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Michelle C. Paraso
Susan E. Ford
Eric N. Powell
Eileen E. Hofmann
Old Dominion University, ehofmann@odu.edu

John M. Klinck
Old Dominion University, jklinck@odu.edu

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MODELING THE MSX PARASITE IN EASTERN OYSTER (CRASSOSTREA VIRGINICA) POPULATIONS. II. SALINITY EFFECTS

MICHELLE C. PARASO,1 SUSAN E. FORD,2 ERIC N. POWELL,2 EILEEN H. HOFMANN,1 AND JOHN M. KLINCK1

1Coastal Ocean Laboratory
National Oceanographic Data Center, NOAA/NESDIS
Silver Spring, Maryland 20910
2Haskin Shellfish Research Laboratory
Rutgers University
Port Norris, New Jersey 08349
3Center for Coastal Physical Oceanography
Old Dominion University
Norfolk, Virginia 23529

ABSTRACT An oyster population model coupled with a model for Haplosporidium nelsoni, the causative agent of the oyster disease MSX, was used with salinity time-series constructed from Delaware River flow measurements to study environmentally-induced variations in the annual cycle of this disease in Delaware Bay oyster populations. Model simulations for the lower Bay (high salinity) site reproduced the annual cycle observed in lower Delaware Bay. Simulations at both upper Bay (low salinity) and lower Bay sites produced prevalences and intensities that were consistent with field observations. At all sites, low freshwater discharge resulted in increased disease levels, whereas high freshwater discharge produced decreased levels. At upper Bay sites, simulated changes in runoff produced high variability in disease prevalence; in the lower Bay, they produced a much lesser effect. Changes in salinity within the 10–20 ppt range produced the greatest changes in disease levels and patterns. Simulated shifts in timing of the spring runoff from March to either February or May affected the mid-Bay (13–19 ppt) only. A February runoff reduced the spring prevalence peak and caused a complete loss of systemic infections. In contrast, a May discharge occurred too late to affect parasite proliferation in the spring so that the spring peak was higher than average. Almost 100% of the infections were systemic by June, which resulted in high oyster mortality during July at this site. Model results indicate that parasite infection intensity under changing salinity is more complex than a simple function of salinity as it affects parasite proliferation and death rates within the oyster, and that the rate of infection is most likely reduced at low salinity. The simulated results demonstrate the ability of the model to reproduce field measurements and its usefulness in elucidating the association between the magnitude and timing of Delaware River discharge, its associated salinity variations, and the H. nelsoni annual cycle.

KEY WORDS: MSX, model, eastern oyster, Crassostrea virginica, Haplosporidium nelsoni, Delaware Bay, salinity

INTRODUCTION

The Eastern oyster, Crassostrea virginica, has been an important economic resource in Delaware Bay (Fig. 1) for at least 2 centuries (Ford 1997). The practice of “farming” oysters, which began in the mid-19th century, involves the transplantation of seed oysters during May and June from natural setting areas (seed beds) in the upper regions of the Bay to privately leased sections (planted grounds) in the lower Bay (Ford and Haskin 1982). Salinity of the water covering the seed beds ranges from 9 to 18 ppt under mid-tide, mean river-flow conditions, which protects the juvenile oysters from major predators (e.g., oyster drills) that are intolerant of low salinities and hence enhances oyster survival. The planted grounds are located in areas of higher salinity (20–23 ppt) that promotes growth and fattening of the oysters. Traditionally, oysters have been marketed the fall following the spring planting or have remained on the planted grounds an additional year or more before harvesting (Ford and Haskin 1982).

In the late 1950s and early 1960s, the causative agent of the Eastern oyster disease MSX (Multinucleated Sphere Unknown), was first observed in Delaware and Chesapeake Bays (Andrews 1966, Haskin et al. 1966). It was described as a hapsporidian by Haskin et al. (1966) and named Haplosporidium nelsoni (Sprague 1978). By 1959, 90%–95% of the planted ground oysters and about 50% of seed bed oysters had died in Delaware Bay. Gradually, in the late 1960s and the 1970s, Delaware Bay seed beds recovered as native oysters developed some resistance to MSX disease (Haskin and Ford 1979, Ford 1997). Following 15 years of modest success, the Delaware Bay oyster industry suffered another setback due to a resurgence of H. nelsoni, which made significant incursions onto the seed beds during a drought in the mid 1980s.

The annual infection cycle (Fig. 2) of H. nelsoni in lower Delaware Bay oysters was described by Ford and Haskin (1982). New infections are acquired beginning in early June and for the remainder of the summer, infections increase in number and intensity, reaching peak infection levels in late fall. Prevalences remain relatively stable through late winter, and then drop as heavily infected oysters die and parasites are lost from the remaining oysters. Prevalence and intensity increase again in April and May, coincident with rising temperatures, and a resurgence of infections that were suppressed by low winter temperatures (Ford 1985b). The spring peak, often having the most intense infections of the year, is typically followed by a rapid loss of infections, the reason for which is not completely understood. By this time, infective particles are once again present in the water and the cycle begins again.

Since poikilothermic and poikilosomatic animals are directly affected by the temperature and salinity of the environment in which they live, interactions between a parasite and a poikilothermic or poikilosomatic host may be strongly influenced by external environmental conditions. For example, temperature and salinity have well-documented effects on the relationship between H. nelsoni and C. virginica (e.g., Andrews 1964, Andrews 1983, Ford 1985a, Ford and Haskin 1988). Early field observations suggested that the parasite was salinity limited (Andrews 1964, Andrews...

Haskin and Ford (1982) proposed two explanations for the observed decrease in H. nelsoni prevalence along a salinity gradient in Delaware Bay. The first hypothesis, which does not involve a direct salinity effect, is that the principal source of infective particles is in the lower Bay. Consequently, the concentration of H. nelsoni infective particles in the upper Bay would be reduced due to increased distance from their source (Haskin and Ford 1982).

The second hypothesis is that reduced prevalence in low salinity areas was the result of a physiological response, either by the host or the parasite. Andrews (1983) suggested that low salinities might enhance the oysters' natural defenses, resulting in active expulsion of the parasite. Fisher and Newell (1986) found that in vitro rates of hemocyte locomotion were greater in low, compared to high, salinity and suggested that enhancement of hemocyte activity at low salinity might play a role in the expulsion of H. nelsoni from oysters in low salinity water. Thus, the parasite may be physiologically incapable of surviving in less than 10 ppt (Andrews 1964, Andrews 1983, Haskin and Ford 1982, Ford 1985a). Ford (1985a) proposed that a physiological limitation may restrict the distribution of H. nelsoni in low salinity water. Tolerance of plasmodial stages to acute in vitro salinity change roughly parallels the parasite's distribution in nature and indicates that its reduced occurrence in low salinity water is most probable due to its physiological inability to tolerate reduced salinity, rather than to enhanced effectiveness of host defense mechanisms (Ford and Haskin 1988).

To investigate the effect of environmental factors, specifically salinity, on the time and space distribution of MSX disease in Delaware Bay oyster populations, simulations were done with a time-dependent numerical model for oyster populations that was coupled with a model for H. nelsoni. The oyster–H. nelsoni model includes the physiological processes that affect the parasite-host interaction and are responsible for the predictable cycle in infection intensity and prevalence observed in Delaware Bay (Ford et al. this volume). Simulations were done to determine how seasonal variations (timing and strength) in Delaware River flow affect H. nelsoni prevalence and intensity at various sites within the Bay and the results from these were then compared with long-term field observations from the same sites.

MATERIALS AND METHODS

The Delaware Bay Study Site

Delaware Bay was chosen as a study site because a long-term database on MSX-disease exists for its oyster population. The Bay (Fig. 1) is a funnel-shaped estuary, extending 215 km from the head at Trenton, New Jersey, to the mouth between Cape Henlopen, Delaware, and Cape May, New Jersey (Wong 1994, Wong
1995, Ford 1997). The Delaware River, gauged at Trenton, New Jersey, contributes approximately 58% of the total freshwater input to the estuary, with an average discharge of 330 m³ s⁻¹ (Sharpe et al. 1986, Wong 1995). The Schuylkill River entering through Philadelphia, Pennsylvania, near the head of the Bay, contributes another 15% of the total freshwater input. No other single freshwater source provides more than 1% of the total discharge (Sharpe et al. 1986). A direct, negative correlation exists between salinity in Delaware Bay and the Delaware River flow (Fegley et al. 1994, Wong 1995).

Five sites in Delaware Bay were selected for this study (Fig. 1). Arnold's, Cohansay, Shell Rock, and Bennies Beds are on natural seed beds, whereas Miah Maull Grounds are located in the planted grounds. The depth of the seed beds ranges from 5 to 7 m. The location and area of each seed bed is described in Fegley et al. (1994). Arnold's Bed is located 64.1 to 67.0 km upbay from the mouth of the Bay and 0.1 to 2.9 km off the New Jersey shore, and has an area of 2.32 km². Cohansay Bed, located 54.4 to 58.5 km upbay and 0.1 to 3.4 km offshore, is 5.45 km². Shell Rock Bed with an area of 4.04 km², is located 50.5 to 53.4 km upbay and 1.4 to 3.6 km offshore. The seed bed site furthest downbay, Bennies Bed, is located 45.4 to 49.7 km upbay and 1.7 to 5.4 km offshore, and has an area of 6.36 km². Miah Maull Grounds is the site closest to the mouth and is representative of the entire leased area. This site is located approximately 25.9-27.8 km from the mouth, 6.5 km from the New Jersey shore, and occupies an area of approximately 32.3 km².

**Oyster Population-Haplosporidium nelsoni Model**

**General Characteristics**

A time-dependent oyster population—H. nelsoni model, described in Ford et al. (this volume), was used to simulate infection prevalence and intensity along the salinity gradient in Delaware Bay. The host-parasite model contains components to simulate the dynamics of a single size-class oyster population (1 g dry wt, in this case), the proliferation and death of H. nelsoni in those oysters (Fig. 5 in Ford et al. this volume), and transmission of H. nelsoni (Powell et al. this volume). The model components are coupled by relationships that describe the infection of unaffected oysters, the removal of oyster energy by the parasite to support its metabolism, and the relationship of parasite density in host tissue to oyster mortality.

**Governing Equation**

The governing equation for the oyster-parasite model moves oysters among H. nelsoni infection classes as parasite densities increase or decrease in the epithelial (r) and systemic (s) tissue of the oyster (O) over time. It is formulated as:

\[
\frac{dO_{rs}}{dt} = -\alpha_{rs}O_{rs} - \beta_{rs}O_{rs} + \alpha_{rs}O_{rs} + \beta_{rs+1}O_{rs+1} + \beta_{rs+1}O_{rs+1} - \alpha_{rs}O_{rs+1} - \alpha_{rs}O_{rs+1} - \delta_{rs}A_{rs}O_{rs} + \delta_{rs}A_{rs}O_{rs+1} + \delta_{rs}A_{rs+1}O_{rs} - \delta_{rs}A_{rs+1}O_{rs+1} + \sum_{i=1}^{N} \sum_{j=1}^{N} \delta_{rs}O_{rs} + \sum_{i=1}^{N} \sum_{j=1}^{N} \delta_{rs}O_{rs+1} + \sum_{i=1}^{N} \sum_{j=1}^{N} \delta_{rs}O_{rs+1} \quad (1)
\]

where the first 6 terms represent the movement of oysters between infection intensity classes through gains or losses of H. nelsoni cells in the epithelial and systemic tissue (Fig. 3 in Ford et al. this volume). The coefficients, \( \alpha \) and \( \beta \), determine the rate at which H. nelsoni cells are gained or lost. The parameterizations used to determine these coefficients are given in Ford et al. (this volume). The seventh term in equation (1) represents the loss of parasites through oyster mortality from lethal infections as determined by the rate of mortality, \( M \). The final 2 terms in equation (1) represent the transfer of oysters from heavy infection classes to lower infection classes due to the formation or attempted formation of spores by H. nelsoni in the oysters with advanced infections, which results in a loss of parasites from infection classes with \( BFU = 4 \) (\( s_4 \) in equation (1)), and a gain of infected oysters into infection class \( [1,0] \). The 6 functions represent a step-function process in which the oysters are introduced into the \([1,0]\) infection class only. The coefficient \( \gamma \) determines the rate of this transfer process.

The establishment of infection in uninfected oysters ([0,0]) class is determined by the equation:

\[
\frac{dO_{00}}{dt} = -\beta_{00}O_{00} + \frac{1}{2} \delta_{00}A_{00} + \sum_{i=0}^{N} \sum_{j=0}^{N} \gamma_{ij}O_{ij}
\]

where the first term represents the acquisition of H. nelsoni infective particles at a rate determined by \( \beta_{00} \). The second term represents addition of oysters to the uninfected class after hypothesized abortive H. nelsoni sporulation events, as described in Ford et al. (this volume), and is given by the eighth term in equation (1). These oysters are divided evenly between the [0,0] and [1,0] infection classes, as given by the last terms in equations (1) and (2).

**The Oyster Population Model**

The model component that describes the time-dependent development of the oyster population model is that described in Powell et al. (1992, 1994, 1995, and 1996) and Hofmann et al. (1992, 1994). For the purpose of this study, only a single size class of oyster (1 g dry wt) was used. The oyster model includes parameterization for the processes that determine the production of somatic and reproductive tissue, which are based on assimilation efficiency, filtration rate, ingestion, and respiration as modified by the environmental conditions of food supply, total seston, temperature and salinity.

**The H. nelsoni Model**

Parameterization of the processes that were used to develop the oyster population—H. nelsoni model, and the biological bases for the mathematical relationships, are described and discussed by Ford et al. (this volume). Processes that influence H. nelsoni interactions with its host include the rate of infection as a function of oyster filtration rate and ambient infective particle concentration; a density-dependent control on parasite proliferation based on oyster accumulated potential growth efficiency; parasite sporulation or attempted sporulation related to oyster accumulated potential growth efficiency, and H. nelsoni mortality due to cold exposure and increased susceptibility to oyster hemocytes, which themselves are influenced by temperature. The processes by which oysters move among infection categories is described in Ford et al. (this volume).

In addition to in vivo processes, the host-parasite model contains a subcomponent for disease transmission, which is dependent on infective dose, the filtration rate by which infective particles reach the oyster, and seasonal and salinity-dependent processes that control the concentration of infective particles in the water column (Powell et al. this volume). As this study is directed at salinity effects on the MSX disease process, the parameterizations involving salinity are discussed in more detail below.
Salinity Parameterizations

The effect of salinity on *H. nelsoni* mortality was parameterized using measurements of acute *in vitro* salinity tolerance of plasmodia, the most common stage in oysters (Ford and Haskin 1988). This mortality effect begins at 15 ppt, increases in intensity from 15 to 5 ppt, and causes rapid *H. nelsoni* mortality below 5 ppt. A sigmoidal function was fit to observations of the fraction of live plasmodia remaining after exposure to a range of salinities and modified to ensure that no parasite mortality occurred above salinities of 15 ppt (Fig. 3). It is formulated by

\[
Smort = \min \left( 1, \frac{0.01 SD_1}{2 + SD_1 - SD_2 e^{-SD_4 S}} \right)
\]

(3)

where *Smort* is the fraction of parasites still alive after exposure, \(SD_1 = 103\), \(SD_2 = 240650\), \(SD_3 = 0.592456\) ppt\(^{-1}\), and *S* is salinity in ppt. The salinity response was assumed to have a time scale of 4 days, which reflects the estimated period required for oyster hemolymph to equilibrate with external salinity after an ambient salinity change and for parasites to respond to the new conditions. The death rate of *H. nelsoni*, *Sdeath*, expressed as \(d^{-1}\) is then given by:

\[
Sdeath = \frac{-\ln(Smort)}{SD_4}
\]

(4)

where \(SD_4 = 4.0\). Under normal circumstances it is assumed that *H. nelsoni* infections, once acquired, are never lost. Therefore, the oysters cannot decrease through *H. nelsoni* cell mortality to a level lower than an initial epithelial infection with no systemic infection (\(c_1, s_0\)). However, for salinity mortality, it is assumed that the infection can be lost at salinities \(\leq 14\) ppt, which is equivalent to *Sdeath* = 0.01787.

It was assumed that salinity effects on parasite proliferation rates occur over the same salinity range producing cell mortality. The effect of salinity on cell growth was parameterized as an exponential relationship that produces reduced growth rates at salinities less than 14 ppt and essentially no growth at salinities less than 5 ppt. The form of the salinity-dependent *H. nelsoni* growth rate, \(Sfactor\), is:

\[
Sfactor = e^{sg(S-S_0)}
\]

(5)

where \(sg\) determines the rate of decrease of parasite proliferation rate with increasing salinity and \(S_0\) is 15 ppt, which represents the threshold above which no reduction in parasite proliferation rate occurs. The salinity effect on cell growth is such that it has the value of zero at \(S \leq 5\) ppt and the value of one at \(S > S_0\). Equation (5) modified the temperature-dependent *H. nelsoni* growth rate that is given in equation (4) of Ford et al. (this volume).

Initial simulations with the above model showed prevalences on the seed beds that were higher than those observed (Haskin and Ford 1982, Fegley et al. 1994). Subsequent simulations indicated that the source of this mismatch was related to the rate at which oysters became infected and suggested that reduced salinity, in some way, reduced infection efficiency. The model thus contains a parameter that varies transmission rate by reducing the number of infective particles at low salinity and is of the form:

\[
IPsal = \frac{1 + \tanh \left( \frac{SM_1 (S - SM_{10})}{SM_2} \right)}{2}
\]

(6)

where *IPsal* is the fractional reduction in spore number that is applied to the calculation of spores filtered given by equation (29) in Ford et al. (this volume). The constants \(SM_1, SM_2\) and \(SM_{10}\) have the values 1.6, 11.0, and 7.0, respectively, and *S* is the ambient salinity. Equation (6) is biologically valid because the salinity range affecting transmission is similar to the range causing cell mortality *in vitro*. However, the effect described by equation (6) has a somewhat wider range, as might be expected by direct exposure to a free-living infective particle. Whether salinity causes cell mortality or just reduced infectivity in infective particles is unknown at present.

The initial simulations for the seed beds sites also showed a decreasing infection intensity at upbay sites, which is inconsistent with observations that showed a single-step drop in infection intensity from the planted grounds to the seed beds, with little additional change related to decreasing salinity (Haskin and Ford 1982). Essentially, the simulations underestimated the systemic infections at low salinity. To include this effect the rate of *H. nelsoni* diffusion from the epithelial to systemic tissue, *Stiff*, was increased at low salinity using the relationship:

\[
Stiff = 1 + SF_1 \left\{ 1 - \tanh \left[ SF_2 \left( \frac{S - S_0}{SF_3} \right) \right] \right\}
\]

(7)

where the reference salinity, \(S_0\), is 15 ppt and the constants \(SF_1, SF_2,\) and \(SF_3\) have the values 9.0, 2.65, and 3.0 ppt, respectively.

---

Figure 3. The relationship between salinity and acute *in vitro* *H. nelsoni* mortality based on observations (asterisks) by Ford and Haskin (1988) of the fraction of *H. nelsoni* plasmodia appearing viable (i.e., excluding Trypan Blue dye) after exposure to a range of salinities.
The value of $S_{diff}$ was set to be 0.955 at 12.0 ppt, 0.5 at 15.0 ppt, and 0.005 at 18.0 ppt. Equation (7) gives the fractional reduction in $H. nelsoni$ cell diffusion rate that modifies equation (6) in Ford et al. (this volume). This relationship is based on the rationale that osmotic stress increases the leakiness of the basement membrane thereby permitting $H. nelsoni$ cells to more easily cross to the oyster circulatory system.

### Environmental Data Sets

The environmental factors that force the oyster population--$H. nelsoni$ model are salinity, temperature, food, and total seston. Data sets for temperature, chlorophyll $a$ (representing food), and total suspended solids (representing total seston), collected over a 5-year period (1981–1986), were obtained from the Haskin Shellfish Research Laboratory of Rutgers University. Collections used for this study were made at Section E (representing the seed beds) and at the Ridge (representing the planted grounds) sites (Fig. 1). Data collected in each month were averaged over all years to construct a representative 1-year time series.

### Temperature Data Sets

Temperatures for both Section E and Ridge time series began to increase during February and reached a maximum of about 26 °C in July and August (Fig. 4A). Temperature decreased with the onset of autumn and a minimum temperature of (2 °C) occurred in February.

### Food Data Sets

Overall, chlorophyll $a$ measurements, representing food available to the oyster, were approximately 5–10 μg L$^{-1}$ higher at Ridge than at Section E (Fig. 4B). Chlorophyll levels began to increase earlier (January) at the Ridge site, compared to the Section E site, where the increase began in February. Maximum chlorophyll concentrations occurred during March and were approximately 55 μg L$^{-1}$ at Ridge and 40 μg L$^{-1}$ at Section E. Chlorophyll then decreased steadily at Section E, with a minimum of approximately 3 μg L$^{-1}$ occurring in October. Levels at Ridge remained relatively high (32 μg L$^{-1}$) through May, then decreased to a minimum of 8 μg L$^{-1}$ in October.

During verification studies of the oyster--$P. marinus$ model in Galveston Bay, Texas (e.g., Powell et al. 1995), agreement between the simulated oyster populations and observed populations was improved if a non-chlorophyll component was added to the chlorophyll measurements to obtain total food supply. This modification to the chlorophyll time series was done using an empirical relationship between chlorophyll and total available food (Sonnat et al. 1998) of the form:

\[ Food = a \times \text{chlorophyll} + b \]  

where $Food$ is given in mg DW L$^{-1}$ and chlorophyll is in μg L$^{-1}$. The constants $a$ and $b$ have the values of 0.088 mg DW μg$^{-1}$ and 0.52 mg DW L$^{-1}$, respectively. This equation recognizes that total available food can include a significant non-chlorophyll component.

Similarly, verification studies with the oyster--$P. marinus$ model for Delaware Bay (Powell et al. 1997) showed that chlorophyll alone did not provide an adequate food supply for the oysters. Therefore, the relationship between chlorophyll and total available food in Delaware Bay was assumed to be of the same form as that for Galveston Bay and the coefficients were determined by an iterative procedure in which simulations and observations were compared (see Powell et al. 1997 for details). This procedure resulted in a conversion to food supply given by equation (7), but with $b$ having the value 0.26 mg DW L$^{-1}$.

### Total Seston Data Sets

Total seston, as measured by total suspended solids, was relatively constant at Section E (10–12 × 10$^3$ μg L$^{-1}$) with a slight increase in May to 16 × 10$^3$ μg L$^{-1}$ (Fig. 4C). The Ridge total seston time series was more variable than in Section E, ranging from 20 × 10$^3$ μg L$^{-1}$ in November to 41 × 10$^3$ μg L$^{-1}$ in August (Fig. 4C). Total seston values were greater at Ridge for all months.

### Salinity Data Sets

Monthly-averaged Delaware River flow for the period 1913 to 1993 was measured at Trenton, New Jersey, by the United States Geological Survey. Salinity time series were then calculated for
1953 to 1993, using a relationship between Delaware River flow and salinity derived by Haskin (1972):

\[ y = c + dx^e \]  

(9)

where \( y \) is salinity in ppt calculated from the preceding mean 30-day river flow \( x \) in \( \text{ft}^3 \text{s}^{-1} \) (1 \( \text{ft}^3 \text{s}^{-1} = 2.83 \times 10^{-2} \text{m}^3 \text{s}^{-1} \)).

Derived values of \( c, d, \) and \( e \) for each of the 5 sites shown in Figure 1 are presented in Table 1. The resultant salinity time series were used as input into the oyster population-\( H. nelsoni \) model.

At each site, the 8 years (20%) between 1953 and 1993 with the highest calculated average monthly salinity were grouped together and a mean salinity calculated for each month at each site. The resultant time series were assumed to be representative of a year with low freshwater runoff (Fig. 5A). Similarly, the 8 years (20%) with lowest monthly mean salinities were also grouped for each site and the salinity time series was taken to represent a year with high freshwater runoff (Fig. 5B). Salinities for the remaining 24 years (60%) were averaged to create a mean freshwater runoff time series (Fig. 4D).

Although the salinity values changed (Table 2), the seasonal pattern for average and low runoff years was similar (Figs. 4D, 5A). Salinity minima occurred in March/April and the maximum values extended from July through October. The pattern for a high runoff year was only slightly different, with a distinct minimum occurring in April and a shortened period of maximum salinities extending from July through September (Fig. 5B).

To simulate a shift in the timing of spring runoff, the maximum freshwater discharge, which occurred during March/April in the mean time series (Fig. 4D), was moved to either February or May (Fig. 5C, D). For these simulations, only the timing was changed; the quantity of freshwater discharge remained that of an average year.

**\( H. nelsoni \) Prevalence and Intensity Data**

Prevalence and intensity of \( H. nelsoni \) in Delaware Bay oysters was measured over the period 1959 to 1992 by personnel from the Haskin Shellfish Research Laboratory (Ford and Haskin 1982, Haskin and Ford 1982, Fegley et al. 1994). Oysters from the lower Bay planted grounds were obtained by dredge at regular intervals during the year. Seed bed oysters were typically collected during late autumn/early winter and the following late spring. These dates were chosen to coincide with peaks in the annual infection cycle (July 1 to June 30 of the following year) (Fig. 2). Stained tissue sections of each oyster were examined to determine the distribution and abundance of \( H. nelsoni \) in the tissues, and these were used to develop a scale, Big Ford Units (BFU, described in Ford et al. this volume), for comparing observed and simulated MSX disease infection and intensity. The BFUs were to define intensity categories as:

- **BFU = 0**: no infections or infections too light for detection by standard histology (hereinafter referred to as "undetectable infections").
- **BFU = 1**: Epithelial infections - \( H. nelsoni \) plasmodia in the gill epithelium only.
- **BFU = 2**: Subepithelial/local infections - \( H. nelsoni \) have broken through the basal lamina between the epithelium and the underlying tissues, but remain largely concentrated near that site.
- **BFU = 3**: Light systemic infections - \( H. nelsoni \) have spread through the tissues via the circulatory system, but parasite abundance remains relatively low.
- **BFU = 4**: Advanced systemic infections - \( H. nelsoni \) have spread throughout the tissues via the circulatory system and parasite abundance is high. These are typically lethal infections.

![Figure 5. Idealized time series of salinity, constructed from Delaware River flow measurements, used as input to the model to simulate A) low freshwater runoff, B) high freshwater runoff, C) a year with an early spring (February) freshwater runoff period, and D) a year with a late spring (May) freshwater runoff period at seed bed (Arnolds, Cohanse, Shell Rock, and Bennies Beds) and planted ground (Miah Maull Grounds) sites.](image)

**TABLE 1.**

Derived constants for the Delaware River flow-Delaware Bay salinity relationship \( (y = a + bx^e) \) for the 5 sites displayed in Figure 1, where \( y \) is the salinity in ppt and \( x \) is river flow in \( \text{ft}^3 \text{s}^{-1} \) (From Haskin 1972).

<table>
<thead>
<tr>
<th>Site</th>
<th>( a ) (ppt)</th>
<th>( b ) (ppt ( \text{ft}^3 \text{s}^{-1} ))</th>
<th>( c ) (non-dimensional)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnolds Bed</td>
<td>-37.85</td>
<td>99.32</td>
<td>-0.08036</td>
</tr>
<tr>
<td>Cohanse Bed</td>
<td>-38.91</td>
<td>102.32</td>
<td>-0.07465</td>
</tr>
<tr>
<td>Shell Rock Bed</td>
<td>-45.91</td>
<td>104.95</td>
<td>-0.06083</td>
</tr>
<tr>
<td>Bennies Bed</td>
<td>-64.25</td>
<td>122.04</td>
<td>-0.04575</td>
</tr>
<tr>
<td>Miah Maull Grounds</td>
<td>19.86</td>
<td>152.16</td>
<td>-0.41722</td>
</tr>
</tbody>
</table>
TABLE 2.
Salinity ranges for high freshwater runoff, average freshwater runoff, and low freshwater runoff time series (Figs. 4D, 5A, and 5B) for the sites displayed in Figure 1. The high value in each range represents the late summer values and the low value represents the spring values.

<table>
<thead>
<tr>
<th>Station</th>
<th>High Runoff (ppt)</th>
<th>Average Runoff (ppt)</th>
<th>Low Runoff (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnolds Bed</td>
<td>5.1–9.5</td>
<td>7.2–12.9</td>
<td>9.6–15.4</td>
</tr>
<tr>
<td>Cohanseay Bed</td>
<td>7.8–12.2</td>
<td>9.9–15.6</td>
<td>12.3–18.2</td>
</tr>
<tr>
<td>Shell Rock Bed</td>
<td>9.5–13.7</td>
<td>11.5–16.9</td>
<td>13.8–19.3</td>
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<tr>
<td>Bennies Bed</td>
<td>11.2–15.5</td>
<td>13.3–18.7</td>
<td>15.6–21.1</td>
</tr>
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<td>Miah Maull Grounds</td>
<td>21.8–23.0</td>
<td>22.4–24.4</td>
<td>23.0–25.7</td>
</tr>
</tbody>
</table>

The distribution of BFUs in relation to epithelial and systemic infections is shown in Figure 4 of Ford et al. (this volume).

Observed infection prevalences and intensities were used to verify model simulations. In the case of the planted ground simulations, comparisons were made with the complete annual cycle. For the seed beds, comparisons were made with the winter (November/December) and spring (late May/early June) prevalence peaks. Observed category 1 and 2 infections were combined to represent the prevalence of localized infections and categories 3 and 4 were combined to represent systemic infections. These data were used to construct a time series of prevalence and intensity (localized vs. systemic infections) in oyster populations at each seed bed and planted ground site (Fig. 6). Simulations for Miah Maull Grounds represent the annual H. nelsoni cycle and infection levels of the entire leased area, since there is relatively little variation within this area as compared to the variation among seed beds. Since measurements at Miah Maull Grounds were sparse, they were supplemented with data from Deepwater and Southwest Line planted grounds (Figs. 1, 7).

Model Implementation

The oyster population—H. nelsoni model was solved numerically using the 2-step pseudo-steady-state approximation scheme (Verwey and van Loon 1994) with a time step of 1 hour. Each simulation began on June 1 and extended for 3 years, with the first 2 years needed for the solution to reach a steady state. The same input data were used for each of the 3 years. The model assumes that the oysters have been continuously exposed to H. nelsoni, as is the natural stock, so that the population starts each cycle with the infection intensity and prevalence remaining at the end of the cycle.

To investigate the effect of salinity variations on the prevalence and intensity of H. nelsoni in Delaware Bay oyster populations, a number of simulations were considered under various environmental conditions (Table 3). A reference simulation was run for each site using mean environmental conditions. A series of simulations to determine the influence of high or low freshwater runoff on infection levels were subsequently run and compared to the reference simulation. Finally, the effect of the timing of the freshwater runoff was investigated.

Statistical Analysis

To analyze variations in prevalence, intensity, and timing of H. nelsoni infections in Delaware Bay oyster populations as a result of the various environmental scenarios, several statistical analyses were performed on the simulated output. The maximum difference, average difference, root mean square (RMS), and correlation coefficient (r) were calculated for each site to compare each simulation to the reference simulation. Comparisons were made for each infection intensity (BFU) category between time series for each hypothetical extreme-condition simulated and the corresponding reference time series.

The maximum difference is the greatest percentage point difference in prevalence for a particular infection category between the simulated extreme and reference annual infection cycles. A high value means that an extreme condition caused a relatively large change in that category of infection compared to the reference simulation; a low value means that the extreme condition caused relatively little change:

$$\text{maximum difference} = \max(A_i - B_i)$$

(10)
Figure 7. Prevalence and intensity of Haplosporidium nelsoni infections in oysters at A) Southwest Line and B) Deepwater planted grounds from records of the Haskin Shellfish Research Laboratory (Fegley et al. 1994). Solid portions of bars represent localized infections (BFU categories 1 and 2); clear portions represent systemic infections (BFU categories 3 and 4). Annual cycles are represented by 2 samples. Bars on the left of the tick mark represent the spring (May/June) measurements; bars on the right represent the early winter (November/December) measurements. Periods when no data were available are indicated by N. Note that after 1990, Perkinsus marinus, cause of Dermo disease in oysters, became prevalent on the New Jersey planted and confined H. nelsoni patterns.

where \( A_{t,i} = A(t) \) or the MSX prevalence time series for a given location (i) and MSX intensity category (i) and \( B_{t,i} = B(t) \) for the reference simulation at the same site and intensity category.

The average difference is the average percentage point difference in prevalence of a particular infection category between simulated extreme and reference annual infection cycles. A high value means that an extreme condition caused a relatively large change in that category of infection; a low value means that the extreme condition caused relatively little change:

\[
\text{average difference} = \frac{1}{T} \sum_{t=1}^{T} (A_{t,i} - B_{t,i})
\]  

(11)

where \( T \) is the length of the simulated time series produced by model simulations.

<table>
<thead>
<tr>
<th>Simulation</th>
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<th>Turbidity (mg (L^{-1} ))</th>
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<td>M, Feb</td>
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<td>B, May</td>
<td>E</td>
<td>E</td>
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<td>M</td>
<td>R</td>
<td>M, May</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

The RMS (root mean square) is a standard measure of the difference between 2 time series. Squaring the prevalence differences between the extreme \( A(t) \) and \( B(t) \) reference time series before taking the average enhances the larger differences and diminishes the smaller differences. Therefore, a relatively large difference will result in a larger value than a smaller difference. Again, a high value means that an extreme condition caused a relatively large change in that category of infection; a low value indicates that the extreme condition caused relatively little change:

\[
\text{RMS} = \sqrt{\frac{1}{T} \sum_{t=1}^{T} (A_{t,i} - B_{t,i})^2}.
\]  

(12)

Spearman’s Rank Correlation Coefficient (r) which is given by:

\[
r = \frac{C_{AB}}{\sqrt{C_{AA} \cdot C_{BB}}}.
\]  

(13)

was used to evaluate correlations of ranked prevalences of each infection category between simulated extreme \( A(t) \) and reference \( B(t) \) annual infection cycles. The coefficient \( C_{AB} \) is defined as the \( \Sigma T_{t=1} \sum_{i=1}^{T} (A_{t,i} - \bar{A})(B_{t,i} - \bar{B}) \), where \( A \) and \( B \) are the mean for time series \( A_{t,i} \) and \( B_{t,i} \), respectively. Values of \( r \) near 1 indicate similar temporal patterns (although absolute values can still vary). Values of \( r \) near zero indicate different temporal patterns.
RESULTS

Model Simulations

Reference Time Series Prevalence

The first set of simulations provided the basic time development of H. nelsoni infection prevalence and intensity at the seed bed and planted ground sites. These reference simulations were forced by average environmental conditions (Table 3) at each location and provided a basis for comparison with simulations produced by extreme environmental conditions.

At Arnolds Bed, the most upbay and least saline bed, mean environmental conditions produce no detectable infections (Fig. 8A). Infections are first detected just downbay at Cohanseky Bed during August, increase in prevalence and intensity during the fall, and reach a winter prevalence maximum of 9% by late October (Fig. 8B). At Shell Rock Bed, a similar pattern is seen with new infections appearing a little earlier, in July, and winter prevalence rising to 20% by November (Fig. 8C). At both sites, little change in total prevalence occurs over the winter. All detectable infections are lost in February and do not reappear in the spring.

Bennies Bed is the first site where the simulation results in an annual cycle more typical of higher salinity regions (with 2 peaks) and retention of some infections throughout the year (Fig. 8D). Prevalence and intensity increase through summer and fall, reaching a maximum by November, when the prevalence is about 45%. Loss of infections in all categories, but primarily systemic, occurs in March; infections re-appear in the spring, coincident with rising water temperatures and salinities. Prevalence reaches 25% in the late May/early June peak.

The reference simulation for Miah Maull, in the planted grounds, produces the highest infection levels (Fig. 8E). From a low of 5% to 10% in July, prevalence increases to 60% to 65%, with a little more than half the infections being systemic by November. A loss of primarily systemic infections causes a total prevalence decline in March and April, followed by a second prevalence peak of 35% in late May, in which most infections are advanced. A rapid loss of infections follows by early summer (see also Ford et al. this volume).

Reference Time Series Intensity

Under average conditions, approximately half the simulated infections in seed bed oysters are systemic at the November/December and late May/early June peaks (Fig. 8A–D). At the Miah Maull Grounds site, about half the winter infections are systemic. In contrast, the spring peak was predominantly systemic, with a large fraction in category 4, advanced systemic infections (Fig. 8E).

Extremes in Freshwater Runoff Simulations

Low Freshwater Runoff. During periods when freshwater runoff is lower than average, salinity in Delaware Bay increases (Fig. 5A) and elevated H. nelsoni infection prevalence results at all sites. Unlike average conditions, low freshwater runoff simulations produce detectable infections at Arnolds Bed, beginning in August (Figs. 8A, 9A). Infections develop slowly through the fall, reaching a winter prevalence peak of 8%. At Cohanseky Bed, detectable infections appear in June and reach a maximum prevalence of about 35% by November. At Arnolds Bed, all detectable infections are lost in January; the decline occurs in February at Cohanseky, where nearly all infections are lost. At neither site do infections re-appear in the spring.

Under the low runoff conditions, the simulated annual cycle for Shell Rock Bed is similar to that for Bennies Bed under average conditions: a 2-peak annual cycle with a substantial fraction of infections persisting throughout the year (Fig. 9C). The winter prevalence peak is approximately 45%. Loss of infections, primarily systemic, occurs in March. The spring peak prevalence is about 30%. Low-flow simulations produce almost identical cycle patterns and levels at Bennies Bed, representing the lower seed beds, as they do at Miah Maull, in the planting grounds (Fig. 9D, E). Both cycles show distinct winter and spring peaks with prevalence maxima of 60% to 65%, a moderate prevalence decrease in late winter, and a major loss of infections after the May peak.

As in the reference simulations, about half the infections are systemic by early winter (Fig. 9). The spring peak, however, produces relatively heavier infections, as well as higher prevalences at Shell Rock and Bennies Beds (Fig. 9C, D). The pattern at Bennies is nearly identical with that of the Miah Maull simulations, with a substantial number of category 4 infections (Fig. 9E).
High Freshwater Runoff. Salinity conditions during periods of high freshwater runoff (Fig. 5B) result in no detectable *H. nelsoni* infections at Arnolds, Coharsen, or Shell Rock Beds. Infections appear at Bennies Bed in July, but reach a maximum prevalence of only 9% from November through January, considerably lower than the 40% to 45% prevalence found under average conditions (Figs. 8D, 10A). Infections disappear in February and do not reappear in spring. Compared to average conditions, there is little change in the Miah Mauil Grounds cycle under high runoff.

Changes in Timing of Freshwater Runoff

Variations in the timing of maximum runoff from early (Fig. 5C) to late spring (Fig. 5D) affects *H. nelsoni* prevalence and intensity at Bennies Bed only. The major differences between the 2 extreme cycles (Fig. 11A, B) and that produced under average conditions (Fig. 8D) are in the degree of late winter infection loss and the extent of the late spring prevalence peak and subsequent decline. A February runoff produces a major loss of infections (from 45% to about 5%) in March followed by a relatively small resurgence, to 20%, in late May, and no subsequent decline (Fig. 11A). Understandably, a May runoff does not affect the late winter prevalence decline, which remains the same as under average conditions. It does, however, allow the late May prevalence peak to become higher than under average conditions (45% vs. 25%). More importantly, most of the infections become systemic (BFUs 3 and 4), resulting in a pronounced decline in prevalence in July (Fig. 11B) which was produced by a larger-than-normal number of heavily infected individuals in June that undergo attempted sporation. After this decline, however, newly acquired infections quickly proliferate so that by late fall, and over the winter, there are no differences in prevalence or intensity among the early, average, and late discharge simulations.

Statistical Comparisons Between Extreme and Average Model Simulations

Because the first 2 years of the 3-year model runs were needed for the solution to reach a steady state, statistical analyses were done using third-year simulations only. The maximum difference,
average difference, root mean square (RMS), and correlation coefficients \( r \) between the reference simulation and each simulation with varied environmental conditions were calculated to quantify the effects of salinity variation of *H. nelsoni* on each infection category.

### Variations in the Magnitude of Runoff

**Low Freshwater Discharge**. Conditions of low freshwater discharge have the largest effect on the undetectable infection category, as shown by high maximum difference, average difference, and RMS values (Table 4). Undetectable infections are, in effect, an inverse measure of percent infection. Thus, the decrease in this category in a high salinity regime simply means an increase in total prevalence. The largest differences associated with low discharge occurs at mid-bay sites, as suggested by the high maximum difference, average difference, and RMS values. Low correlation coefficients (<0.50) and relatively large maximum difference values were also calculated at Shell Rock and Bennies Beds for infection categories 1, 3, and 4, reflecting increased intensity as infections move from category 1 to categories 3 and 4 under high salinity conditions. Correlation coefficients of 1.0 for every infection category at Miah Mauil Grounds, as well as low values for the other 3 measures, indicate that there was almost no difference in prevalence or intensity associated with low runoff at this site.

**High Freshwater Discharge**. The greatest variation in simulated prevalences at all sites during high freshwater runoff occurs in the undetectable infection category (Table 5). This is consistent with the results of low freshwater discharge conditions and reflects the loss of parasites under low salinity conditions and a decrease in total prevalence. The greatest variability in maximum and total difference, and in RMS, is at Bennies Bed. Overall, correlation coefficients are positive and high (≥0.50 in all but one comparison, at Bennies Bed), and are 0.99 in all infection categories at Miah Mauil Grounds. Values for the other three measures are lowest at the Miah Mauil site, indicating little change in infection intensity and prevalence associated with high freshwater discharge at this location.

#### Table 4

Results of statistical analysis of the differences between low freshwater runoff and reference simulations at the seed bed and planted ground sites. *Haplosporidium nelsoni* infection intensity categories are UD = undetectable infections, 1 = epithelial, 2 = subepithelial local, 3 = light systemic, and 4 = systemic infections. “Maximum” and “Average” represent the maximum and average prevalence differences over an annual infection cycle. RMS represents the root mean square error, and \( r \) is the correlation coefficient. Bold is used to emphasize extreme differences.

<table>
<thead>
<tr>
<th>Site</th>
<th>Intensity category</th>
<th>Maximum (%)</th>
<th>Average (%)</th>
<th>RMS (%)</th>
<th>( r )</th>
</tr>
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<td>Arnolds Bed</td>
<td>UD</td>
<td>8.03</td>
<td>2.42</td>
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<td>0.03</td>
<td></td>
</tr>
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**Figure 11**. Simulated, time evolution of *Haplosporidium nelsoni* prevalence and intensity at Bennies Bed for A) early (February) and B) late (May) high freshwater discharge events. Numbers 1 through 4 refer to the cumulative percentage of total oysters (prevalence) with epithelial (1), subepithelial local (2), light systemic (3), and advanced systemic (4) infections. The area above the category 4 line represents the portion of oysters with undetectable infections. Arrows show the timing of the freshwater discharge.
TABLE 5.

Results of statistical analysis of the differences between high freshwater runoff and reference simulations at the seed bed and planted ground sites. *Haemoplagorion nelsoni* infection intensity categories are UD = undetectable infections, 1 = epithelial, 2 = subepithelial local, 3 = light systemic, and 4 = systemic infections. “Maximum” and “Average” represent the maximum and average prevalence differences over an annual infection cycle. RMS represents the root mean square error, and $r$ is the correlation coefficient. Bold is used to emphasize extreme differences.

<table>
<thead>
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<th>Station</th>
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<th>Average (%)</th>
<th>RMS (%)</th>
<th>$r$</th>
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<td>11.77</td>
<td>1.65</td>
<td>3.42</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>0.70</td>
<td>0.11</td>
<td>0.23</td>
<td></td>
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<td>Bennies Bed</td>
<td>UD</td>
<td>44.43</td>
<td>28.04</td>
<td>29.35</td>
<td>0.81</td>
</tr>
<tr>
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<td>26.06</td>
<td>13.43</td>
<td>14.28</td>
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</tr>
<tr>
<td></td>
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<td>13.50</td>
<td>5.60</td>
<td>6.70</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
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<td>12.93</td>
<td>4.56</td>
<td>5.55</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10.04</td>
<td>4.46</td>
<td>5.92</td>
<td>0.79</td>
</tr>
<tr>
<td>Miah Maull Grounds</td>
<td>UD</td>
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<td>4.14</td>
<td>4.40</td>
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</tr>
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<td></td>
<td>1</td>
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<td>1.47</td>
<td>1.75</td>
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<tr>
<td></td>
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<td>3.25</td>
<td>0.93</td>
<td>1.19</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.27</td>
<td>0.55</td>
<td>0.64</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.89</td>
<td>1.20</td>
<td>1.71</td>
<td>1.00</td>
</tr>
</tbody>
</table>

TABLE 6.

Results of statistical analysis of the differences between early spring freshwater runoff, late spring freshwater runoff and reference simulations at Bennies Bed. *Haemoplagorion nelsoni* infection intensity categories are UD = undetectable infections, 1 = epithelial, 2 = subepithelial local, 3 = light systemic, and 4 = systemic infections. “Maximum” and “Average” represent the maximum and average prevalence differences over an annual infection cycle. RMS represents the root mean square error, and $r$ is the correlation coefficient. Bold is used to emphasize extreme differences.

<table>
<thead>
<tr>
<th>Station</th>
<th>Intensity category</th>
<th>Maximum (%)</th>
<th>Average (%)</th>
<th>RMS (%)</th>
<th>$r$</th>
</tr>
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<tr>
<td>Early freshwater</td>
<td>UD</td>
<td>14.46</td>
<td>6.01</td>
<td>7.21</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.26</td>
<td>5.07</td>
<td>6.39</td>
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<tr>
<td></td>
<td>3</td>
<td>9.30</td>
<td>1.58</td>
<td>2.78</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.45</td>
<td>1.01</td>
<td>1.37</td>
<td>0.96</td>
</tr>
<tr>
<td>Late freshwater</td>
<td>UD</td>
<td>18.40</td>
<td>5.01</td>
<td>7.60</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10.45</td>
<td>3.53</td>
<td>4.66</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.73</td>
<td>1.66</td>
<td>2.64</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
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<td>23.74</td>
<td>4.01</td>
<td>8.20</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.74</td>
<td>1.37</td>
<td>2.04</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Rock Beds and at Miah Maull Grounds do not differ significantly from those obtained with the reference simulations (RMS $< 0.37\%$ and $r > 0.98$). The only significant modifications of the annual infection cycle associated with early or late discharge occur at Bennies Bed. Under early (February) runoff conditions, the greatest deviation from average was produced in undetectable and category 1 infections, as indicated by the maximum difference value at these categories (Table 6). A comparatively low correlation (0.59) in category 1 reflects the light infections present during the late spring and early summer, which never progress to higher levels (Fig 11A) as they do under average conditions (Fig 8D). The undetectable and category 1 infections were also affected by late runoff, as indicated by the maximum difference values. The greatest maximum difference value was in category 2 infections (Table 6), reflecting the high percentage of light systemic infections in the late spring peak (Fig 11B) compared to the average cycle (Fig 8D).

**DISCUSSION**

**Comparison of Model Simulations with Field Observations**

A test of the accuracy of the oyster population—*H. nelsoni* model is to compare simulated with observed infection prevalence and intensity patterns and levels under different environmental conditions. This was done in two ways. For the high salinity region of lower Delaware Bay, where considerable data are available on *H. nelsoni* infections throughout the year (Fig. 2), model simulations for Miah Maull in the planted grounds were compared with the annual infection cycle for that region, as described by Ford and Haskin (1982). On the other hand, data from the low salinity seed beds consist primarily of samples taken at winter and spring infection peaks (Haskin and Ford 1982, Fegley et al. 1994). Consequently, model output for this region of the bay was tested by comparing simulated prevalences and intensities at the peaks with observed values under similar ambient conditions.

The **Annual H. nelsoni Infection Cycle in a High-Salinity Location**

The onset of the simulated infection cycle at the Miah Maull Grounds begins in June. Prevalences and intensities increase, reaching a maximum prevalence of 65% in December (Fig 8E). This value is consistent with prevalences reported by Ford and Haskin (1982) for oysters in the planted ground area, which averaged 40% to 60% in most years, with minimum and maximum prevalences of 15% and 100%, respectively. As discussed by Ford et al. (this volume) the simulated proliferation of parasites during warm weather is primarily determined by temperature, but the reduction in proliferation rates apparent by late fall is caused by a combination of falling temperatures and high parasite density in oyster tissue. The latter inhibits proliferation because parasites are competing for a limited and declining nutrient resource within the host.

In late winter, the simulated infection cycle shows a marked loss of infected oysters, as does the observed cycle (Figs. 2, 8E). Observed mortality of severely infected oysters occurs at this time, reducing both prevalence and intensity. In addition, intensity lessens because parasites do not tolerate overwinter conditions well. Perhaps this is due to a direct low temperature intolerance or other unfavorable influences facing the parasite under the prolonged anaerobic conditions that occur inside the oyster, over the winter (Ford and Haskin 1982). For instance, *H. nelsoni* plasmodia contain numerous mitochondria (Muller 1967, Perkins 1968), which suggests that the parasite is well equipped for aerobic metabolism and may not be able to tolerate low oxygen well. The model
simulates the degeneration and disappearance of parasites at this time by including a susceptibility factor, which is applied to para-
sites and which increases as a function of degree days below 5 °C
(Ford et al. this volume). As susceptibility increases, the parasites
become more vulnerable to attack by host hemocytes. Hemocytes
themselves are becoming more active with increasing spring tem-
peratures and, as a consequence, can readily eliminate the dam-
aged parasites from the host.

Both simulated and observed annual cycles contain a distinct
second peak, reached in late May or early June. This peak contains
many very heavily infected oysters due to increasing temperatures
that cause a swift resurgence of those infections that were sup-
pressed over the winter (Andrews 1966, Ford 1985b, Ford and
Haskin 1982). The model, however, was unable to reproduce the
very rapid infection development by temperature alone. A second
factor was added, which eased the density-dependent limits on
parasite growth as a consequence of improving conditions within
the oyster (Ford et al. this volume). The increasing quality of the
parasite's environment, hypothesized to occur at this time, is due
to the spring bloom, increased feeding rates of the host, and the
cleansing of waste products that have accumulated in the host over
the winter.

Finally, both simulated and observed cycles show a marked
decline in prevalence following the spring peak (Figs. 2, 8E). Heavily
infected oysters die at this time, accounting for some of the
decline. Additionally, parasites are lost from living oysters for
reasons that are not clear, but may have to do with attempted, but
unsuccessful, sporulation and consequently a failure on the part of
the parasite to complete its life cycle (Ford and Haskin 1982, Ford
et al. this volume).

Peak Infection Levels at Low Salinity Seed Bed Locations

To compare simulations for the seed bed sites (Fig. 1) with field
measurements, the latter were grouped, by site, according to years
of high, average, and low freshwater runoff. The grouping criteria
were the same as those used to produce salinity time series for
these conditions. That is, the average years were those in the
middle 60% of the range; the high and low runoff years were the
20% at either extreme. For each flow regime and site, the mean,
median, and range of observed prevalences at both late fall and
spring peaks were obtained (Table 7). Because of the limited num-
ber of observations (Fig. 6) Shell Rock Bed was not included in this
comparison.

Comparison of initial simulations with observations showed
that the timing of infections was correct, but that prevalences at the
seed bed sites were too high. The relationship used to model the
host-parasite relationship under varying salinity was derived from a
study in which parasites were subjected to acute in vitro salinity
change (Ford and Haskin 1988), modified to simulate more gradual
in vivo changes in salt content of host fluids (equation 3). The
inability of the model to reproduce observed prevalences using
this relationship alone suggested that prevalence was influ-
enced by an additional factor or factors.

Although temperature from only a single site, Section E (Fig.
4A), were used in reference simulations for all seed bed sites,
monthly-averaged temperatures varied by a maximum of only
1.4 °C from the planted grounds to the seed bed areas (Fig. 4A).
Further, temperature variations within the seed bed area are rela-
tively small (Fegley et al. 1994). Thus, temperature differences are
unlikely to be responsible for the disparity.

Another possible factor could be salinity-associated differences
in the rate of infection. The infective stage of H. nelsoni remains
unknown. Spores, typically an important element in parasite trans-
mission, are rare in adult oysters and their absence has led to the
hypothesis that another host may be involved in the life cycle
(Burresson 1988, Haskin and Andrews 1988). On the other hand,
spores appear to be produced regularly in juvenile oysters with
advanced infections (Barber and Ford 1992, Burresson 1994), so

### Table 7.

Comparison of simulated total prevalence with observed mean, median, and range of prevalences on Delaware Bay sites (Fig. 1) under High,
Average, and Low Delaware River runoff conditions. PG indicates various planted grounds (Fig. 7).

<table>
<thead>
<tr>
<th></th>
<th>High runoff</th>
<th>Average runoff</th>
<th>Low runoff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% MSX Fall</td>
<td>% MSX Spring</td>
<td>% MSX Fall</td>
</tr>
<tr>
<td>Arnold's Model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>observed mean</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>observed median</td>
<td>1</td>
<td>&lt;1</td>
<td>13</td>
</tr>
<tr>
<td>observed range</td>
<td>0-5</td>
<td>0-5</td>
<td>0-60</td>
</tr>
<tr>
<td>Cohanscy Model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>observed mean</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>observed median</td>
<td>5</td>
<td>&lt;1</td>
<td>16</td>
</tr>
<tr>
<td>observed range</td>
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<td>0-5</td>
<td>0-52</td>
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<td>Shell Rock Model</td>
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<td>0</td>
<td>20</td>
</tr>
<tr>
<td>observed median</td>
<td>9</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
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<td>0-30</td>
<td>0-65</td>
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<tr>
<td>observed mean</td>
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<tr>
<td>observed range</td>
<td>5-95</td>
<td>0-80</td>
<td>10-95</td>
</tr>
</tbody>
</table>

(continued on next page)
direct transmission between oysters remains a possibility. Further, Barber and Ford (1992) found that haplosporidian spores, morphologically similar to those of *H. nelsoni*, are present in the water column in Delaware Bay in approximately equal abundance in upper and lower Bay sites. These authors speculated that if these spores are, in fact, *H. nelsoni* and are the infective element, the observed disparity between their concentration and infection prevalence in the upper and lower Bay could be explained by reduced infectivity under low salinity conditions. Alternatively, if another host is involved, the disparity could be explained by a salinity effect on another host or on its relationship with *H. nelsoni*.

The discrepancy was resolved in the model by adding an infection efficiency factor (equation 6), which reduces infection rates with decreasing salinity. This relationship implies nothing about the source or form of the infective particle; however, it does suggest that some element in the life cycle other than the plasmoidal stage in the oyster, is sensitive to low salinity. This includes, but is not limited to, an intermediate or reservoir (alternate) host, the abundance of infective stages in the water, or the ability of infective stages to invade and establish themselves in oysters.

Addition of infection efficiency to the model resulted in simulated prevalences that largely matched those observed (Table 7). The simulated levels were most like the median prevalences, which they produced almost exactly in many cases. Notably, the model produced no detectable infections on Arnold's Bed except under low flow conditions, as observed, and reproduced the elimination of the spring peak in cases where it was observed in the field. The model also accurately simulated the relative insensitivity to river flow changes of the lower Bay infection cycle that is observed in nature.

Only on Bennies Bed for average conditions and at Bennies and Cohasney Beds for low flow conditions, did the simulations not closely reproduce the mean or median observed prevalences (Table 7). The simulated prevalences were higher, especially under the low flow conditions, although they were clearly within the range of observations. Similarly, the modeled prevalence for Miaw Maull Grounds tended to be at the high end of the observed mean or median range for the lower Bay planted grounds. It is clear that observed prevalence in the moderate to high salinity region of the bay varies widely and in some years no infected oysters are found, regardless of salinity (Figs. 6, 7; Table 7). Multiyear cycles in *H. nelsoni* prevalence, totally independent of river flow and salinity, were described by Ford and Haskin (1982) for lower Delaware Bay. The salinity in lower Delaware Bay, in fact, is always high enough to support full *H. nelsoni* activity so that year-to-year variations must be due to some other condition. The fact that disparities between observed and simulated prevalences were maximum at mid-Bay sites under low flow, high salinity conditions, which produce infection levels and patterns similar to the lower Bay, suggests that they are influenced by factors other than the local salinity. The possible factors are included in a more complex disease transmission model as described in Powell et al. (this volume).

Another element of the basic model that was modified to simulate observed conditions involved infection intensity. Long-term observed averages show that despite decreasing prevalence, the ratio of systemic to localized infections remains essentially 1:1 along the salinity gradient in the seed bed regions of Delaware Bay (Ford and Haskin 1982, Fegley et al. 1994). However, the simulated intensity decreased upbay. To improve the model, the transmission rate of parasites from the epithelium to the systemic tissue was increased with decreasing salinity. The addition of this formulation to the model caused about half of the infections to remain systemic at peak prevalence on seed bed sites, while still allowing more intense infections to develop at the spring peak under the normally high salinity conditions of the lower bay and, at low river flow, on the lower seed beds. When a strong spring peak occurs, which it does only under high salinity situations, oysters often have the most advanced infections of the year, so that simulations reproduce observed intensities under high salinity conditions also.

The biological basis for the maintenance of high systemic infection levels under conditions of decreasing prevalence along the salinity gradient is unclear. Further, the mechanism used by parasites to penetrate the basal lamina between the epithelium and the underlying tissue is not known (Scribner and Ford 1990). Thus, there is no direct evidence that low salinity enhances the transfer rate between the two tissues. On the other hand, model simulations were run for a population in which all oysters were the same size (1 g dry weight). However, adult size generally decreases along the salinity gradient in Delaware Bay, and along with it occurs an increase in the surface (epithelium) to volume (systemic) ratio. A given concentration of parasites per unit area of epithelium in a small oyster is actually a larger total parasite load in the surface layer than it is in a larger oyster. In some way, this large overall burden may speed the movement of parasites into the underlying tissue and thus help explain the observed relatively high intensity levels on the seed beds. The addition of multiple oyster size classes to the model is needed to test this hypothesis.

Simulations were also run with variations in food availability in the spring and with warm winter conditions (≥5 °C) to determine the relative effects of salinity compared to food and temperature on oyster populations infected with *H. nelsoni*. Variations in salinity had a larger effect on the simulated infection levels than did variations in food availability and winter temperatures in a population of 1-g oysters. However, the simulated time development of infections did demonstrate the importance of cold winters in producing a reduction in infections the following spring, and therefore modulating the annual cycle.

When absolute differences between average and extreme conditions were compared statistically, the mid-Bay locations of Shell Rock and Bennies Beds showed the greatest variations. The explanation lies in the fact that mean mid-tide salinities at these locations range from 9.5–11.2 ppt to 19.3–21.1 ppt (Table 2), and it is in this range that a shift in salinity causes the greatest change in parasite survival (Fig. 3). In addition, Bennies Bed (salinity range 13.3 to 18.7 under average conditions) was the only site at which simulated variation in the timing of "spring" runoff caused a significant change from the reference annual cycle. This is because Bennies Bed, under average conditions, was the only seed bed to exhibit a late spring prevalence peak (Fig. 8D) and thus the only bed that had any infections to be altered by differences in timing. Salinity was always low enough to permit a spring MSX prevalence peak at the upper bay sites regardless of the timing of the spring runoff and salinity in the planted grounds was high enough (>20 ppt) nearly all the time to support infections so that changes in timing, like changes in volume of discharge, would have little effect on the spring peak at Miaw Maull Grounds.

**Summary and Conclusions**

The simulations obtained with the oyster population—*H. nelsoni* model were able to reproduce observed infection patterns and lev-
els along the salinity gradient in Delaware Bay. The base relationship used by the model was developed from in vitro parasite survival data. The inability of initial simulations, using this direct salinity-parasite function, to produce infection levels that adequately represented field observations, indicate a more complex host-parasite relationship at low salinity. Two additional functions, both based on assumptions, were added to the model to improve simulations. One allowed infection intensity to remain stable along the decreasing salinity gradient by increasing the transfer rate of parasites from epithelial to systemic tissues. The biological rationale may be linked to decreasing size of oysters and increasing surface-to-volume ratios along the salinity gradient. It is also possible that the transfer rate change may simply be a substitute for some other mechanism that allows intense infections to occur at low prevalence. The second modification was to diminish infection efficiency with decreasing salinity. Because the mode of transmission of {\em H. nelsoni} and the form of its infective stage remain unknown, this assumption cannot be verified. Reduced infection efficiency may also be a surrogate for another mechanism, such as abundance of a potential intermediate or alternate host, the abundance of infective stages, or both.

The importance of these modifications is not necessarily the assumptions that were made, but that they show that factors other than a simple, direct salinity effect on {\em H. nelsoni} proliferation and death rates in the oyster are required to explain field observations. The model results emphasize that in the {\em H. nelsoni} relationship, salinity is a permissive/restrictive element, modifying the degree and pattern of infections, during transmission as well as after oysters become infected.

Another, overriding factor, is the presence and abundance of infective particles, which varies from year to year. Model simulations show that in low salinity environments, infection levels are clearly correlated with river flow and that salinity changes over the 10–20 ppt range have the greatest effect on infection patterns and level. This association, however, breaks down at high salinity. Infection levels in high salinity (>20 ppt) regions of Delaware Bay are uncorrelated with river flow, as was noted earlier (Haskin and Ford 1982), and is attributed to fluctuations in infective particle abundance. The model shows that under drought conditions, the same factor or factors override local salinity to control infections in mid-Bay locations.

The ability to investigate {\em H. nelsoni}-oyster-salinity relationships with relative ease using the coupled model demonstrates its usefulness as a practical tool. In many regions other than Delaware Bay, low salinity areas are critical as setting and storage areas for seed oysters. The capacity of the model to simulate, with only minor modifications, observed {\em H. nelsoni} levels in mid-Chesapeake Bay demonstrates that it is robust and can be employed in other areas (Powell et al. this volume).

ACKNOWLEDGMENTS

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LITERATURE CITED


Haskin, H. H. & S. E. Ford. 1982. \textit{Haplosporidium nelsoni} (MSX) on


ABSTRACT An oyster population model coupled with a model for Haplosporidium nelsoni, the causative agent of
the oyster disease

MSX, was used with salinity time-series constructed from Delaware River flow measurements to study environmentally-induced variations in the annual cycle of this disease in Delaware Bay oyster populations. Model simulations for the lower Bay (high salinity) site reproduced the annual cycle observed in lower Delaware Bay. Simulations at both upper Bay (low salinity) and lower Bay sites produced prevalences and intensities that were consistent with field observations. At all sites, low freshwater discharge resulted in increased disease levels, whereas high freshwater discharge produced decreased levels. At upper Bay sites, simulated changes in runoff produced high variability in disease prevalence; in the lower Bay, they produced a much lesser effect. Changes in salinity within the 10-20 ppt range produced the greatest changes in disease levels and patterns. Simulated shifts in timing of the spring runoff from March to either February or May affected the mid-Bay (13-19 ppt) only. A February runoff reduced the spring prevalence peak and caused a complete loss of systemic infections. In contrast, a May discharge occurred too late to affect parasite proliferation in the spring so that the spring peak was higher than average. Almost 100% of the infections were systemic by June, which resulted in high oyster mortality during July at this site. Model results indicate that parasite infection intensity under changing salinity is more complex than a simple function of salinity as it affects parasite proliferation and death rates within the oyster, and that the rate of infection is most likely reduced at low salinity. The simulated results demonstrate the ability of the model to reproduce field measurements and its usefulness in elucidating the association between the magnitude and timing of Delaware River discharge, its associated salinity variations, and the H. nelsoni annual cycle.

KEY WORDS: MSX, model, eastern oyster. Crassostrea virginica, Haplosporidium nelsoni. Delaware Bay, salinity

INTRODUCTION

The Eastern oyster, Crassostrea virginica, has been an impor-
tant economic resource in Delaware Bay (Fig. 1) for at least 2
centuries (Ford 1997). The practice of "farming" oysters, which
began in the mid-19th century, involves the transplantation of seed
oysters during May and June from natural setting areas (seed beds)
in the upper regions of the Bay to privately leased sections (planted
grounds) in the lower Bay (Ford and Haskin 1982). Salinity of the
water covering the seed beds ranges from 9 to 18 ppt under mid-
tide, mean river-flow conditions, which protects the juvenile oys-
ters from major predators (e.g., oyster drills) that are intolerant of
low salinities and hence enhances oyster survival. The planted
grounds are located in areas of higher salinity (20-23 ppt) that
promotes growth and fattening of the oysters. Traditionally, oys-
ters have been marketed the fall following the spring planting or
have remained on the planted grounds an additional year or more
before harvesting (Ford and Haskin 1982).

In the late 1950s and early 1960s, the causative agent of the
Eastern oyster disease MSX (Multinucleated Sphere Unknown),
was first observed in Delaware and Chesapeake Bays (Andrews
1966. Haskin et al. 1966). It was described as a haplosporidian by
Haskin et al. (1966) and named Haplosporidium nelsoni (Sprague
1978). By 1959, 90%-95% of the planted ground oysters and
about 50% of seed bed oysters had died in Delaware Bay. Gradu-
ally, in the late 1960s and the 1970s, Delaware Bay seed beds
recovered as native oysters developed some resistance to MSX
disease (Haskin and Ford 1979. Ford 1997). Following 15 years of
modest success, the Delaware Bay oyster industry suffered another
setback due to a resurgence of H. nelsoni, which made significant incursions onto the seed beds during a drought in the mid 1980s.

The annual infection cycle (Fig. 2) of H. nelsoni in lower Delaware Bay oysters was described by Ford and Haskin (1982). New infections are acquired beginning in early June and for the remainder of the summer, infections increase in number and intensity, reaching peak infection levels in late fall. Prevalences remain relatively stable through late winter, and then drop as heavily infected oysters die and parasites are lost from the remaining oysters. Prevalence and intensity increase again in April and May, coincident with rising temperatures, and a resurgence of infections that were suppressed by low winter temperatures (Ford 1985b). The spring peak, often having the most intense infections of the year, is typically followed by a rapid loss of infections, the reason for which is not completely understood. By this time, infective particles are once again present in the water and the cycle begins again.

Since poikilothermic and poikilosmotic animals are directly affected by the temperature and salinity of the environment in which they live, interactions between a parasite and a poikilothermic or poikilosmotic host may be strongly influenced by external environmental conditions. For example, temperature and salinity have well-documented effects on the relationship between H. nelsoni and C. virginica (e.g., Andrews 1964, Andrews 1983, Ford 1985a, Ford and Haskin 1988). Early field observations suggested that the parasite was salinity limited (Andrews 1964, Andrews
Paraso et al.

Figure 1. Location of Delaware Bay. Inset shows locations of the New Jersey oyster seed beds (sites 1—1) and the planted ground (sites 5-7) stations. The solid line indicates the area designated as planted grounds. The (*) indicates the locations where time series of temperature, chlorophyll a, and total seston were acquired. The dashed line distinguishes between shoal and channel areas as designed by Pennock and Sharp (1986).


Haskin and Ford (1982) proposed two explanations for the observed decrease in H. nelsoni prevalence along a salinity gradient in Delaware Bay. The first hypothesis, which does not involve
a direct salinity effect, is that the principal source of infective particles is in the lower Bay. Consequently, the concentration of H. nelsoni infective particles in the upper Bay would be reduced due to increased distance from their source (Haskin and Ford 1982).

The second hypothesis is that reduced prevalence in low salinity areas was the result of a physiological response, either by the host or the parasite. Andrews (1983) suggested that low salinities might enhance the oysters' natural defenses, resulting in active expulsion of the parasite. Fisher and Newell (1986) found that in vitro rates of hemocyte locomotion were greater in low compared to high, salinity and suggested that enhancement of hemocyte activity at low salinity might play a role in the expulsion of H. nelsoni from oysters in low salinity water. On the other hand, because the oyster does not osmoregulate, the parasite is exposed to salinities that mirror the ambient water. Thus, the parasite may be physiologically incapable of surviving in less than 10 ppt (Andrews 1964, Andrews 1983, Haskin and Ford 1982, Ford 1985a). Ford (1985a) proposed that a physiological limitation may restrict the distribution of H. nelsoni in low salinity water. Tolerance of plasmodial stages to acute in vitro salinity change roughly parallels
Infection Period

Figure 2. Schematic of the annual Haplosporidium nelsoni infection cycle reproduced from Figure 4 in Ford and Haskin 1982. Filled arrows represent the percentage of oysters in each infection level (prevalence), with epithelial infections representing the lightest oysters (BFU category 1) and general systemic infections representing the heaviest infections (BFU category 4). The infection period designates the time in which infections begin in uninfected oysters and are designated by the unfilled arrows. The length of the arrows represents the relative intensity of infection pressure. The area above the general systemic infection line represents the portion of oysters in the undetectable category.

The parasite’s distribution in nature and indicates that its reduced occurrence in low salinity water is most probable due to its physiological inability to tolerate reduced salinity, rather than to enhanced effectiveness of host defense mechanisms (Ford and Haskin 1988).
To investigate the effect of environmental factors, specifically salinity, on the time and space distribution of MSX disease in Delaware Bay oyster populations, simulations were done with a time-dependent numerical model for oyster populations that was coupled with a model for H. nelsoni. The oyster-//, nelsoni model includes the physiological processes that affect the parasite-host interaction and are responsible for the predictable cycle in infection intensity and prevalence observed in Delaware Bay (Ford et al. this volume). Simulations were done to determine how seasonal variations (timing and strength) in Delaware River flow affect //. nelsoni prevalence and intensity at various sites within the Bay and the results from these were then compared with long-term field observations from the same sites.

MATERIALS AND METHODS

The Delaware Bay Study Site

Delaware Bay was chosen as a study site because a long-term database on MSX-disease exists for its oyster population. The Bay (Fig. 1) is a funnel-shaped estuary, extending 215 km from the head at Trenton, New Jersey, to the mouth between Cape Henlopen, Delaware, and Cape May, New Jersey (Wong 1994. Wong

[Begin Page: Page 503]
1995, Ford 1997). The Delaware River, gauged at Trenton, New
Jersey, contributes approximately 589<- of the total freshwater input
to the estuary, with an average discharge of 330 nr s~' (Sharp et
Philadelphia, Pennsylvania, near the head of the Bay, contributes
another 15% of the total freshwater input. No other single fresh-
water source provides more than 1% of the total discharge (Sharp
et al. 1986). A direct, negative correlation exists between salinity
in Delaware Bay and the Delaware River flow (Fegley et al. 1994.
Wong 1995).

Five sites in Delaware Bay were selected for this study (Fig. 1 ).
Arnolds, Cohansey, Shell Rock, and Bennies Beds are on natural
seed beds, whereas Miah Maull Grounds are located in the planted
grounds. The depth of the seed beds ranges from 5 to 7 m. The
location and area of each seed bed is described in Fegley et al.
(1994). Arnolds Bed is located 64.1 to 67.0 km upbay from the
mouth of the Bay and 0.1 to 2.9 km off the New Jersey shore, and
has an area of 2.32 km 2 . Cohansey Bed, located 54.4 to 58.5 km
upbay and 0.1 to 3.4 km offshore, is 5.45 km 2 . Shell Rock Bed
with an area of 4.04 km 2 , is located 50.5 to 53.4 km upbay and 1.4
to 3.6 km offshore. The seed bed site furthest downbay. Bennies
Bed, is located 45.4 to 49.7 km upbay and 1.7 to 5.4 km offshore,
and has an area of 6.36 km 2 . Miah Maull Grounds is the site
closest to the mouth and is representative of the entire leased area.
This site is located approximately 25.9-27.8 km from the mouth.
6.5 km from the New Jersey shore, and occupies an area of approximately 32.3 km$^2$.

Oyster /Vputaio/i-Haplosporidium nelsoni Model

General Characteristics

A time-dependent oyster population-//, nelsoni model, described in Ford et al. (this volume), was used to stimulate infection prevalence and intensity along the salinity gradient in Delaware Bay. The host-parasite model contains components to simulate the dynamics of a single size-class oyster population (1 g dry wt. in this case), the proliferation and death of//, nelsoni in those oysters (Fig. 5 in Ford et al. this volume), and transmission of H. nelsoni (Powell et al. this volume). The model components are coupled by relationships that describe the infection of uninfected oysters, the removal of oyster energy by the parasite to support its metabolism, and the relationship of parasite density in host tissue to oyster mortality.

Governing Equation

The governing equation for the oyster-parasite model moves oysters among H. nelsoni infection classes as parasite densities increase or decrease in the epithelial (e) and systemic (s) tissue of the oyster (O) over time. It is formulated as:
where the first 6 terms represent the movement of oysters between infection intensity classes through gains or losses of \( H. \) nelsoni cells in the epithelial and systemic tissue (Fig. 3 in Ford et al. this volume). The coefficients, \( a \) and \( S \), determine the rate at which \( H. \) nelsoni cells are gained or lost. The parameterizations used to determine these coefficients are given in Ford et al. (this volume). The seventh term in equation (1) represents the loss of parasites through oyster mortality from lethal infections as determined by the rate of mortality, \( M \). The final 2 terms in equation (1) represent the transfer of oysters from heavy infection classes to lower infection classes due to the formation or attempted formation of spores by \( H. \) nelsoni in the oysters with advanced infections, which results in a loss of parasites from infection classes with BFU = 4 (s 4 in equation 1), and a gain of infected oysters into infection class [1.0]. The 8 functions represent a step-function process in which the oysters are introduced into the [1,0] infection class only. The coefficient 7 determines the rate of this transfer process.
The establishment of infection in uninfected oysters ([0.0] class) is determined by the equation:

\[
\frac{dO_t}{dt} = -P_0 f A_i.o + 2 8 f .o8(U 2 2 1e,sO).
\]

(2)

where the first term represents the acquisition of H. nelsoni infective particles at a rate determined by \( B_0 \). The second term represents addition of oysters to the uninfected class after hypothesized abortive H. nelsoni sporulation events, as described in Ford et al. (this volume), and is given by the eighth term in equation (1). These oysters are divided evenly between the [0.0] and [1.0] infection classes, as given by the last terms in equations (1) and (2).

The Oyster Population Model

The model component that describes the time-dependent development of the oyster population model is that described in Powell et al. (1992, 1994, 1995, and 1996) and Hofmann et al. (1992, 1994). For the purpose of this study, only a single size class of oyster (1 g dry wt) was used. The oyster model includes parameterization for the processes that determine the production of
somatic and reproductive tissue, which are based on assimilation efficiency, filtration rate, ingestion, and respiration as modified by the environmental conditions of food supply, total seston, temperature and salinity.

The H. nelsoni Model

Parameterization of the processes that were used to develop the oyster population-/, nelsoni model, and the biological bases for the mathematical relationships, are described and discussed by Ford et al. (this volume). Processes that influence H. nelsoni interactions with its host include the rate of infection as a function of oyster filtration rate and ambient infective particle concentration; a density-dependent control on parasite proliferation based on oyster accumulated potential growth efficiency; parasite sporulation or attempted sporulation related to oyster accumulated potential growth efficiency; and H. nelsoni mortality due to cold exposure and increased susceptibility to oyster hemocytes, which themselves are influenced by temperature. The processes by which oysters move among infection categories is described in Ford et al. (this volume).

In addition to in vivo processes, the host-parasite model contains a subcomponent for disease transmission, which is dependent on infective dose, the filtration rate by which infective particles reach the oyster, and seasonal and salinity-dependent processes that control the concentration of infective particles in the water column (Powell et al. this volume). As this study is directed at salinity effects on the MSX disease process, the parameterizations
Salinity Parameterizations

The effect of salinity on H. nelsoni mortality was parameterized using measurements of acute in vitro salinity tolerance of Plasmodia, the most common stage in oysters (Ford and Haskin 1988). This mortality effect begins at 15 ppt, increases in intensity from 15 to 5 ppt, and causes rapid H. nelsoni mortality below 5 ppt. A sigmoidal function was fit to observations of the fraction of live Plasmodia remaining after exposure to a range of salinities and modified to ensure that no parasite mortality occurred above salinities of 15 ppt (Fig. 3). It is formulated by

\[
\text{Smort} = \min \left(1, -0.01 \cdot \text{SD}, 1 + \frac{-\text{SD}}{\text{SD}}\right)
\]
where Smort is the fraction of parasites still alive after exposure.

\[
SD, \quad 103. \quad SD, \\
.240650. \quad SD, = 0.592456 \text{ ppt} - \cdot \quad \text{and } S \text{ is}
\]

salinity in ppt. The salinity response was assumed to have a time scale of 4 days, which reflects the estimated period required for oyster hemolymph to equilibrate with external salinity after an ambient salinity change and for parasites to respond to the new conditions. The death rate of H. nelsoni, Sdeath, expressed as \( d^{-} x \) is then given by:

\[
S_{\text{death}} = \frac{1}{SD 4} \ln(\text{Smort})
\]

where \( SD 4 = 4.0 \). Under normal circumstances it is assumed that H. nelsoni infections, once acquired, are never lost. Therefore, the
oysters cannot decrease through H. nelsoni cell mortality to a level lower than an initial epithelial infection with no systemic infection (e., s.). However, for salinity mortality, it is assumed that the infection can be lost at salinities <14 ppt. which is equivalent to S_{death} = 0.01787.

It was assumed that salinity effects on parasite proliferation rates occur over the same salinity range producing cell mortality. The effect of salinity on cell growth was parameterized as an exponential relationship that produces reduced growth rates at salinities less than 14 ppt and essentially no growth at salinities less than 5 ppt. The form of the salinity-dependent H. nelsoni growth rate, S_{factor}, is:

\[
S_{factor} = \frac{S_{g}}{S} \cdot \exp\left(-\frac{S_{g}}{S_{\text{threshold}}} \cdot (S - S_{\text{threshold}})\right)
\]

where S_{g} determines the rate of decrease of parasite proliferation rate with increasing salinity and S is 15 ppt, which represents the threshold above which no reduction in parasite proliferation rate occurs. The salinity effect on cell growth is such that it has the value of zero at S < 5 ppt and the value of one at S > S_{\text{threshold}}. Equation (5) modified the temperature-dependent H. nelsoni growth rate that is given in equation (4) of Ford et al. (this volume).

Initial simulations with the above model showed prevalences on the seed beds that were higher than those observed (Haskin and
Ford 1982, Fegley et al. 1994). Subsequent simulations indicated that the source of this mismatch was related to the rate at which oysters became infected and suggested that reduced salinity, in some way, reduced infection efficiency. The model thus contains a parameter that varies transmission rate by reducing the number of infective particles at low salinity and is of the form:

\[
\begin{array}{cccc}
10 & 20 & 30 \\
\end{array}
\]

Salinity (ppt)

Figure 3. The relationship between salinity and acute in vitro H. nelsoni mortality based on observations (asterisks) by Ford and Haskin (1988) of the fraction of H. nelsoni plasmodia appearing viable (i.e., excluding Trypan Blue dye) after exposure to a range of salinities.

\[
\text{IP}_{\text{sal}} = \frac{(S - SM)}{SM_c - SM_f} \left(1 - \frac{S - SM}{SM_c - SM_f}\right)
\]

(6)

where IP_{sal} is the fractional reduction in spore number that is applied to the calculation of spores filtered given by equation (29) in Ford et al. (this volume). The constants SM_c, SM_f, and SM, have
the values 1.6, 11.0, and 7.0, respectively, and S is the ambient salinity. Equation (6) is biologically valid because the salinity range affecting transmission is similar to the range causing cell mortality in vivo. However the effect described by equation (6) has a somewhat wider range, as might be expected by direct exposure to a free-living infective particle. Whether salinity causes cell mortality or just reduced infectivity in infective particles is unknown at present.

The initial simulations for the seed bed sites also showed a decreasing infection intensity at upbay sites, which is inconsistent with observations that showed a single-step drop in infection intensity from the planted grounds to the seed beds, with little additional change related to decreasing salinity (Haskin and Ford 1982). Essentially, the simulations underestimated the systemic infections at low salinity. To include this effect the rate of H. nelsoni diffusion from the epithelial to systemic tissue, $S_{diff}$, was increased at low salinity using the relationship:

$$S_{diff} = S_{diff} + SF, S - S,$$

$$1 - tank$$

$SF,$
where the reference salinity, S is 15 ppt and the constants SF, SF 2, and SF, have the values 9.0, 2.65, and 3.0 ppt, respectively.

The value of Sdiff was set to be 0.955 at 12.0 ppt, 0.5 at 15.0 ppt, and 0.005 at 18.0 ppt. Equation (7) gives the fractional reduction in H. nelsoni cell diffusion rate that modifies equation (6) in Ford et al. (this volume). This relationship is based on the rationale that osmotic stress increases the leakiness of the basement membrane thereby permitting H. nelsoni cells to more easily cross to the oyster circulatory system.

Environmental Data Sets

The environmental factors that force the oyster population-//. nelsoni model are salinity, temperature, food, and total seston. Data sets for temperature, chlorophyll <; (representing food), and total suspended solids (representing total seston), collected over a 5-year period (1981-1986), were obtained from the Haskin Shell-
fish Research Laboratory of Rutgers University. Collections used for this study were made at Section E (representing the seed beds) and at the Ridge (representing the planted grounds) sites (Fig. 1). Data collected in each month were averaged over all years to construct a representative 1-year time series.

Temperature Data Sets

Temperatures for both Section E and Ridge time series began to increase during February and reached a maximum of about 26 °C in July and August (Fig. 4A). Temperature decreased with the onset of autumn and a minimum temperature of (2 °C) occurred in February.

Food Data Sets

Overall, chlorophyll a measurements, representing food available to the oyster, were approximately 5-10 p.g L⁻¹ higher at Ridge than at Section E (Fig. 4B). Chlorophyll levels began to increase earlier (January) at the Ridge site, compared to the Section E site, where the increase began in February. Maximum chlorophyll concentrations occurred during March and were approximately 55 (xg L⁻¹) at Ridge and 40 p.g L⁻¹ at Section E. Chlorophyll then decreased steadily at Section E, with a minimum of approximately 3 p.g L⁻¹ occurring in October. Levels at Ridge remained relatively high (32 p.g L⁻¹) through May, then decreased to a minimum of 8 p.g L⁻¹ in October.
During verification studies of the oyster-P. marinus model in Galveston Bay, Texas (e.g., Powell et al. 1995), agreement between the simulated oyster populations and observed populations was improved if a non-chlorophyll component was added to the chlorophyll measurements to obtain total food supply. This modification to the chlorophyll time series was done using an empirical relationship between chlorophyll and total available food (Soniat et al. 1998) of the form:

\[
\text{Food} = a \times \text{chlorophyll} + h
\]

where \(\text{Food}\) is given in mg DW L\(^{-1}\) and chlorophyll is in p.g L\(^{-1}\). The constants \(a\) and \(b\) have the values of 0.088 mg DW u.g \(\_1\) and 0.52 mg DW L\(_{-1}\), respectively. This equation recognizes that total available food can include a significant non-chlorophyll component.

Similarly, verification studies with the oyster-P. marinus model for Delaware Bay (Powell et al. 1997) showed that chlorophyll alone did not provide an adequate food supply for the oysters. Therefore, the relationship between chlorophyll and total available food in Delaware Bay was assumed to be of the same form:

. Arnolds Bed
. Cohanseey Bed
Figure 4. Time series of environmental data measured by the Haskin Shellfish Research Laboratory (monthly averages for 1981 to 1986), used as input to the model to simulate average conditions. Panels A, B, and C show time series of temperature, food, and total seston (TSP = total suspended particulates) data, respectively, for seed bed (Section E) and planted ground (Ridge) sites. Panel D shows mean salinity time series, estimated from freshwater inflow data, for the seed bed (Arnolds, Cohanseyy, Shell Rock, and Bennies Beds) and planted ground (Miah Mauull Grounds) sites.

form as that for Galveston Bay and the coefficients were determined by an iterative procedure in which simulations and observations were compared (see Powell et al. 1997 for details). This procedure resulted in a conversion to food supply given by equation (7), but with b having the value 0.26 mg DW L\(^{-1}\).

Total Seston Data Sets

Total seston, as measured by total suspended solids, was rela-
tively constant at Section E (10-12 x 10^3 p.g L^-1) with a slight increase in May to 16 x 10^3 pg L^-1 (Fig. 4C). The Ridge total seston time series was more variable than in Section E, ranging from 20 x 10^3 p.g L^-1 in November to 41 x 10^3 p.g L^-1 in August (Fig. 4C). Total seston values were greater at Ridge for all months.

Salinity Data Sets

Monthly-averaged Delaware River flow for the period 1913 to 1993 was measured at Trenton, New Jersey, by the United States Geological Survey. Salinity time series were then calculated for

\[ v = c + dx'' \]

where \( y \) is salinity in ppt calculated from the preceding mean 30-day river flow \( x \) in ft^3 s^-1 (1 ft^3 s^-1 = 2.83 x 10^2 m^3 s^-1).

Derived values of \( c \), \( d \), and \( e \) for each of the 5 sites shown in Figure
1 are presented in Table 1. The resultant salinity time series were used as input into the oyster population-W. nelsoni model.

At each site, the 8 years (20%) between 1953 and 1993 with the highest calculated average monthly salinity were grouped together and a mean salinity calculated for each month at each site. The resultant time series were assumed to be representative of a year with low freshwater runoff (Fig. 5A). Similarly, the 8 years (20%) with lowest monthly mean salinities were also grouped for each site and the salinity time series was taken to represent a year with high freshwater runoff (Fig. 5B). Salinities for the remaining 24 years (60%) were averaged to create a mean freshwater runoff time series (Fig. 4D).

Although the salinity values changed (Table 2), the seasonal pattern for average and low runoff years was similar (Figs. 4D, 5A). Salinity minima occurred in March/April and the maximum values extended from July through October. The pattern for a high runoff year was only slightly different, with a distinct minimum occurring in April and a shortened period of maximum salinities extending from July through September (Fig. 5B).

To simulate a shift in the timing of spring runoff, the maximum freshwater discharge, which occurred during March/April in the mean time series (Fig. 4D), was moved to either February or May (Fig. 5C, D). For these simulations, only the timing was changed: the quantity of freshwater discharge remained that of an average year.
Prevalence and intensity of *H. nelsoni* in Delaware Bay oysters was measured over the period 1959 to 1992 by personnel from the Haskin Shellfish Research Laboratory (Ford and Haskin 1982, Haskin and Ford 1982, Fegley et al. 1994). Oysters from the lower Bay planted grounds were obtained by dredge at regular intervals during the year. Seed bed oysters were typically collected during late autumn/early winter and the following late spring. These dates were chosen to coincide with peaks in the annual infection cycle (July 1 to June 30 of the following year) (Fig. 2). Stained tissue sections of each oyster were examined to determine the distribution and abundance of *H. nelsoni* in the tissues, and these were

### TABLE 1.

Derived constants for the Delaware River flow-Delaware Bay salinity relationship \( y = a + bx' \) for the 5 sites displayed in Figure 1, where \( v \) is the salinity in ppt and \( x \) is river flow in ft \( 3 \) s\(^{-1} \) (From Haskin 1972).
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Figure 5. Idealized time series of salinity, constructed from Delaware River flow measurements, used as input to the model to simulate A) low freshwater runoff, B) high freshwater runoff. C) a year with an early spring (February) freshwater runoff period, and D) a year with a late spring (May) freshwater runoff period at seed bed (Arnolds. Cohansey, Shell Rock, and Bennies Beds) and planted ground (Miah Maull Grounds) sites.

used to develop a scale. Big Ford Units (BFU, described in Ford et al. this volume), for comparing observed and simulated MSX disease infection and intensity. The BFUs were to define intensity categories as:

BFU = 0; no infections or infections too light for detection by standard histology (hereinafter referred to as "undetectable infections").

BFU = 1; Epithelial infections - H. nelsoni plasmodia in the gill epithelium only.
BFU = 2: Subepithelial/local infections - H. nelsoni have broken through the basal lamina between the epithelium and the underlying tissues, but remain largely concentrated near that site.

BFU = 3; Light systemic infections - H. nelsoni have spread through the tissues via the circulatory system, but parasite abundance remains relatively low.

BFU = 4; Advanced systemic infections - H. nelsoni have spread throughout the tissues via the circulatory system and parasite abundance is high. These are typically lethal infections.

TABLE 2. Salinity ranges for high freshwater runoff, average freshwater runoff, and low freshwater runoff time series (Figs. 4D, 5A, and 5B) for the sites displayed in Figure 1. The high value in each range
represents the late summer values and the low value represents the
spring values.

High Runoff Average Runoff Low Runoff
Station (ppt) (pptt (ppt)

The distribution of BFUs in relation to epithelial and systemic
infections is shown in Figure 4 of Ford et al. (this volume).

Observed infection prevalences and intensities were used to
verify model simulations. In the case of the planted ground simu-
lations, comparisons were made with the complete annual cycle.
For the seed beds, comparisons were made with the winter (No-
ember/December) and spring (late May/early June) prevalence
peaks. Observed category 1 and 2 infections were combined to
represent the prevalence of localized infections and categories 3
and 4 were combined to represent systemic infections. These data
were used to construct a time series of prevalence and intensity
(localized vs. systemic infections) in oyster populations at each
seed bed and planted ground site (Fig. 6). Simulations for Miah
Maull Grounds represent the annual H. nelsoni cycle and infection
levels of the entire leased area, since there is relatively little varia-
tion within this area as compared to the variation among seed beds.
Since measurements at Miah Maull Grounds were sparse, they
were supplemented with data from Deepwater and Southwest Line
planted grounds (Figs. 1,7).

Model Implementation
The oyster population-\textit{nelsoni} model was solved numerically using the 2-step pseudo-steady-state approximation scheme (Verwer and van Loon 1994) with a time step of 1 hour. Each simulation began on June 1 and extended for 3 years, with the first 2 years needed for the solution to reach a steady state. The same input data were used for each of the 3 years. The model assumes that the oysters have been continuously exposed to \textit{H. nelsoni}, as is the natural stock, so that the population starts each cycle with the infection intensity and prevalence remaining at the end of the cycle.

To investigate the effect of salinity variations on the prevalence and intensity of \textit{H. nelsoni} in Delaware Bay oyster populations, a number of stimulations were considered under various environmental conditions (Table 3). A reference simulation was run for each site using mean environmental conditions. A series of simulations to determine the influence of high or low freshwater runoff on infection levels were subsequently run and compared to the reference simulation. Finally, the effect of the timing of the freshwater runoff was investigated.

Statistical Analysis

To analyze variations in prevalence, intensity, and timing of \textit{H. nelsoni} infections in Delaware Bay oyster populations as a result of the various environmental scenarios, several statistical analyses

Solid portions of bars represent localized infections (BFU categories 1 and 2); clear portions represent systemic infections (BFU categories 3 and 4). Annual cycles are represented by 2 samples. Bars on the left of the tick mark represent the spring (May/June) measurements; bars on the right represent the early winter (November/December) measurements. Periods when no data were available are indicated by N. Note that after 1992, Perkinsus marinus, cause of Dermo disease in oysters, became prevalent on the seed beds and confounded H. nelsoni patterns.

were performed on the simulated output. The maximum difference, average difference, root mean square (RMS), and correlation coefficient (r) were calculated for each site to compare each simulation to the reference simulation. Comparisons were made for each infection intensity (BFU) category between time series for
each hypothetical extreme-condition simulated and the corre-
sponding reference time series.

The maximum difference is the greatest percentage point dif-
ference in prevalence for a particular infection category between
the simulated extreme and reference annual infection cycles. A
high value means that an extreme condition caused a relatively
large change in that category of infection compared to the refer-
ence simulation; a low value means that the extreme condition
causd relatively little change:

\[
\text{maximum difference} = \max(A, -B) \]

(10)
Figure 7. Prevalence and intensity of Haplosporidium nelsoni infections in oysters at A) Southwest Line and B) Deepwater planted grounds from records of the Haskin Shellfish Research Laboratory (Fegley et al. 1994). Solid portions of bars represent localized infections (BFU categories 1 and 2); clear portions represent systemic infections (BFU categories 3 and 4). Annual cycles are represented by 2 samples. Bars on the left of the tick mark represent the spring (May/June) measurements; bars on the right represent the early winter (November/December) measurements. Periods when no data were available are indicated by N. Note that after 1990, Perkinsus marimis, cause of Dermo disease in oysters, became prevalent on the New Jersey planted and confounded H. nelsoni patterns.

where $A_{\lambda} = A(t)$ or the MSX prevalence time series for a given location ($\lambda$) and MSX intensity category ($\lambda$) and $B_{\lambda} = B(t)$ for the
The average difference is the average percentage point difference in prevalence of a particular infection category between simulated extreme and reference annual infection cycles. A high value means that an extreme condition caused a relatively large change in that category of infection; a low value means that the extreme condition caused relatively little change:

\[
\text{average difference} = -\frac{1}{T} \sum_{t=1}^{T} (A_t - B_t),
\]

where \(T\) is the length of the simulated time series produced by model simulations.

**TABLE 3.**

Environmental time series for model simulations. Salinity for Anrolds, Cohansey, Shell Rock, and Bennies Beds, and Miah Maull Grounds are indicated by A, C, S, B, and M, respectively. "Dry" and "wet" indicate low and high freshwater runoff, respectively. "Dry" and "wet" indicate low and high freshwater runoff, respectively.

Timing of freshwater discharge is indicated by the month to which the spring discharge event was moved. R and E represent time
series constructed from field measurements at Ridge and Section E

sites, respectively.

Simulation Site

Temperature salinity Food Supply Turbidity

(°C> (ppt) (ugchlaL 1 ) (mgl 1 )

The RMS (root mean square) is a standard measure of the
difference between 2 time series. Squaring the prevalence differences between the extreme A(f) and B(t) reference time series before taking the average enhances the larger differences and diminishes the smaller differences. Therefore, a relatively large difference will result in a larger value than a smaller difference.

Again, a high value means that an extreme condition caused a relatively large change in that category of infection; a low value indicates that the extreme condition caused relatively little change:

\[ \text{RMS}; \]

\[ f^\oplus(Au \text{ B U Y}) \]

\[(12)\]
Spearman's Rank Correlation Coefficient (r) which is given by:

\[
Car
\]

\( (13) \)

\[-AA V "-SB\]

was used to evaluate correlations of ranked prevalences of each infection category between simulated extreme (A(t)) and reference (B(r)) annual infection cycles. The coefficient C_{AB} is defined as the
\[
I, 7 !,(A, , - A)(fi, , - S), \text{ where } A \text{ and } B \text{ are the mean for time series A,, and B,, respectively. Values of } r \text{ near 1 indicate similar temporal patterns (although absolute values can still vary). Values of } r /- \text{ near zero indicate different temporal patterns.}
\]

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Modeling the MSX in Eastern Oyster Populations II

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RESULTS

Model Simulations

Reference Time Series Prevalence
The first set of simulations provided the basic time development of *H. nelsoni* infection prevalence and intensity at the seed bed and planted ground sites. These reference simulations were forced by average environmental conditions (Table 3) at each location and provide a basis for comparison with simulations produced by extreme environmental conditions.

At Arnolds Bed, the most upbay and least saline bed, mean environmental conditions produce no detectable infections (Fig. SA). Infections are first detected just downbay at Cohansey Bed during August, increase in prevalence and intensity during the fall, and reach a winter prevalence maximum of 9% by late October (Fig. 8B). At Shell Rock Bed, a similar pattern is seen with new infections appearing a little earlier, in July, and winter prevalence rising to 20% by November (Fig. 8C). At both sites, little change in total prevalence occurs over the winter. All detectable infections are lost in February and do not reappear in the spring.

Bennies Bed is the first site where the simulation results in an annual cycle more typical of higher salinity regions (with 2 peaks) and retention of some infections throughout the year (Fig. 8D). Prevalence and intensity increase through summer and fall, reaching a maximum by November, when the prevalence is about 45%. Loss of infections in all categories, but primarily systemic, occurs in March: infections re-appear in the spring, coincident with rising water temperatures and salinities. Prevalence reaches 25% in the late May/early June peak.
The reference simulation for Miah Maull, in the planted grounds, produces the highest infection levels (Fig. 8E). From a low of 5% to 107r in July, prevalence increases to 60% to 65%, with a little more than half the infections being systemic by November. A loss of primarily systemic infections causes a total prevalence decline in March and April, followed by a second prevalence peak of 55% in late May, in which most infections are advanced. A rapid loss of infections follows by early summer (see also Ford et al. this volume).

Month

Reference Time Series Intensity

Under average conditions, approximately half the simulated
infections in seed bed oysters are systemic at the November/December and late May/early June peaks (Fig. 8A-D). At the Miah Maull Grounds site, about half the winter infections are systemic. In contrast, the spring peak was predominantly systemic, with a large fraction in category 4, advanced systemic infections (Fig. 8E).

Extremes in Freshwater Runoff Simulations

Low Freshwater Runoff. During periods when freshwater runoff is lower than average, salinity in Delaware Bay increases (Fig. 5A) and elevated H. nelsoni infection prevalence results at all sites. Unlike average conditions, low freshwater runoff simulations produce detectable infections at Arnolds Bed, beginning in August (Figs. 8A, 9A). Infections develop slowly through the fall, reaching a winter prevalence peak of 8%. At Cohansey Bed, detectable infections appear in June and reach a maximum prevalence of about 35% by November. At Arnolds Bed, all detectable infections are lost in January; the decline occurs in February at Cohansey, where nearly all infections are lost. At neither site do infections re-appear in the spring.

Under the low runoff conditions, the simulated annual cycle for Shell Rock Bed is similar to that for Bennies Bed under average conditions: a 2-peak annual cycle with a substantial fraction of infections persisting throughout the year (Fig. 9C). The winter prevalence peak is approximately 45%. Loss of infections, primarily systemic, occurs in March. The spring peak prevalence is about 30%. Low-flow simulations produce almost identical cycle pat-
terns and levels at Bennies Bed, representing the lower seed beds, as they do at Miah Maull. in the planting grounds (Fig. 9D, E).

Both cycles show distinct winter and spring peaks with prevalence maxima of 60% to 65%. A moderate prevalence decrease in late winter, and a major loss of infections after the May peak.

As in the reference simulations, about half the infections are systemic by early winter (Fig. 9). The spring peak, however, produces relatively heavier infections, as well as higher prevalences at Shell Rock and Bennies Beds (Fig. 9C, D). The pattern at Bennies is nearly identical with that of the Miah Maull simulations, with a substantial number of category 4 infections (Fig. 9E).
Figure 9. Simulated time evolution of Haplosporidium nelsoni prevalence and intensity produced by low freshwater conditions at A) Arnolds Bed, B) Cohansey Bed, C) Shell Rock Bed, D) Bennies Bed, and E) Miah Maull Grounds. Numbers 1 through 4 refer to the cumulative percentage of total oysters (prevalence) with epithelial (1), subepithelial local (2), light systemic (3), and advanced systemic (4) infections. The area above the category 4 line represents the portion of oysters in the undetectable infection category.

Figure 10. Simulated, time evolution of Haplosporidium nelsoni prevalence and intensity produced by high freshwater discharge conditions at A) Bennies Bed and B) Miah Maull (irounds). Numbers 1 through 4 refer to the cumulative percentage of total oysters (prevalence) with epithelial (1), subepithelial local (2), light systemic (3), and advanced systemic (4) infections. The area above the category 4 line represents the portion of oysters in the undetectable infection category.

High Freshwater Runoff. Salinity conditions during periods of high freshwater runoff (Fig. 5B) result in no detectable H. nelsoni infections at Arnolds, Cohansey, or Shell Rock Beds. Infections appear at Bennies Bed in July, but reach a maximum prevalence of only 9% from November through January, considerably lower than
the 40% to 45% prevalence found under average conditions (Figs. 8D, 10A). Infections disappear in February and do not reappear in spring. Compared to average conditions, there is little change in the Miah Maull Grounds cycle under high runoff.

Changes in Timing of Freshwater Runoff

Variations in the timing of maximum runoff from early (Fig. 5C) to late spring (Fig. 5D) affects H. nelsoni prevalence and intensity at Bennies Bed only. The major differences between the 2 extreme cycles (Fig. 11A, B) and that produced under average conditions (Fig. 8D) are in the degree of late winter infection loss and the extent of the late spring prevalence peak and subsequent decline. A February runoff produces a major loss of infections (from 45% to about 5%) in March followed by a relatively small resurgence, to 20%, in late May, and no subsequent decline (Fig. 11A). Understandably, a May runoff does not affect the late winter prevalence decline, which remains the same as under average conditions. It does, however, allow the late May prevalence peak to become higher than under average conditions (45% vs. 25%).

More importantly, most of the infections become systemic (BFUs 3 and 4), resulting in a pronounced decline in prevalence in July (Fig. 11B) which was produced by a larger-than-normal number of heavily infected individuals in June that undergo attempted sporulation. After this decline, however, newly acquired infections quickly proliferate so that by late fall, and over the winter, there are no differences in prevalence or intensity among the early, average, and late discharge simulations.
Statistical Comparisons Between Extreme and Average Model Simulations

Because the first 2 years of the 3-year model runs were needed for the solution to reach a steady state, statistical analyses were done using third-year simulations only. The maximum difference.

Figure 11. Simulated, time evolution of Haplosporidium nelsoni prevalence and intensity at Bennies Bed for A) early (February) and B) late (May! high freshwater discharge events. Numbers 1 through 4 refer to the cumulative percentage of total oysters (prevalence) with epithelial (1), subepithelial local (2), light systemic (3), and advanced systemic (4) infections. The area above the category 4 line represents the portion of oysters with undetectable infections. Arrows show the timing of the freshwater discharge.
average difference, root mean square (RMS), and correlation coefficients ($\rho$) between the reference simulation and each simulation with varied environmental conditions were calculated to quantify the effects of salinity variation of H. nelsoni on each infection category.

Variations in the Magnitude of Runoff

Low Freshwater Discharge. Conditions of low freshwater discharge have the largest effect on the undetectable infection category, as shown by high maximum difference, average difference, and RMS values (Table 4). Undetectable infections are, in effect, an inverse measure of percent infection. Thus, the decrease in this category in a high salinity regime simply means an increase in total prevalence. The largest differences associated with low discharge occurs at mid-bay sites, as suggested by the high maximum difference, average difference, and RMS values. Low correlation coefficients (<0.50) and relatively large maximum difference values were also calculated at Shell Rock and Bennies Beds for infection

TABLE 4.

Results of statistical analysis of the differences between low freshwater runoff and reference simulations at the seed bed and planted ground sites. Haplosporidium nelsoni infection intensity
categories are UD = undetectable infections, 1 = epithelial, 2 = subepithelial local, 3 = light systemic, and 4 = systemic infections.

"Maximum" and "Average" represent the maximum and average prevalence differences over an annual infection cycle. RMS represents the root mean square error, and r is the correlation coefficient. Bold is used to emphasize extreme differences.

Site

Intensity Maximum Average RMS
category (%) (%) (%) r

categories 1, 3, and 4. reflecting increased intensity as infections move from category 1 to categories 3 and 4 under high salinity conditions. Correlation coefficients of 1.0 for every infection category at Miah Maull Grounds, as well as low values for the other 3 measures, indicate that there was almost no difference in prevalence or intensity associated with low runoff at this site.

High Freshwater Discharge. The greatest variation in simulated prevalences at all sites during high freshwater runoff occurs in the undetectable infection category (Table 5). This is consistent with the results of low freshwater discharge conditions and reflects the
loss of parasites under low salinity conditions and a decrease in total prevalence. The greatest variability in maximum and total difference, and in RMS, is at Bennies Bed. Overall, correlation coefficients are positive and high (0.50 in all but one comparison, at Bennies Bed), and are 0.99 in all infection categories at Miah Maull Grounds. Values for the other three measures are lowest at the Miah Maull site, indicating little change in infection intensity and prevalence associated with high freshwater discharge at this location.

Variation in Timing of Freshwater Discharge

The simulated prevalence and intensity obtained for early and late freshwater runoff conditions at Arnolds, Cohansey, and Shell

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TABLE 5.

Results of statistical analysis of the differences between high freshwater runoff and reference simulations at the seed bed and planted ground sites. Haplosporidium ielsoni infection intensity
categories are UD = undetectable infections, 1 = epithelial, 2 =
subepithelial local, 3 = light systemic, and 4 = systemic infections.

"Maximum" and "Average" represent the maximum and average
prevalence differences over an annual infection cycle. RMS
represents the root mean square error, and r is the correlation
coefficient. Bold is used to emphasize extreme differences

TABLE 6.

Results of statistical analysis of the differences between early spring
freshwater runoff, late spring freshwater runoff and reference
simulations at Bennies Bed. Haplosporidium nelsoni infection
intensity categories are UD = undetectable infections, I = epithelial,
2 = subepithelial local, 3 = light systemic, and 4 = systemic
infections. "Maximum" and "Average" represent the maximum and
average prevalence differences over an annual infection cycle. RMS
represents the root mean square error, and \( r \) is the correlation coefficient. Bold is used to emphasize extreme differences.

Intensity Maximum Average RMS

Station category (%) (%) (%)

Simulation

Intensity Maximum Average RMS
category (\( c \), I) (%) (%)

Cohansey Bed

Shell Rock Bed

Bennies Bed

Miah Maull Grounds

US

1

2

3
<table>
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<tr>
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</tr>
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</tr>
<tr>
<td>4.46</td>
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<td>4.14</td>
</tr>
</tbody>
</table>
Early freshwater runoff

Late freshwater runoff

UD

1

2
tions for Miah Maull in the planted grounds were compared with
the annual infection cycle for that region, as described by Ford and
Haskin (1982). On the other hand, data from the low salinity seed
beds consist primarily of samples taken at winter and spring in-
fection peaks (Haskin and Ford 1982. Fegley et al. 1994). Conse-
quently, model output for this region of the bay was tested by
comparing simulated prevalences and intensities at the peaks with
observed values under similar ambient conditions.

Rock Beds and at Miah Maull Grounds do not differ significantly
from those obtained with the reference simulations (RMS < 0.37%
and r > 0.98). The only significant modifications of the annual
infection cycle associated with early or late discharge occur at
Bennies Bed. Under early (February) runoff conditions, the great-
est deviation from average was produced in undetectable and cat-
egory 1 infections, as indicated by the maximum difference value at these categories (Table 6). A comparatively low correlation (0.59) in category 1 reflects the light infections present during the late spring and early summer, which never progress to higher levels (Fig. 11A) as they do under average conditions (Fig. 8D). The undetectable and category 1 infections were also affected by late runoff, as indicated by the maximum difference values. The greatest maximum difference value was in category 3 infections (Table 6), reflecting the high percentage of light systemic infections in the late spring peak (Fig. 11B) compared to the average cycle (Fig. 8D).

DISCUSSION

Comparison of Model Simulations with Field Observations

A test of the accuracy of the oyster population-/*, nelsoni model is to compare simulated with observed infection prevalence and intensity patterns and levels under different environmental conditions. This was done in two ways. For the high salinity region of lower Delaware Bay, where considerable data are available on H. nelsoni infections throughout the year (Fig. 2), model simu-

The Annual H. nelsoni Infection Cycle in a High-Salinity Location

The onset of the simulated infection cycle at the Miah Maull Grounds begins in June. Prevalences and intensities increase, reaching a maximum prevalence of 65% in December (Fig. 8E).
This value is consistent with prevalences reported by Ford and Haskin (1982) for oysters in the planted ground area, which averaged 40% to 60% in most years, with minimum and maximum prevalences of 15% and 100%, respectively. As discussed by Ford et al. (this volume) the simulated proliferation of parasites during warm weather is primarily determined by temperature, but the reduction in proliferation rates apparent by late fall is caused by a combination of falling temperatures and high parasite density in oyster tissue. The latter inhibits proliferation because parasites are competing for a limited and declining nutrient resource within the host.

In late winter, the simulated infection cycle shows a marked loss of infected oysters, as does the observed cycle (Figs. 2. 8E). Observed mortality of severely infected oysters occurs at this time, reducing both prevalence and intensity. In addition, intensity lessens because parasites do not tolerate overwinter conditions well. Perhaps this is due to a direct low temperature intolerance or other unfavorable influences facing the parasite under the prolonged anaerobic conditions that occur inside the oyster, over the winter (Ford and Haskin 1982). For instance, H. nelsoni plasmodia contain numerous mitochondria (Muller 1967, Perkins 1968), which suggests that the parasite is well equipped for aerobic metabolism and may not be able to tolerate low oxygen well. The model
simulates the degeneration and disappearance of parasites at this time by including a susceptibility factor, which is applied to parasites and which increases as a function of degree days below 5 °C (Ford et al. this volume). As susceptibility increases, the parasites become more vulnerable to attack by host hemoeytes. Hemocytes themselves are becoming more active with increasing spring temperatures and, as a consequence, can readily eliminate the damaged parasites from the host.

Both simulated and observed annual cycles contain a distinct second peak, reached in late May or early June. This peak contains many very heavily infected oysters due to increasing temperatures that cause a swift resurgence of those infections that were suppressed over the winter (Andrews 1966, Ford 1985b. Ford and Haskin 1982). The model, however, was unable to reproduce the very rapid infection development by temperature alone. A second factor was added, which eased the density-dependent limits on parasite growth as a consequence of improving conditions within the oyster (Ford et al. this volume). The increasing quality of the parasite's environment, hypothesized to occur at this time, is due to the spring bloom, increased feeding rates of the host, and the cleansing of waste products that have accumulated in the host over the winter.

Finally, both simulated and observed cycles show a marked decline in prevalence following the spring peak (Figs. 2, 8E).
Heavily infected oysters die at this time, accounting for some of the decline. Additionally, parasites are lost from living oysters for reasons that are not clear, but may have to do with attempted, but unsuccessful, sporulation and consequently a failure on the part of the parasite to complete its life cycle (Ford and Haskin 1982. Ford et al. this volume).

Peak Infection Levels at Low Salinity Seed Bed Locations

To compare simulations for the seed bed sites (Fig. 1) with field measurements, the latter were grouped, by site, according to years of high, average, and low freshwater runoff. The grouping criteria were the same as those used to produce salinity time series for these conditions. That is, the average years were those in the middle 60% of the range; the high and low runoff years were the 20% at either extreme. For each flow regime and site, the mean, median, and range of observed prevalences at both late fall and spring peaks were obtained (Table 7). Because of the limited number of observations (Fig. 6) Shell Rock Bed was not included in this comparison.

Comparison of initial simulations with observations showed that the timing of infections was correct, but that prevalences at the seed bed sites were too high. The relationship used to model the host-parasite relationship under varying salinity was derived from a study in which parasites were subjected to acute in vitro salinity change (Ford and Haskin 1988). modified to simulate more
gradual in vivo changes in salt content of host fluids (equation 3).
The inability of the model to reproduce observed prevalences using this relationship alone suggested that prevalence was influenced by an additional factor or factors.

Although temperatures from only a single site, Section E (Fig. 4A), were used in reference simulations for all seed bed sites, monthly-averaged temperatures varied by a maximum of only 1.4 °C from the planted grounds to the seed bed areas (Fig. 4A). Further, temperature variations within the seed bed area are relatively small (Fegley et al. 1994). Thus, temperature differences are unlikely to be responsible for the disparity.

Another possible factor could be salinity-associated differences in the rate of infection. The infective stage of H. nelsoni remains unknown. Spores, typically an important element in parasite transmission, are rare in adult oysters and their absence has led to the hypothesis that another host may be involved in the life cycle (Burreson 1988, Haskin and Andrews 1988). On the other hand, spores appear to be produced regularly in juvenile oysters with advanced infections (Barber and Ford 1992, Burreson 1994), so

TABLE 7.

Comparison of simulated total prevalence with observed mean, median, and range of prevalences on Delaware Bay sites (Fig. 1) under High, Average, and Low Delaware River runoff conditions. PG indicates various planted grounds (Fig. 7).
direct transmission between oysters remains a possibility. Further. Barber and Ford (1992) found that haplosporidian spores, morphologically similar to those of H. nelsoni, are present in the water column in Delaware Bay in approximately equal abundance in upper and lower Bay sites. These authors speculated that if these spores are, in fact, H. nelsoni and are the infective element, the observed disparity between their concentration and infection prevalence in the upper and lower Bay could be explained by reduced infectivity under low salinity conditions. Alternatively, if another host is involved, the disparity could be explained by a salinity effect on another host or on its relationship with H. nelsoni.

The discrepancy was resolved in the model by adding an infection efficiency factory (equation 6), which reduces infection rates with decreasing salinity. This relationship implies nothing about the source or form of the infective particle; however, it does suggest that some element in the life cycle other than the plasmodial stage in the oyster, is sensitive to low salinity. This includes, but is not limited to, an intermediate or reservoir (alternate) host, the abundance of infective stages in the water, or the ability of infective stages to invade and establish themselves in oysters.
Addition of infection efficiency to the model resulted in simulated prevalences that largely matched those observed (Table 7). The simulated levels were most like the median prevalences, which they reproduced almost exactly in many cases. Notably, the model produced no detectable infections on Arnolds Bed except under low flow conditions, as observed, and reproduced the elimination of the spring peak in cases where it was observed in the field. The model also accurately simulated the relative insensitivity to river flow changes of the lower bay infection cycle that is observed in nature.

Only on Bennies Bed for average conditions and at Bennies and Cohansey Beds for low flow conditions, did the simulations not closely reproduce the mean or median observed prevalences (Table 7). The simulated prevalences were higher, especially under the low flow conditions, although they were clearly within the range of observations. Similarly, the modeled prevalence for Miah Maull Grounds tended to be at the high end of the observed mean or median range for the lower Bay planted grounds. It is clear that observed prevalence in the moderate to high salinity region of the bay varies widely and in some years no infected oysters are found, regardless of salinity (Figs. 6, 7; Table 7). Multiyear cycles in *H. nelsoni* prevalence, totally independent of river flow and salinity, were described by Ford and Haskin (1982) for lower Delaware Bay. The salinity in lower Delaware Bay, in fact, is always high enough to support full *H. nelsoni* activity so that year-to-year variations must be due to some other condition. The fact that disparities between observed and simulated prevalences were
maximum at mid-Bay sites under low flow, high salinity conditions, which produce infection levels and patterns similar to the lower Bay. suggests that they are influenced by factors other than the local salinity. The possible factors are included in a more complex disease transmission model as described in Powell et al. (this volume).

Another element of the basic model that was modified to simulate observed conditions involved infection intensity. Long-term observed averages show that despite decreasing prevalence, the ratio of systemic to localized infections remains essentially 1:1 along the salinity gradient in the seed bed regions of Delaware Bay (Ford and Haskin 1982, Fegley et al. 1994). However, the simulated intensity decreased upbay. To improve the model, the transfer rate of parasites from the epithelium to the systemic tissue was increased with decreasing salinity. The addition of this formulation to the model caused about half of the infections to remain systemic at peak prevalence on seed bed sites, while still allowing more intense infections to develop at the spring peak under the normally high salinity conditions of the lower bay and. at low river flow, on the lower seed beds. When a strong spring peak occurs, which it does only under high salinity situations, oysters often have the most advanced infections of the year, so that simulations reproduce observed intensities under high salinity conditions also.

The biological basis for the maintenance of high systemic infection levels under conditions of decreasing prevalence along the salinity gradient is unclear. Further, the mechanism used by para-
sites to penetrate the basal lamina between the epithelium and the underlying tissue is not known (Scro and Ford 1990). Thus, there is no direct evidence that low salinity enhances the transfer rate between the two tissues. On the other hand, model simulations were run for a population in which all oysters were the same size (1 g dry weight). However, adult size generally decreases along the salinity gradient in Delaware Bay, and along with it occurs an increase in the surface (epithelium) to volume (systemic) ratio. A given concentration of parasites per unit area of epithelium in a small oyster is actually a larger total parasite load in the surface layer than it is in a larger oyster. In some way, this large overall burden may speed the movement of parasites into the underlying tissue and thus help explain the observed relatively high intensity levels on the seed beds. The addition of multiple oyster size classes to the model is needed to test this hypothesis.

Simulations were also run with variations in food availability in the spring and with warm winter conditions (>5 °C) to determine the relative effects of salinity compared to food and temperature on oyster populations infected with H. nelsoni. Variations in salinity had a larger effect on the simulated infection levels than did variations in food availability and winter temperatures in a population of 1-g oysters. However, the simulated time development of infections did demonstrate the importance of cold winters in producing a reduction in infections the following spring, and therefore modulating the annual cycle.

When absolute differences between average and extreme con-
ditions were compared statistically, the mid-Bay locations of Shell
Rock and Bennies Beds showed the greatest variations. The ex-
planation lies in the fact that mean mid-tide salinities at these
locations range from 9.5-11.2 ppt to 19.3-21.1 ppt (Table 2), and
it is in this range that a shift in salinity causes the greatest change
in parasite survival (Fig. 3). In addition, Bennies Bed (salinity
range 13.3 to 18.7 under average conditions) was the only site at
which simulated variation in the timing of "spring" runoff caused
a significant change from the reference annual cycle. This is be-
cause Bennies Bed, under average conditions, was the only seed
bed to exhibit a late spring prevalence peak (Fig. 8D) and thus the
only bed that had any infections to be altered by differences in
timing. Salinity was always low enough to permit a spring MSX
prevalence peak at the upper bay sites irregardless of the timing of
the spring runoff and salinity in the planted grounds was high
enough (>20 ppt) nearly all the time to support infections so that
changes in timing, like changes in volume of discharge, would
have little effect on the spring peak at Miah Maull Grounds.

Summary and Conclusions

The simulations obtained with the oyster population-//, nelsoni
model were able to reproduce observed infection patterns and lev-
The inability of initial simulations, using this direct salinity-parasite function, to produce infection levels that adequately represented field observations, indicate a more complex host-parasite relationship at low salinity. Two additional functions, both based on assumptions, were added to the model to improve simulations. One allowed infection intensity to remain stable along the decreasing salinity gradient by increasing the transfer rate of parasites from epithelial to systemic tissues. The biological rationale may be linked to decreasing size of oysters and increasing surface-to-volume ratios along the salinity gradient. It is also possible that the transfer rate change may simply be a substitute for some other mechanism that allows intense infections to occur at low prevalence. The second modification was to diminish infection efficiency with decreasing salinity. Because the mode of transmission of H. nelsoni and the form of its infective stage remain unknown, this assumption cannot be verified. Reduced infection efficiency may also be a surrogate for another mechanism, such as abundance of a potential intermediate or alternate host, the abundance of infective stages, or both.

The importance of these modifications is not necessarily the assumptions that were made, but that they show that factors other than a simple, direct salinity effect on H. nelsoni proliferation and death rates in the oyster are required to explain field observations. The model results emphasize that in the H. nelsoni relationship,
salinity is a permissive/restrictive element, modifying the degree and pattern of infections, during transmission as well as after oysters become infected.

Another, overriding factor, is the presence and abundance of infective particles, which varies from year to year. Model simulations show that in low salinity environments, infection levels are clearly correlated with river flow and that salinity changes over the 10-20 ppt range have the greatest effect on infection patterns and level. This association, however, breaks down at high salinity. Infection levels in high salinity (>20 ppt) regions of Delaware Bay are uncorrected with river flow, as was noted earlier (Haskin and Ford 1982), and is attributed to fluctuations in infective particle abundance. The model shows that under drought conditions, the same factor or factors override local salinity to control infections in mid-Bay locations.

The ability to investigate H. He/.wm'-oyster-salinity relationships with relative ease using the coupled model demonstrates its usefulness as a practical tool. In many regions other than Delaware Bay, low salinity areas are critical as setting and storage areas for seed oysters. The capacity of the model to simulate, with only minor modifications, observed H. nelsoni levels in mid-Chesapeake Bay demonstrates that it is robust and can be employed in other areas (Powell et al. this volume).

ACKNOWLEDGMENTS
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LITERATURE CITED


Ford, S. E. 1985a. Effects of salinity on survival of the MSX parasite
Haplosporidium nelsoni (Haskin, Stauber, and Mackin) in oysters. J.

Shellfish Res. 5:85-90.

Ford, S. E. 1985b. Chronic infections of Haplosporidium nelsoni (MSX) in


Ford, S. E. 1997. History and present status of molluscan shellfisheries

from Barnegat Bay to Delaware Bay. NOAA Tech. Rep. NMFS-127.

Ford, S. E. & H. H. Haskin. 1982. History and epizootiology of Haplospo-

ridium nelsoni (MSX). an oyster pathogen in Delaware Bay. 1957-


of the oyster parasite, Haplosporidium nelsoni (MSX) and hemocytes

from the host, Crassostrea virginica. Comp. Biochem. Physiol. 90A:

183-187.


the MSX in Eastern Oyster (Crassostrea virginica) populations. I.

Model development, implementation and verification. /. Shellfish Res.

18:473^198


the life cycle of the Eastern oyster pathogen Haplosporidium nelsoni


nelsoni (MSX) mortality in laboratory-reared and native oyster stocks

in Delaware Bay. Mar. Fish. Rev. 41:54—63.

Haskin, H. H. & S. E. Ford. 1982. Haplosporidium nelsoni (MSX) on

[Begin Page: Page 516]

516

Paraso et al.

Delaware Bay seed oyster beds: A host-parasite relationship along a


sp. (Haplosporida. haplosporidiidae): Causative agent of the Delaware


Modeling oyster populations II. Adult size and reproductive effort. J.


