestation and a measure of marketability were all inversely proportional to aerial exposure. There was no detectable relationship between *H. nelsoni* incidence or intensity of infection with aerial exposure at the end of the experiment. Due to the heavy infestation of *Polydora* blisters at low levels, we suggest that more aerially exposed ("hardened") oysters grown at lower intertidal levels for a second growing season would lead to a higher overall yield.

**KEY WORDS:** intertidal, *Crassostrea virginica*, *Haplosporidium nelsoni*, MSX, hardening

### INTRODUCTION

The oyster parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin 1966), commonly known as MSX, has been responsible for extensive mass mortalities of the native American oyster *Crassostrea virginica* (Gmelin), leading to the steady decline of oyster populations along the eastern seaboard of the United States since the early 1950s (Andrews 1968, Farley 1968, Ford and Haskin 1982, Andrews 1984). Despite its recognition as a major pathogen, the full details of the life cycle of this ascetosporidan protozoan remains unclear. The MSX infection cycle is seasonal (Andrews 1982, 1984) yet our knowledge of the life cycle is incomplete largely because neither *C. virginica* tissue nor *H. nelsoni* can be cultured *in vitro* and there is no direct evidence of there being an intermediate host or host-to-host transfer (Haskin and Ford 1979). The presence of *H. nelsoni* in *C. virginica* poses an energetic burden on the oyster host (Barber et al. 1988a, Newell and Barber 1988, Barber et al. 1991), debilitating its feeding capacity (Newell 1985), reducing the biochemical energy store (Barber et al. 1988b) and lessening its reproductive capacity (Ford and Figueras 1988). The combined effects of parasitism and fluctuations in natural and anthropogenic environmental factors are also considered to increase the energetic burden imposed by MSX disease (Littlewood and Ford 1990).

Ever since MSX epizootic mortalities have occurred in the Delaware Bay (Haskin et al. 1965), selective breeding programs have been implemented to develop stocks of *C. virginica* resistant to MSX induced mortality (Ford and Haskin 1987). There is also evidence that similar disease tolerance has evolved in native oyster populations (Haskin and Ford 1979, Ford 1988) although these populations remain too devastated to support the ailing oyster in-

dustry. Aquaculture of *C. virginica*, as an alternative to fishing wild stocks, is in its relative infancy in the Delaware Bay (Aprill and Maurer 1976). Furthermore, aquaculture of disease resistant strains has been largely untested. Resistant strains are limited in quantity and are not presently commercially available.

Of the wide variety of oyster culture techniques available, intertidal cultivation may be the most pertinent method in the Delaware Bay region for the following reasons. Extensive tidal flats enable relatively easy access to intertidally cultivated stocks, and oysters which are regularly exposed to air are comparatively free from fouling and predation pressure (Arakawa 1980, Gibbons and Chu 1989). Thus, oysters held in the intertidal zone may be expected to experience greater survival than oysters held subtidally unless fouling and predation is controlled (e.g. see Ogasawara et al. 1962, Wisely et al. 1979, Littlewood 1988, Gibbons and Chu 1989).

The proportion of time an intertidally held animal is exposed to air, is dependent on its position (vertical height) relative to the tide and the amplitude of the tide. These parameters may be integrated into a proportion of aerial exposure (AE). Oysters held in the subtidal zone experience 0% AE and have the maximum time available to feed. Consequently, with increased aerial exposure, growth and the total yield of oysters is expected to be reduced as feeding time becomes limiting.

MSX is thought to be a water-born parasite (Ford and Haskin 1982) and we were interested in whether infection prevalence and intensity were affected by intertidal height, and how growth, mortality and yield of cultivated *C. virginica* might be affected at different exposure levels in a bay experiencing an MSX epizootic. Our overall goal was to investigate the viability of an aquaculture technique in the context of MSX disease. Although we also aim to shed light on the effects of aerial exposure on the parasitism of *C. virginica* by *H. nelsoni* it must be recognised that we used only MSX mortality resistant stock.

### MATERIALS AND METHODS

Three multi-level racks constructed from tubular steel were anchored firmly on the tidal flats at the Rutgers Shellfish Research

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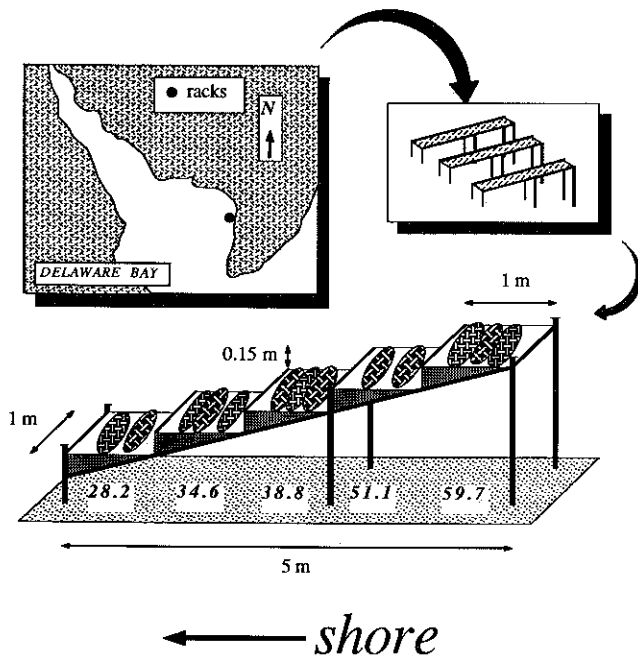


Figure 1. Location, position of racks and their dimensions. Mesh bags were held at five levels; italicized numbers represent estimates of percent aerial exposure (see Fig. 2).

Laboratory's Cape Shore field station in the Delaware Bay. Each rack consisted of a step-wise series of horizontal PVC coated metal mesh tiers which in turn supported the experimental animals. The location, orientation of each rack relative to the shore and approximate dimensions of each rack are illustrated in Figure 1.

Only hatchery reared oysters, bred in this laboratory for resistance to MSX induced mortality, were used in the experiment although comparisons are later made with unselected stocks grown by this laboratory at the same site during the same months. Two to three nylon mesh (25 × 25 mm) bags, each with 100 cultchless oysters (9 months old) were placed at each level on each rack on 2 May 1988 and were sampled periodically until the experiment was terminated on 6 December 1988. Bags were weighed, cleaned and reweighed to determine fouling and siltation load between sampling periods. Dead oysters were counted to calculate mortality and the shell height of 25 oysters from each bag was measured ( $\pm 1$  mm) to estimate growth.

At the end of the experiment 50 oysters were collected from each level, equally distributed between the bags on each rack. Shell height, shell length, and total weight was determined for each animal. Oysters were shucked, dried shells were weighed and oyster tissue was fixed in Davidson's fixative and later stored in 70% ethanol. Whole fixed meats were weighed and sections of tissue were removed and processed for histology according to standard methods (Haskin et al. 1966, Douglass and Haskin 1976) to determine MSX presence and intensity. MSX disease in oyster body and gills was scored to give a single discrete scale of infection intensity using a standard, repeatable method described by Barber et al. (1988a), where 0 represents no infection, 1 represents light epithelial gill infections, 2 represents light systemic infections, 3 represents a heavy gill infection, and 4 represents an advanced systemic infection. The remainder fixed meat was dried to constant weight and reweighed to estimate total dry meat

weight. Condition index was calculated both as the ratio of total dry meat weight to shell weight multiplied by 100 (CWT) and total dry meat weight to shell cavity volume multiplied by 100 (CVOL). For our purposes shell cavity volume was calculated as the difference between whole animal and shell weight and then converting this weight to a volume assuming the density of oyster meat is equal to that of sea water (see Lawrence and Scott 1982). The total number of *Polydora* mudworm blisters on the inside of each upper valve was recorded. A previous study has shown that there is no significant difference in *Polydora* infestation between upper and lower valves of an oyster, either in terms of number or area of shell affected (Wargo and Ford unpublished data).

The vertical height of each rack level above datum was recorded for the field site. The proportion of time each rack level was exposed to air during the experiment, i.e. % aerial exposure (AE), was determined from tide tables (NOAA 1987) using the method described by Littlewood (1988). Condition index ratios and mortality data were arcsine transformed prior to statistical analyses. Data were analyzed with the SAS (SAS Institute Inc. 1987) and SuperANOVA (Abacus Concepts Inc. 1989) statistical packages.

## RESULTS

### Tide and Aerial Exposure

The height above datum, the tidal cycle and the proportion each rack level was aerially exposed are shown in Figure 2. All subsequent results are presented as a function of aerial exposure. This should allow comparison of data between sites where, for example, beach profiles, tidal cyclicity and tidal amplitude may differ.

### Survival, Growth, Fouling and Condition

Figure 3 illustrates survival, growth and the rate of fouling as a function of aerial exposure during the course of the growing season. 95% confidence intervals about the mean values in Figures 3a, b are so small they are occluded by the symbols. Solely for illustrative purposes and in order to clarify the effects of aerial exposure we chose to pool the data within each rack level for these figures.

Oysters held at the 28.2% AE (lowest) level suffered highest mortalities, and the differences in survival between levels were

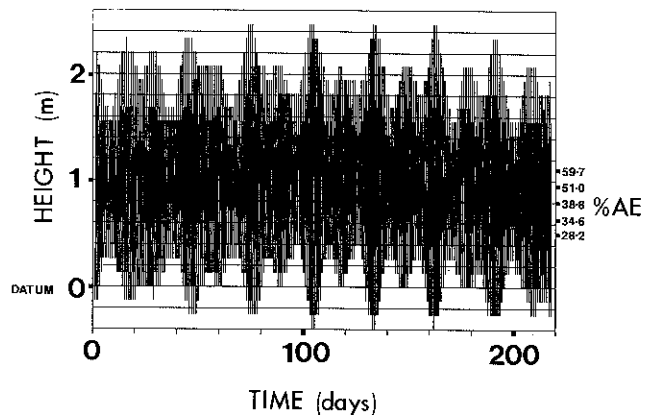


Figure 2. Tidal cycle based on highs and lows relative to datum; data were taken from published tide tables (NOAA, 1987). Estimate of the proportion of time each rack level was exposed to air during the experiment (% AE) calculated according to Littlewood (1988).

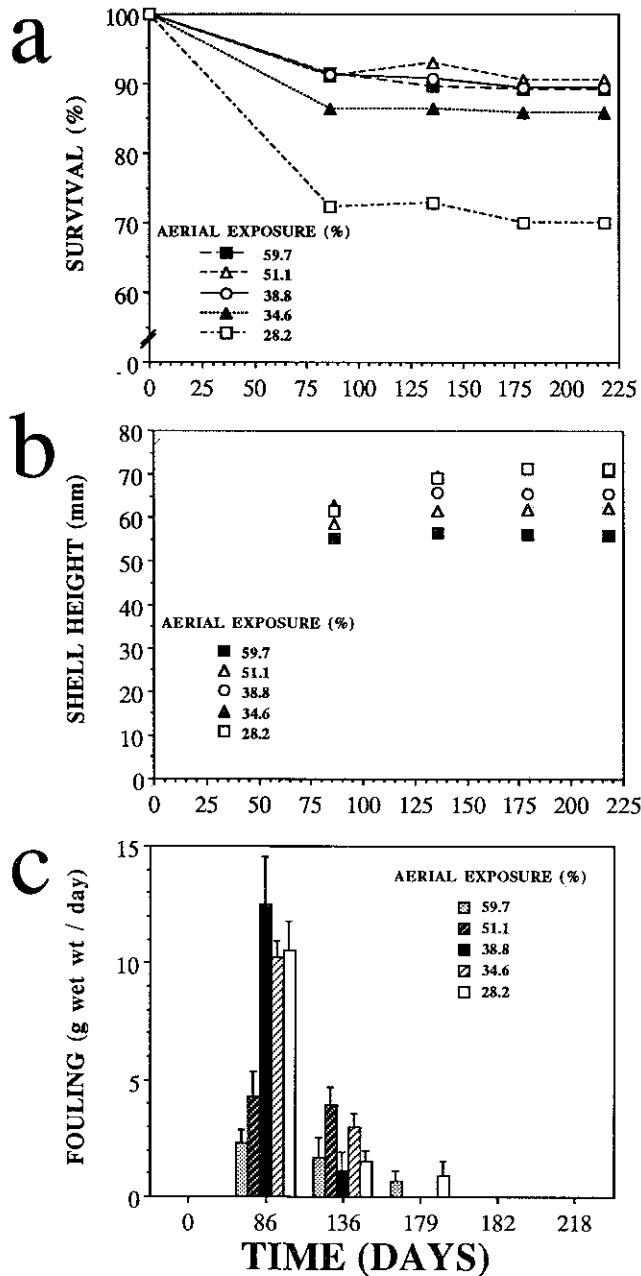


Figure 3. a. Survival, b. shell height and c. rate of fouling accumulation at each of five intertidal levels during one growing season. All data are presented as means  $\pm$  95% confidence intervals; confidence intervals are too narrow in a. or b. to be seen.

established by day 86 (August 10, 1988; see Fig. 3a). There were significantly fewer oysters at 28.2% AE level from this date until the end of the experiment ( $\chi^2$ -test;  $P < 0.01$ ). Mean shell height was significantly different between levels at each sampling date (ANOVA,  $P < 0.001$ ) with shell height inversely proportional to aerial exposure (Fig. 3b).

The major component of fouling was mudworm infestation, probably *Polydora ligni*, and the large volume of silt associated with the species (Stauber and Nelson 1940). In general, fouling accumulated at a greater rate at levels experiencing less aerial exposure (Fig. 3c). The rate of fouling was not consistent during

the growing period. The greatest weight of fouling was recorded during the first three months of the growth period, irrespective of exposure level.

Table 1 illustrates the results of a nested analysis of variance conducted on data accumulated on the last day of the experiment. Although there were strong bag and rack effects, indicated by low P values, we were particularly interested in the effects of aerial exposure (among levels component). The strong influence of intertidal exposure is demonstrated in the ANOVA table, where all growth and condition variables were significantly different among levels, and also in Figures 4a, b where mean values  $\pm$  confidence intervals do not overlap. The trend towards decreased growth and condition with increased aerial exposure is clear.

Oysters grown on the lowest intertidal exposure level (28.2% aerial exposure) had the greatest number of *Polydora* blisters on the upper valve. The mean number of blisters diminished with greater aerial exposure (Fig. 4b). ANOVA indicated a statistically significant difference in numbers of blisters between levels (Table 1) with oysters grown on the lowest level experiencing the highest levels of infestation (mean = 1.88 blisters per valve).

**MSX Infection**

Fewer than 20% of the oysters sampled at each level (from 2 to 8 individuals) were infected with *Haplosporidium nelsoni* (Fig. 4c). Again, for clarity, only the overall means in Figures 4a, b are illustrated. Aerial exposure had no statistically significant effect on MSX scores (Table 1). A  $\chi^2$ -test indicated that there was no significant difference in the proportion of MSX infected and uninfected oysters between exposure levels ( $\chi^2 = 6.816$ , 4 df,  $P = 0.146$ ). Although neither MSX incidence nor mean MSX intensity amongst infected oysters follow regular or similar trends with aerial exposure (Fig. 4c) each tended to be higher with greater aerial exposure.

**Marketability**

Figure 5 illustrates the proportion of the initial number of oysters attaining marketable quality as a function of intertidal height. We have chosen two criteria for marketability to discuss. Those

TABLE 1.

Results of nested analysis of variance determining the effects of bag, rack and level on oyster growth variables, oyster condition, MSX infection, and *Polydora* infestation; probabilities (italicized) and F ratios [df].

Dependent Variable	Source of Variation					
	Among Levels		Among Racks Within Levels		Among Bags Within Racks Within Levels	
	F <sub>[4,206]</sub>	(P)	F <sub>[9,206]</sub>	(P)	F <sub>[25,206]</sub>	(P)
shell height	46.18	(<0.01)	3.40	(<0.01)	1.60	(0.04)
shell length	33.79	(<0.01)	1.06	(0.39)	1.80	(0.15)
whole wt.	20.56	(<0.01)	1.67	(0.10)	1.53	(0.06)
shell wt.	14.31	(<0.01)	1.64	(0.10)	1.43	(0.09)
dry meat wt.	31.14	(<0.01)	1.74	(0.08)	2.06	(<0.01)
condition	7.89	(<0.01)	1.53	(0.14)	1.22	(0.22)
MSX rating	1.26	(0.29)	1.67	(0.10)	1.29	(0.17)
<i>Polydora</i>	67.95	(<0.01)	4.18	(<0.01)	0.94	(0.55)

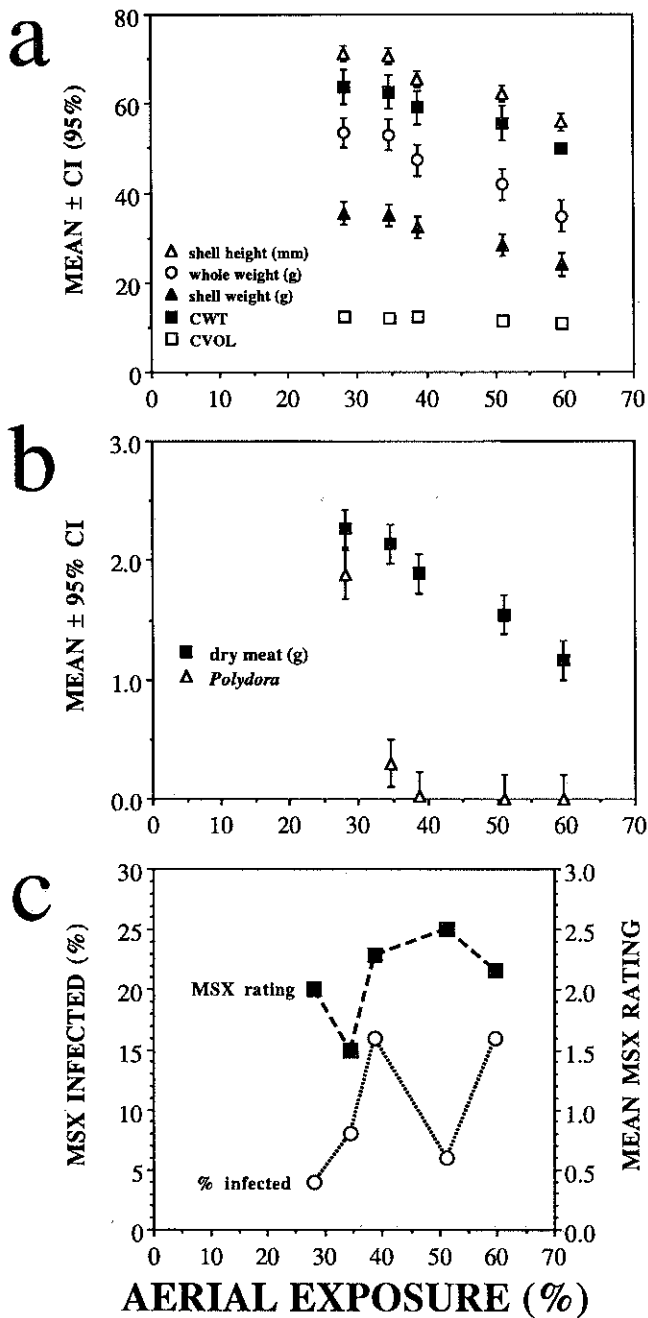


Figure 4. a. Final oyster dimensions and condition index, b. MSX intensity, dry meat weight and *Polydora* infection; values are means ( $n = 50$ )  $\pm$  95% confidence intervals, c. proportion of oysters parasitized with MSX, and intensity of infection of oysters with MSX. All variables are presented as a function of aerial exposure.

oysters with a shell height of 70 mm or more are considered to be of marketable size, and of those, oysters without mudworm blisters are considered to be preferable for the half-shell trade (Fig. 5). Approximately 50% of the oysters grown at each intertidal height below 35% exposure reached marketable size, whereas fewer than 25% were marketable at each level above 35% exposure. Indeed, based on size alone there is a direct relationship between aerial exposure and marketability. However, when those oysters ( $\geq 70$  mm) with mudworm blisters are eliminated, the marketability of

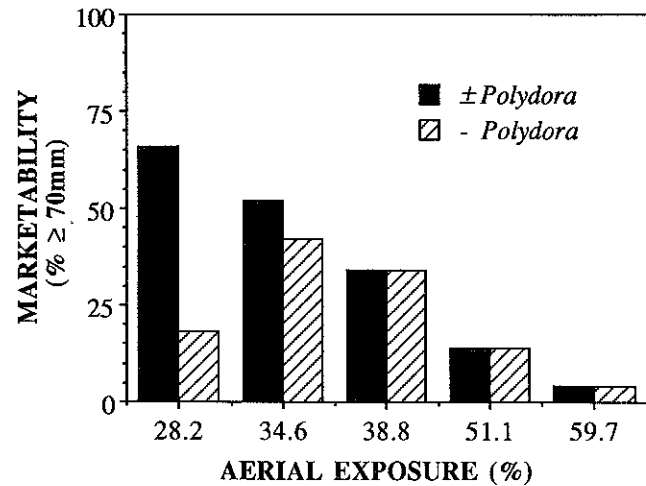


Figure 5. Marketability of oysters as a function of aerial exposure. Marketability is defined as the proportion of the initial number of oysters attaining a shell height of  $\geq 70$  mm; i. regardless of mudworm blisters ( $\pm$  *Polydora*), and ii. without blisters ( $-$  *Polydora*).

oysters grown at the lowest level (28.2% AE) is severely reduced, with those oysters grown at 34.6% and 38.8% AE yielding the highest proportion of oysters suitable for the half-shell trade.

## DISCUSSION

### Condition Index

Based on shell weight, condition index (CWT) is recognised as a useful index of physiological condition as it is essentially a ratio of tissue to shell growth (Lucas and Beninger 1985). In retrospect however, it is inappropriate to compare CWT between levels, as shell weight itself changes as a function of aerial exposure. Although both shell and dry meat weight change in a similar fashion with aerial exposure, they do so at a different rate so that increased aerial exposure results in a reduction in CWT. This is probably due to the considerable increase in shell thickness with greater exposure, observed in this experiment and others (e.g. Littlewood 1988).

Condition, based on shell cavity volume (CVOL), is a more appropriate index when comparing levels as it deals more specifically with meat, rather than whole oyster, condition (Mann 1978). Although statistically significant, the reduction in CVOL with increased aerial exposure is less dramatic than with CWT. Meats were of high quality at all levels and we consider any differences in CWT between levels to be negligible. Therefore, we consider aerial exposure to have little effect on meat quality, although it is recognised that increased aerial exposure considerably reduced meat growth.

### Fouling and *Polydora*

The difference in the rate of fouling during the experiment corresponds with the settlement of the mudworm *Polydora ligni* which occurred predominantly between June and July of 1988 (personal observations, RNW). The large volumes of silt which also accumulated up to August 10 (day 86) were attributable to the presence and characteristic burrow forming behaviour of *P. ligni*

(Stauber and Nelson 1940). After day 86, when bags were cleaned, relatively less fouling collected; from this time few *P. ligni*, or indeed any other epibiont, settled. We conclude that the fouling (recorded mainly as silt) was seasonally coincident with the settlement of *P. ligni* and that increased aerial exposure may have limited the recruitment of mudworms as well as having limited the time available for siltation. The temporal and spatial incidence of *P. ligni* in the present study coincides with previously recorded settlement patterns of *P. ligni* (Green and Hobson 1970, Orth 1971).

Mudworm blisters in the experimental oysters are believed to have been caused exclusively by *Polydora websteri* (Blake 1969, Zottoli and Carriker 1974). Both *P. ligni* and *P. websteri* infection intensity may be limited by controlling aerial exposure, and blisters may be avoided by holding oysters at levels experiencing greater than 40% aerial exposure, at least during times of mudworm settlement.

#### MSX Parasitism

The incidence and intensity of *Haplosporidium nelsoni* parasitism of *Crassostrea virginica* at the termination of this experiment had not been significantly affected by aerial exposure (between 28 and 60%), although there were indications that greater exposure may increase both infection and intensity of infection (Fig. 4c). As MSX infection was determined only at the end of the experiment it is difficult to determine its role in growth and mortality directly from this experiment, where aerial exposure *per se* incorporates so many variables. Nonetheless, a continuing monitoring program at this laboratory enables us to discuss the progress of the MSX infection cycle during the course of the present experiment. In 1988 Delaware Bay experienced a major MSX epizootic. During the first 86 days of the growth period in our experiment (from May to August) each of two stocks of 1988 year class oysters imported from Virginia and Maryland (unselected MSX-susceptible), and held adjacent to oysters in this experiment, experienced MSX mediated mortalities greater than 90% (Ford et al. 1989 unpublished report). Mortality of the resistant stock used in the present study was less than 30% at any aerial exposure, with less mortality at higher levels.

Despite the high infectivity and transmissibility of *Perkinsus marinus* (Ray 1954, Andrews 1984), another water-borne parasite pathogenic to *C. virginica*, it does not seem to be affected by tidal exposure either (Burrell et al. 1984, Gibbons and Chu 1989). However, there still exists the possibility that the infectivity and intensity of infection of some water-borne protozoan parasites are directly affected by aerial exposure. For instance, *Mikrocytos roughleyi* which has been implicated as the causal agent of winter mortality in *Saccostrea cucullata* (Farley et al. 1988, R. J. G. Lester pers. comm. 1990) has been shown to be limited when oysters are held high in the intertidal zone (P. H. Wolf and A. J. Collins pers. comm. in Wisely et al. 1979). This suggests that disease may be controlled by limiting the time oysters are exposed to infective particles or agents in the water column. In the present study the lack of a relationship between exposure and disease may be explained by a number of possibilities. One such scenario may be that there are too few infective particles or intermediate hosts in the water column, so that the probability of the pathogen, or its vector, coming into contact with and infecting the host is very small. This is unlikely in the present study considering the high mortalities experienced by the adjacent, unselected stocks. Alter-

natively, infection may be temporally or spatially constrained within a specific tidal cycle(s) such that oysters at all intertidal levels are equally as likely to be infected.

#### Marketability

The yield of marketable *Crassostrea virginica* grown in mesh bags within the intertidal zone of a bay with MSX was clearly dependent on the degree of aerial exposure. Unlike other oysters, *C. virginica* was capable of growing at intertidal levels exceeding 50% AE (c.f. *Ostrea edulis* which reportedly fails to grow above 30% AE, Walne, 1958), indicating further its remarkable adaptation to an intertidal existence (Galtsoff 1964). Gillmor (1982) showed that intertidally grown *C. virginica* grew better at certain intertidal levels than subtidally, even in the absence of fouling organisms. This suggested that the oyster has the ability to supplement energy input within the intertidal zone (see also Littlewood 1988). Our experiment did not include subtidally held oysters, but we do show an additional advantage in holding oysters intertidally, at least during their first growing season.

The occurrence of mudworms, which reduce oyster condition (Wargo and Ford unpublished data), and mudworm blisters, which weaken oyster shells and thereby interfere with shucking, reduce the market value of oysters (Haigler 1969, Kennedy and Breisch 1981). As a consequence, those oysters grown at the lowest exposure level were not the most suitable for the half shell trade, despite their larger shell size and whole weight.

This experiment followed oysters through to the end of their first full growing season. For commercial purposes oysters would probably best be grown for all or part of a second growing season to maximize the number of marketable, blister-free oysters. However, despite the size advantage of oysters grown in the low intertidal range, the high survival and mudworm free condition of oysters grown in the high intertidal range justifies their use as suitable stock to be grown for a second season. Oysters with mudworm blisters, i.e. those grown at low aerial exposure, are best sold at the end of the first growing season.

Keeping oysters high in the intertidal zone during the first growing season is known to enhance survival and increase shell thickness in *Crassostrea gigas* (Ogasawara et al. 1962). In Japan, *C. gigas* are cultured for these reasons because they become "hardened". A "hardened" oyster is one held intertidally for its first year; it is small and thick shelled. In their second growing season growth rate is so high that they attain the size of oysters of the same age held subtidally (Ventilla 1984). The advantage of this technique with *C. gigas*, and with *C. virginica* in the present study, is that there are a greater number of "hardened" oysters than those originally less exposed or subtidal. In the light of our results and considering the economic advantages of "hardening" *C. gigas* (Ogasawara et al. 1962, Ventilla 1984) we recommend a thorough investigation of the technique as applied to *C. virginica*.

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