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THE POTENTIAL FOR OYSTERS, *CRASSOSTREA VIRGINICA*, TO DEVELOP RESISTANCE TO DERMO DISEASE IN THE FIELD: EVALUATION USING A GENE-BASED POPULATION DYNAMICS MODEL

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ABSTRACT Today, populations of eastern oysters, *Crassostrea virginica*, are commonly limited by disease mortality. Resistance to MSX disease has developed in a number of cases, but the development of resistance to Dermo disease would appear to be limited, despite the high mortality rates and frequency of epizootics. Can aspects of the host's genetics or population dynamics limit the response to the disease despite the apparent opportunity afforded by alleles conferring disease resistance or tolerance? To answer this question, we use a gene-based population dynamics model, configured for *C. virginica*, to simulate the development of disease resistance using mortality as the agent of selection. Simulated populations were exposed to 4 levels of mortality covering the range in mortality observed in Delaware Bay in the 1990s. In each case, disease resistance increased in the simulated population over time, normally proportional to the increase in mortality rate imposed by the disease. However, simulations show that the population responds even at its most rapid rate on multidecadal to half-century timescales. As the mortality rate declines with increasing disease resistance, the rate of further improvement in disease resistance likewise declines. Thus, disease resistance develops over decadal timescales at a 40%-per-year mortality rate, but, as mortality rate falls to 25% per year, the rate of further development of disease resistance extends to half-century timescales. The discouraging profundity is that a mortality rate of 25% per year, yielding a rate of selection profoundly slow, is still very high. In northern climes, significant decrements in oyster abundance will occur. Evidence from fisheries retrospectives suggests that oysters cannot withstand a constant removal at this scale without compromising population integrity noticeably. So, a mortality rate that grievously limits the development of disease resistance still sorely strains the species' ability to maintain a vibrant population necessary to its long-term survival.

KEY WORDS: oyster, resistance, Dermo, model

INTRODUCTION

Populations of eastern oysters, *Crassostrea virginica*, are commonly limited by disease mortality (Mann et al. 2009b, Powell et al. 2009a, Powell et al. 2009b). Two diseases are most significant, MSX and Dermo caused, respectively, by the protistans *Haplosporidium nelsoni* and *Perkinsus marinus* (e.g., Andrews 1979, Ford & Haskin 1982, Andrews 1988, Ford & Tripp 1996). Of the two, Dermo is by far the most geographically widespread and, thus, a dominant factor in the population dynamics of the oyster over much of its latitudinal range (e.g., Wilson et al. 1990, Ray 1996, Cook et al. 1998, Ford & Smolowitz 2007, Gullian-Klanian et al. 2008, Pecher et al. 2008, but see Ulrich et al. 2007). Dermo is routinely epizootic throughout much of the Gulf of Mexico (e.g., Powell et al. 1992, Kim & Powell 1998, Soniat et al. 2009), the southeastern coast of the United States (e.g., Burrell et al. 1984, White et al. 1998, Kim & Powell 2006), and north of Cape Hatteras at least to Delaware Bay (Jordan 1995, Ragone Calvo et al. 2001, Powell et al. 2008), with sporadic outbreaks farther north (Brousseau 1996, Brousseau et al. 1998, Ford & Smolowitz 2007). *Perkinsus marinus* was first observed in the Gulf of Mexico during the late 1940s (Mackin 1953, Ray 1954, Mackin & Hopkins 1962) and soon thereafter in Chesapeake Bay (Andrews 1954, Andrews 1996). The parasite is limited by winter temperatures, thus its northern range limit remained the Chesapeake Bay for some decades thereafter. However, warming of the Mid-Atlantic Bight has permitted

P. marinus to expand its range rapidly northward during the past two decades (Ford 1996, Cook et al. 1998).

Whether *P. marinus* has always been present in the Gulf of Mexico and southern United States is an open question (Ray 1996). What is clear is that the parasite has been an important contributor to the mortality rate[1] of adult oysters in this region since its first observation (e.g., Mackin et al. 1950, Mackin 1959, Soniat & Brody 1988, Soniat et al. 1989, Soniat et al. 2006). Farther north, in Delaware Bay, the mortality rate is lessened; however, even here, Dermo disease at least doubles the natural mortality rate of the adult animals in epizootic years (Powell et al. 2008, Powell et al. 2009a). Mortality rates of this order should result in rapid development of disease resistance in the naïve population (e.g., Duffy & Sivars-Becker 2007, Zbinden et al. 2008, Duffy et al. 2009). A similar challenge by MSX during the late 1950s through early 1960s resulted in documented increases in disease resistance over a few generations (Haskin & Ford 1979, Ford & Haskin 1982, Ford 1988, Burreson 1991). Nevertheless, for Dermo, the increase in disease resistance, if anything, has been extremely limited (Encomio et al. 2005, but see Brown et al. 2005a, Brown et al. 2005b), if the continuing high infection intensities and mortalities throughout much of its range can be inferred as an affirmation (e.g., Soniat & Gauthier 1989, Crosby & Roberts 1990, Hofmann et al. 1995, Volety et al. 2000, and others heretofore referenced). Directed breeding programs anecdotally have produced marginally better results, although published affirmations are few, but even here the differential between MSX and Dermo would seem to be dramatic, as intensive breeding programs have successfully developed oyster strains that are substantially MSX resistant (Haskin & Ford 1979,

Ford & Haskin 1987, Allen et al. 1993). The conundrum we address in this contribution is the incongruity between the impact of the parasite *Perkinsus marinus* on the population dynamics of the host *C. virginica* relative to the trifling response of the host to this challenge.

Some resolutions to this enigma may be postulated. First, the oyster's immune system is limited in its response or easily compromised by the environment (e.g., Ford & Tripp 1996, Hégaret et al. 2004). Although the development of disease resistance has been documented for a number of pathogens (Ford & Tripp 1996, Barber et al. 1998, Oliver et al. 2003, Gómez León et al. 2008), in the case of *P. marinus*, although immune responses have been documented (e.g., Anderson et al. 1992, Chu & LaPeyre 1993, Volety & Fisher 2000; Gauthier & Vasta 2002, Earnhart et al. 2005), these simply may be inadequate (e.g., Chu et al. 1993, Anderson & Beaven 2001, Earnhart & Kaattari 2003, Villalba et al. 2004). *P. marinus* can reside intracellularly in hemocytes (Mackin 1951, Mackin 1962, Goedken et al. 2005) as well as extracellularly and throughout the tissues (Bushek et al. 1994, Anderson 1996, Gauthier & Vasta 2002, Nickens et al. 2002), and the cell coat may contain antigens unrecognized by the oyster's immune system (see Choi et al. 1991, Dungan & Roberson 1993, Montes et al. 2005). Second, development of resistance or tolerance to Dermo disease may be countered rapidly by changing virulence. Competition between the development of host resistance or tolerance and the progression of parasite virulence is a well-documented component of host–parasite evolutionary models (e.g., Lenski & May 1994, Frank 1996, Boots et al. 2004, Schneider & Ayres 2008, Duffy & Forde 2009). Different strains of *P. marinus* have been observed (Bushek & Allen 1996, Gaffney & Bushek 1996, Reece et al. 2001, Panko et al. 2008), and they may be associated with a range of virulence (Brown et al. 2005a, Brown et al. 2005b, Earnhart et al. 2004), although confirmatory data remain limited. Nevertheless, some evidence suggests that *P. marinus* can circumvent part of the oyster's immune capacity (e.g., Winstead & Couch 1988, Anderson et al. 1992, Cheng & Manzi 1996, Goedken et al. 2005), and virulence is known to be lost rapidly during *in vitro* culture (Ford et al. 2002). Third, *P. marinus* is a disease that dominantly kills mature animals, typically after at least one spawning cycle. Impact on oyster reproduction does not occur until infections are near lethal levels (Choi et al. 1989, Paynter 1996, Dittman et al. 2001). Normally, spawning occurs prior to disease intensification (Ford & Tripp 1996). The timing of reproduction and the tendency for a number of reproductive events to occur prior to death should limit the effectiveness of selection for disease resistance or increased tolerance in the population. Fourth, other aspects of the population dynamics may limit the host response as well, including rapid growth in the southern portion of the range (e.g., Butler 1952, Ingle & Dawson 1952, Hayes & Menzel 1981), the density dependency of *P. marinus* doubling times at higher infection levels (Saunders et al. 1993), and the apparently limited physiological impact on the oyster except at the highest infection intensities (e.g., Paynter & Burreson 1991, Paynter 1996, Dittman et al. 2001).

The potential for the development of disease resistance would appear to exist, however. Oyster strains are observed to vary in their response to Dermo disease (Ray & Chandler 1955, Andrews & Hewatt 1957, Bushek & Allen 1996, Gaffney & Bushek 1996, Brown et al. 2005a, Brown et al. 2005b), suggesting a potential for disease resistance, although associations with environmental variables that might degrade the immune response (Craig et al. 1989, Chu & Hale 1994, Lenihan et al. 1999, Bushek et al. 2007, Gray et al. 2009), also well documented, limit the inference based on currently available data. Of more significance, perhaps, alleles associated with resistance or tolerance are known, so that an inherent capability would seem present. In what follows, we conflate the concepts of resistance and tolerance, although they are distinctive (Schneider & Ayres 2008, Hasu et al. 2009), because information currently available does not distinguish a reduction in mortality resulting from limitation in the proliferation of the disease from a reduction in mortality resulting from a lessoning of the systemic impact of that proliferation. We also discount the concept of immunity (e.g., Hoshen et al. 2000, Harding et al. 2005, Duffy & Sivars-Becker 2007, Duffy et al. 2009) and the importance of susceptibility limiting transmission. No evidence exists that oysters can divest themselves completely of the pathogen once infected, and essentially all oysters in areas down-estuary of the lowest salinity reaches, at latitudes where winter temperatures are not limiting, are infected within their first 1–2 y of life. Although slowing the rate of infection may offer an explanation for the low infection intensities in other oyster species (Meyers et al. 1991, Chu et al. 1996), for *C. virginica*, it is more likely that a decrease in the rate of parasite proliferation or the ability to tolerate a given infection level better will be responsible for any improvement in survival.

It is the incongruity of the potential for the development of disease resistance contrasted to the ostensible minimality of the result that we seek to investigate in this contribution. Can aspects of the host population dynamics limit the response to the disease despite the apparent opportunity afforded by alleles conferring disease resistance? To examine this question, we configure a gene-based population dynamics model for the genetic structure of *C. virginica*, imbuing the simulated animals with the known complement of loci with alleles conferring disease resistance and the relative selective advantage thought to be conferred through dominance. Then we ask a series of questions concerning the interaction of genotype, pathogen virulence, host population abundance, and host population dynamics to evaluate potential impediments to the development of disease resistance that might be present.

METHODS

The Model DyPoGEn: Model Structure and Flow

The Dynamic Population Genetics Engine (DyPoGen) is a numerical model configured for this implementation to simulate the genetic structure and population dynamics of *C. virginica*. The model simulates a population composed of multiple cohorts, each composed of multiple individuals. However, the age, sex, and genotype of each individual is stored independently. The genetic structure of each oyster is defined by 10 pairs of chromosomes (Wang et al. 1999, Wang et al. 2005), each with 4 genes, each with 2 alleles. Thus, each animal is specified by 40 genes and 80 alleles, and the genotypes permitted are *AA, AB,* and *BB*.

To initiate a simulation, an initial population numbering `NewAnimals`[2] is created with a biased random genetic structure. Alleles on loci not involved in disease resistance are assigned to the *A* or *B* genotype randomly. Alleles identified as conferring Dermo disease resistance were assigned to a 1:9 *A:B* probability. Thus, initial allelic frequency for alleles

conferring disease resistance, all given the *A* genotype, approximates 10%. This initially low allelic frequency invokes the assumption that alleles conferring disease resistance are rare in naïve populations because such alleles are likely to be deleterious if the disease is not present (e.g., Cotter et al. 2004, Zbinden et al. 2008, Hasu et al. 2009, Duffy & Forde 2009). The allelic frequency at initialization, however, is relatively high in comparison with some other models simulating the onset of disease in naive populations (e.g., Wilhoit 1991, MacKenzie & Bishop 1999, Galvani & Slatkin 2004, Abell et al. 2005).

Each simulated mating season creates a cohort of individuals. For simplicity, most simulations were run under the assumption that animals born in one year do not spawn in the same year, a reproductive pattern typical of all but the most southern climes (Stauber 1950, Hayes & Menzel 1981, Kennedy & Krantz 1982, Barber et al. 1991). Each year, after incrementing the age of all individuals by 1, the population suffers age-dependent mortality, and the functional sex changes for some. Then potential parents are chosen and reproduction occurs. Recombination is implemented during the formation of each new offspring by the random choice of a location for each chromosome pair for each parent, and the genetic information is crossed over at this point. Gametes are formed through the process of meiosis, and each set of haploid chromosomes is obtained by randomly choosing one strand from each pair of chromosomes. Last, one gamete is chosen at random from each parent for each offspring. The fate of each offspring is controlled by random larval mortality at a rate set to permit establishment of a relatively stable population.

Many of the processes in the model depend on a random draw. Unless otherwise indicated, a number is drawn from a uniform distribution with a range from 0–1. These uniform deviates (*R*) are obtained from the pseudo-random generator function `ran3` described by Press et al. (1986). Whenever a normal deviate (*N*) is required, the `gasdev` routine of Press et al. (1986) is used to obtain a random deviate from a 0 mean, unit variance normal distribution. Repeat simulations using different sequences of random numbers returned results with only modest variations in scale and trend in initial trials. Consequently, results are provided only for single simulations for each set of parameter values.

The model can be described as the marriage of three components: a postsettlement population dynamics submodel that contains parameterizations for growth, mortality, and reproduction; a larval submodel that contains parameterizations for larval mortality; and a gene submodel that describes each animal in terms of its genetic structure and that tracks genotype through time from one cohort to the next. Alleles can be lost through drift and, for some, through selection. A genotype–phenotype interface interprets genotype in terms of fitness that then influences the larval and postsettlement submodels. This permits the feedback between genotype and phenotype that drives selection.

Postsettlement Population Dynamics

Sex Determination

Oysters are protandric (Kennedy 1983, Morton 1990, Guo et al. 1998). Gender is specified by a recessive protandric allele (*P*) and a dominant male allele (*M*) (Guo et al. 1998). The homozygous male animal (*MM*) is not allowed. The heterozygote male (*PM*) acts only as a male. The protandric individual (*PP*) is male at an early age and converts to female at some later age.

The *M* allele is inherently unstable over a range of population dynamics (Powell et al. 2011a). Short generation times, equivalent to high mortality rates or small population sizes, promote loss of the *M* allele. Alternative hypotheses for the genetic determination of sex provide protandry under conditions in which permanent males must persist, a characteristic possibly evolutionarily preferred (Powell et al. 2011a). These hypotheses necessarily also include permanent females, the presence of which has not been demonstrated, however. Regardless, the outcome of simulations in this study depend little on the specifics of sex determination, as simulated populations composed solely of protandric individuals return results illustrative of those with the more complex mixture of protandric animals and permanent males.

Each generation, a protandric male is given the chance to convert to a functional female. A conversion probability was obtained from empirical data from Delaware Bay (Powell, unpubl. data) using age–length relationships recorded by Kraeuter et al. (2007). This relationship between the fraction female, *Ff,* and age can be modeled as a Gompertz curve:

$$Ff = \alpha\, e^{\beta\, e^{(\gamma \cdot Age)}} \tag{1}$$

where *Age* is the age of the animal in years. Eq 1 can be used to estimate a probability for any animal changing from male to female based on its derivative:

$$Df = \frac{d\,Ff}{d\,Age} = \alpha\beta\gamma\, e^{((\gamma \cdot Age) + (\beta\, e^{(\gamma \cdot Age)}))} \tag{2}$$

The probability is then calculated as

$$P = \min\!\left(1, \frac{Df}{1 - Ff}\right) \tag{3}$$

For the simulations used here, the parameter values for the high-mortality beds in Delaware Bay (for descriptions of bed region, see Fig. 1 in Powell et al. 2008) have been used: $\alpha = 0.79$, $\beta = -3.9$, and $\gamma = -0.653$. Because of the age dependency of the probability of sex change, all long-lived protandric individuals eventually become functional females. All oysters that are protandric begin life as male. Hence, all recruits are male. However, some recruits convert to female prior to first spawning, as appears to be the case in the field (Powell, unpubl. data).

Reproduction

The fraction of the population parenting each generation is derived from a predefined fraction of parents reproducing each mating season (`FracParents`), based on estimates of effective population number for oysters (Hedgecock et al. 1992, Hedgecock 1994):

$$FrParents = \texttt{FracParents}10^{(N \cdot FracParentsVar)} \tag{4}$$

`FracParentsVar` permits variability to exist in the fraction of parents reproducing. *FrParents* is used to determine the number of parental pairs as

$$nParents = \max(0.5 \cdot FrParents \cdot LastAnimal, \texttt{minParent}) \tag{5}$$

where *LastAnimal* is the count of adult animals in the population. A minimal number of parents, `minParents`, is allowed

to reproduce, thus guaranteeing some, albeit low, level of reproduction when abundance becomes low.

Potential parents are drawn randomly, without replacement, from a list of all animals. Drawing stops when enough males and females accrue to provide *nParents*, or until the list of animals is exhausted. Each pair of parents, taken randomly, without replacement, from the parents list, produces a number of offspring, `MaxOffspring`, decreed at the beginning of the simulation. However, oyster fecundity varies with size (Choi et al. 1993, Hofmann et al. 1994, Kobayashi et al. 1997). Consequently, the number of offspring is affected by parental age through a weight-based von Bertalanffy process (Fabens 1965, Vakily 1992, Mancera & Mendo 1996, Jensen 1997) relating size and fecundity to age:

$$W = W_\infty \left(1 - e^{-kt}\right)^b \qquad (6)$$

where k and L_∞ are the von Bertalanffy parameters, and W_∞ is obtained from L_∞ by the allometric equation $W = aL^b$, with $a = 0.0003$ and $b = 2$. We note that for oysters, weight scales more nearly to the square of the length rather than the more typical cube (Yoo & Yoo 1973, Powell & Stanton 1985, Powell, unpubl. data).

In the simulations presented here, three von Bertalanffy curves have been used (Fig. 1), covering a range of growth rates typical of oysters in temperate latitudes. The von Bertalanffy parameters are as follows: for fast growth, parameters were fit from data obtained from oysters in Snail Bay and Hackberry Bay, LA (Addison 2006) ($k = 1.2$, $L_\infty = 100$); for intermediate growth, parameters were obtained for *C. virginica* in Chesapeake Bay (Mann, pers. comm.) ($k = 0.4$, $L_\infty = 100$ mm). For slower growth, parameters for the high-mortality beds of Delaware Bay were obtained from Kraeuter et al. (2007) ($k = 0.23$, $L_\infty = 140$).

We recognize that the values of L_∞ are likely biased low relative to the prehistoric populations little affected by fishing or disease. These 3 cases are representative of literature values that cover a relatively wide range of growth dynamics (e.g., Rothschild et al. 1994, Arizpa 1996, Mancera & Mendo, 1996, Mann & Evans 2004). Slower growth rates are known from these latitudes (e.g., the low-mortality region of Delaware Bay (Kraeuter et al. 2007)); however, these populations typically live under conditions not conducive to Dermo disease proliferation and so are not considered further in this study.

Eq 6 is applied to fecundity by assuming that oyster spawn is a standard fraction of biomass (Hofmann et al. 1992, Hofmann et al. 1994, Powell et al. 2011b). Hence, the fecundity equivalent of W_∞, *MaxOffspring*, is scaled to animal size by a von Bertalanffy correction factor, *AgeFactor*, defined as

$$AgeFactor = \left(1 - e^{-k(age - age_0)}\right)^b \qquad (7)$$

The age factor for reproduction is the same for males and females of the same age. The number of eggs produced is

$$nOff = 1 + \left(2^{(2N\eta)} \cdot AgeFactorF \cdot \texttt{MaxOffspring}\right) \qquad (8)$$

and the number of sperm produced is

$$nOffM = 1 + \left(2^{(2N\eta)} \cdot AgeFactorM\right.$$
$$\left. \cdot \left(\texttt{MaxOffspring} \cdot 2^{11}\right)\right) \qquad (9)$$

where η takes the value 0 or 1 so that variability can be imposed when desired, and the factor 2^{11} represents the greater number of sperm made from a given amount of gonadal mass (compare Dong (2005) and Gallager and Mann (1986)). The value of 2
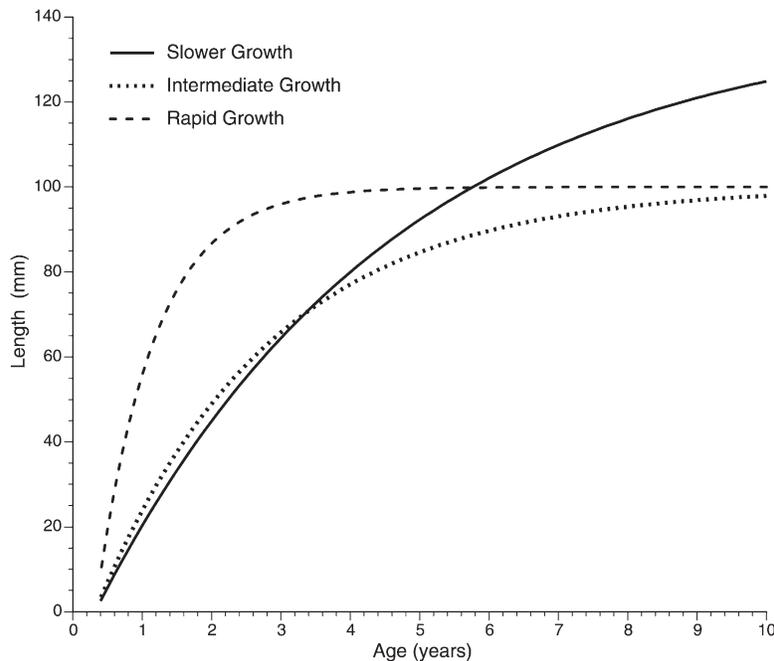

Figure 1. The 3 von Bertalanffy curves used. The rapid-growth curve is from Addison (2006) for oysters from Hackberry Bay and Snail Bay, LA. The intermediate-growth and slow-growth curves come from Chesapeake Bay and Delaware Bay populations, respectively.

multiplying *N* approximates the factor of 2 difference in spawning potential observed in the genus *Crassostrea* between, for example, *C. virginica* and *Crassostrea gigas* (Héral & Deslous-Paoli 1983, Choi et al. 1993, Choi et al. 1994, Kang et al. 2003, Ngo et al. 2006). The partner producing the fewest gametes determines the total number of fertilized eggs per mating pair. This is usually the female. Fertilization is assumed never to be sperm-concentration limited.

Adult Mortality

Adult mortality is specified as age dependent. Although juvenile mortality is high in bivalves, this portion of mortality is subsumed into larval mortality, as the purpose of the simulations was to examine the influence of mortality factors acting on sexually mature animals. Age-dependent mortality is an inherent attribute of oyster populations impacted by Dermo disease (Hofmann et al. 1995, Powell et al. 1996) and is characteristic of some other bivalves such as hard clams, *Mercenaria mercenaria* (Hofmann et al. 2006, Kraeuter et al. 2008). Whether mortality in oyster populations existing prior to the onset of disease or human exploitation was age dependent is unknown. However, the mismatch in life expectancy based on observed adult mortality rates prior to disease onset of 10–13% per year (Powell et al. 2008, Powell et al. 2009a) inferred from an assumption of constant mortality (e.g., Hoenig 1983) and the few pertinent direct estimates of adult age frequency (e.g., Harding et al. 2008) supports such a formulation, as the former would predict the observation of older animals than is observed.

The probability of dying is derived from the age of the animal according to the formulas

$$AM = \texttt{AvgAgeMort} - ((1 - \texttt{FitFac}) \cdot \texttt{dAvgAgeMort}) \quad (10)$$

$$ASM = \texttt{AvgSpreadMort} - ((1 - \texttt{FitFac}) \\ \cdot \texttt{dAvgSpreadMort}) \quad (11)$$

$$P = 0.5 \left[1 + \tanh\left(\frac{Age - AM}{ASM}\right) \right] \quad (12)$$

where *P* is the probability of death, `AvgAgeMort` is the average age of mortality (*P* is 0.5 at this age), and `AvgSpreadMort` controls how rapidly the mortality approaches 1. `FitFac`, a fitness factor to be described later, permits mortality to increase by reducing the average age of mortality by the factors `dAvgAgeMort` and `dAvgSpreadMort`.

Larval Mortality

Larval survival is controlled by an estimated population reproductive capacity that depends on `MaxOffspring`, *nParents* (the number of parent pairs), and *LastAnimal*. The estimated reproductive capacity, standardized to the number of individuals is

$$ReprPerAdult = \frac{\texttt{MaxOffspring} \cdot nParents}{4 \cdot LastAnimal} \quad (13)$$

where the factor of 4 includes the average of the uniform random deviates (0.5) and the fact that the number of offspring per parent is one-half the number of offspring per female.

The probability of a larva surviving is

$$LarvalSurv = (0.5 + 1.5R)\frac{\texttt{CarryCapacity}}{4 \cdot ReprPerAdult \cdot LastAnimal} \quad (14)$$

where `CarryCapacity` regulates the number of animals in the population. This relationship incorporates a logistic process in which average recruitment per adult declines as population abundance increases with respect to the environmental carrying capacity. A compensatory relationship between broodstock and recruitment has been identified in a number of molluscan stocks (e.g., Hancock 1973, Peterson & Summerson 1992, McGarvey et al. 1993, Kraeuter et al. 2005), including oysters (Powell et al. 2009b) and the ability of oysters to filter water more rapidly than its resupply generating food limitation downstream (Wilson-Ormond et al. 1997) provides a theoretical basis for this expectation.

The probability of death for each larva is calculated as

$$P = 1 - LarvalSurv \quad (15)$$

For a random draw, if *R* < *P*, then the larva dies. If the larva recruits to the population, it is given an identifying number, a birth date, and an age of 0. All protandric individuals become functional males at age 1 and pass into the postsettlement population as potentially reproductively active animals.

Genetic Structure and Fitness

Definition of Fitness

Ultimately, fitness of any potential parent is established by the number of progeny that live to reproduce. Because the fraction of the population successfully spawning is small, many recruits fail to spawn successfully before they die. Discounting the probabilistic aspects of the model, the factors that control the number of progeny that reproduce include the growth rate that controls lifetime spawning potential by influencing size at age and the mortality rate that is age dependent. The first is imputed to the model by means of a von Bertalanffy process. The second, in this study, is influenced by selection. For simplicity, we use adjectival modifiers to the term "fitness" to refer to 3 subsets of this overall process. The term "adult fitness" will be applied to the genetic complement of any adult that influences the probability of death at age.[3] The term "population fitness" will refer to the arithmetic average of the adult fitness values for the individuals in the population. The term "allele fitness" will be applied to the contribution of any individual locus to adult fitness.

Guo (unpubl. data) identified 14 loci with alleles that may confer some degree of resistance to mortality from Dermo disease. These loci were identified as having significant shifts in genotype frequency within families after disease caused mortality. The exact mechanisms are unknown. Certain of these alleles may confer a greater increment in survival than others; however, the data currently available are insufficient to provide more than a crude and relatively uncertain ranking. For the purposes of this study, the importance of this information is to establish (1) that a rather large number of alleles may be involved in the selection process and (2) that these loci are distributed among the majority of the chromosomes (Table 1). Most models of genetically based disease resistance rely on 1-locus (e.g., Wilhoit 1991, MacKenzie & Bishop 1999, Abell et al. 2005) or 2-loci (e.g., Galvani & Slatkin 2004) configurations. For Dermo disease, a multilocus

TABLE 1.

The allele fitnesses (`PhysioFuncWeight`) for each of the 14 loci associated with resistance to Dermo disease.

Locus	Chromosome	Assigned Position on Chromosome	Relative Fitness		
			AA	*AB*	*BB*
1	1	1	1.0	0.424	0.152
2	2	1	1.0	0.726	0.506
3	3	1	1.0	0.556	0.162
4	4	1	1.0	0.554	0.390
5	4	3	1.0	0.561	0.325
6	7	1	1.0	0.584	0.289
7	7	2	1.0	0.596	0.289
8	7	4	1.0	0.390	0.628
9	8	1	1.0	0.579	0.335
10	8	3	1.0	0.566	0.089
11	9	1	1.0	0.141	0.339
12	9	3	1.0	0.385	0.179
13	9	2	1.0	0.531	0.439
14	10	1	1.0	0.528	0.504

In each case, the *AA* genotype is given a value of 1.0 and the remaining genotypes are valued relative to that ranking (Guo, unpubl. data). Chromosomes are numbered according to Wang et al. (2005). Locus number is simply an ordinal assignment. Position on the chromosome was assigned for convenience to site 1 for a single locus contributing to disease resistance; positions 1 and 3 for dual loci; and positions 1, 2, and 3 or 1, 2, and 4 for a triplex of loci. Adult fitness is defined between 0 and 1, inclusive, by standardizing the maximal adult fitness, all participating loci homozygous *AA*, to 1.0, and the minimal adult fitness, all participating loci homozygous *BB*, ignoring underdominance, to 0.0. Thus, some rare and unlucky individuals might have an adult fitness less than 0.0.

model is clearly required. For these simulations, DyPoGEn is configured with 14 loci undergoing selection with one allele, *A*, conferring increased disease resistance; 1 locus handling the animal's sex; and 25 loci with neutral alleles.

Implementation of Allele Fitness and Adult Fitness

The value for allele fitness for each genotype potentially present at a locus, *AA, AB,* or *BB*, is provided by the first, second, or third value, respectively, of `PhysioFuncWeight` (Table 1). Each locus on a chromosome pair is assigned a set of allele fitnesses through the array `PhysioFuncClass`. Thus, one locus may be characterized by dominance and another by underdominance, for example. For these simulations, the 14 loci have been assigned allele fitnesses as described in Table 1, based on the designation of *A* for the allele conferring disease resistance and the designation of *B* for the remaining alleles. Note that each is given a weight relative to 1.0, which is assigned to the *AA* homozygote, in keeping with the earlier caveat that only the relative ranking of effect on disease resistance among genotypes can now be assigned with any degree of confidence. Note that most of these loci are characterized by some degree of dominance; however, 2 are characterized by underdominance (loci 8 and 11) and several are characterized by a limited differential between the *BB* and the *AB* genotypes (e.g., loci 13 and 14). The array `FitnessClass` then determines which of the genes influences adult fitness. Positions of each such gene on each chromosome are reported in Table 1.

Thus, adult fitness is determined for each individual as follows. Each allele pair at a locus is identified as *AA, AB,* or *BB*. The entry for that locus in `FitnessClass` identifies whether it is among those potentially conferring disease resistance, and the entry in `PhysioFuncClass` identifies which set of allele fitnesses to use; the `PhysioFuncWeight` for the allele pair gives the value contributing to adult fitness by that locus. The adult fitness for the animal is determined as the average of the 14 values from each of the 14 loci conferring disease resistance. Most oyster loci have more than 2 alleles (Launey & Hedgecock 2001, Wang & Guo 2007). For these simulations, we assume that only one of these alleles is associated with disease resistance, so that a 2-allele configuration can be used, with the second allele representing the host of alleles having no influence on disease resistance. We assume no epistasis, having limited information to the contrary (e.g., Sokolova et al. 2006), although epistasis is a common occurrence in *Crassostrea* (Hedgecock et al. 1995, Hedgecock et al. 1996). In some cases, the simple average of the maximum or minimum values of allele fitness for the designated loci may define a range more narrow than 0 to 1, inclusive. From Table 1, an animal with all *BB* genotypes at the 14 loci would have an adult fitness value well above 0, for example. This is an inherent outcome of the relative rankings provided by genetic analyses (Guo, unpubl. data). To retain the important distinction between the most and least fit animals within this specified 0-to-1 range for adult fitness, the minimal and maximal fitness values obtained from sums of the individual fitness values for each of the designated alleles are standardized to values of 0 and 1, respectively, and any value between 0 and 1 is standardized within the 0-to-1 continuum by interpolation. The final adult fitness for each animal, then, has a value between 0 and 1, inclusive. We recognize that an animal with *AB* genotype at the 2 loci showing underdominance and otherwise *BB* would have an adult fitness value less than 0.0; such rare animals are redefined with an adult fitness of 0.0 for convenience.

Genotype–Phenotype Interface

Selection is controlled by a variation in the probability of death at age based on an individual's value of adult fitness, `FitFac`, as specified in Eqs 10–12. Each simulation is referenced against a base case configured for a mortality rate thought to be characteristic of oyster populations before the onset of significant mortality by Dermo disease. This value is of the order of 10–15% of the adult population per year (Powell et al. 2008, Powell et al. 2009a). Base cases were run for 200 generations to permit drift to modify allelic frequencies from the initial random, for neutral loci, or biased random, for loci with alleles conferring disease resistance, state. The population after 200 generations is defined as the naïve population. Dermo disease is introduced in generation 201 by increasing the value of `dAvgAgeMort` and `dAvgSpreadMort` in Eqs 10 and 11 from 0 to some value between 0 and the value of `AvgAgeMort` and `AvgSpreadMort`. This increases the rate of adult mortality to the degree permitted by the fitness value of the individual as determined by `FitFac`. An example is shown in Figure 2, in which a naïve population with a yearly mortality of about 13% of the stock without the disease (labeled F = 1 in Fig. 2) is exposed to a yearly mortality of about 24% of the stock at `FitFac = 0` (labeled F = 0 in Fig. 2). After developing complete resistance to the disease at `FitFac = 1`, the stock attains the natural mortality rate of the unexposed naïve population (labeled F = 1 in Fig. 2).
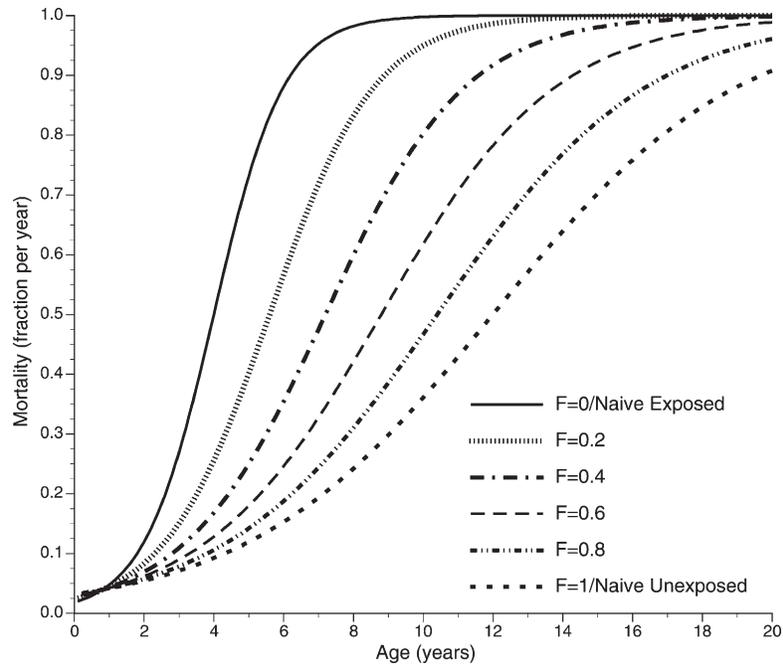
Figure 2. An example of the mortality trends with age for adult fitness varying by 0.2 units from a minimal value of 0 to a maximal value of 1. The adult-fitness-of-0 case (F = 0, or naïve exposed) yields a population mortality rate of about 25% for this parameterization of Eqs 8 and 9. The adult-fitness-of-1 case (F = 1, or naïve unexposed) yields a population mortality rate of about 13%.

Simulation Constraints

Preliminary simulations indicated that simulations of 200 generations in length were adequate to reveal how adult mortality influenced the final distribution of genotypes in the population. In some cases, a stable ending genotype frequency was obtained; in other cases, the trajectory for future selection was well established. Simulations of more than 200 generations in length were deemed unnecessary as a consequence. Carrying capacity was set so that population abundance remained high enough that genetic drift never resulted in the loss of neutral alleles during the 200-generation base-case simulations. Parameterizations varying by simulation are summarized in Table 2.

RESULTS

Influence of Mortality Level at the Onset of Disease

Except where noted, simulations were configured to approximate the population dynamics of oysters in the Chesapeake and Delaware bays. A case for Delaware Bay shows a stable population of about 400,000 individuals with a mortality rate for populations unencumbered by disease of about 13%, consistent with observations reported by Powell et al. (2008, 2009a) (Fig. 3). No alleles are lost over 200 generations. Some alleles conferring disease resistance increase in frequency; others decrease (Fig. 4). The female-to-male ratio is about 0.75, with a ratio nearing 1.5 for the older adults (Fig. 5). These ratios are somewhat higher than observed today in Delaware Bay (Powell, unpubl. data), probably because of the shorter generation time in the present-day population relative to this simulation. However, a simulation with a mortality rate near the present day returns female-to-male ratios near 0.5, as observed, and for older adults near 1.3, also as observed (Fig. 5). Spat-to-adult ratios vary from 0.2–0.5, lower

than observed on average. However, once again, the higher adult mortality rate generates higher spat-to-adult ratios greater than 0.5, consistent with observations. Thus, the simulated population dynamics are representative of observed populations at defined mortality rates.

The naïve population at generation 200 contains animals in a range of low values of adult fitness with the expected mode of 0.1 based on the initial frequency of alleles conferring disease resistance of 10% and with very few animals with values more than 0.3 (Fig. 6; generations 1, 91, and 191). Such a population would be expected to be susceptible to epizootic mortality on onset of a new disease challenge. Dermo-induced epizootic mortality is inherently modulated by the local environment, particularly temperature and salinity (Andrews 1988, Powell et al. 1996, Ragone Calvo et al. 2001). Thus, we considered 4 levels of mortality at the onset of disease, interpretable as relative measures of the virulence of the pathogen or conduciveness of the environment: ~40% of the population yearly, ~25%, ~22%, and ~17% (Fig. 7), referred to as mortality levels 4, 3, 2, and 1, hereafter. Levels 2 and 3 are representative of epizootic mortality rates often observed in Delaware Bay and Chesapeake Bay. Level 4 is a minimal value for the Gulf of Mexico (Mackin & Sparks 1962, Mackin & Hopkins 1962) and a level observed in the initial epizootics after onset of Dermo disease in the highest mortality regions of Delaware Bay. Each of these simulations shows a decline in oyster abundance on onset of the disease, with the expected greater declines at ever higher mortality rates (Fig. 7). Note that the higher mortality rates result in much increased mortality for the older adults (Fig. 8), which then leads to a decline in population average age and generation time (Fig. 7).

Fitness of the population increased over 200 generations for each of the levels of initial pathogen virulence (Fig. 9). An example shift in population fitness frequency shows that the

TABLE 2.

Parameterizations defining model configurations for examples provided in the text and figures.

Simulation Series	No. of Disease-Resistant Alleles	Carrying Capacity Scale (CarryCapacity)	Fraction Parents (FracParents)	Variation in FracParents	Mortality Parameters		Offspring Variance	Bertalanffy-k	Bertalanffy-L$_\infty$
					AvgAgeMort AvgSpreadMort	dAvgAgeMort dAvgSpreadMort			
Delaware Bay (Figs. 3–7, 9, 10)									
Base case	14	100,000	0.0005	Off	12,7	0,0	Off	0.23	140
Level 1 mortality	14	100,000	0.0005	Off	12,7	5,3	Off	0.23	140
Level 2 mortality	14	100,000	0.0005	Off	12,7	7,4	Off	0.23	140
Level 3 mortality	14	100,000	0.0005	Off	12,7	8,5	Off	0.23	140
Level 4 mortality	14	100,000	0.0005	Off	12,7	10,6	Off	0.23	140
Chesapeake Bay (Figs. 9, 11–14)									
Base case	14	100,000	0.0005	Off	12,7	0,0	Off	0.40	100
Level 1 mortality	14	100,000	0.0005	Off	12,7	5,3	Off	0.40	100
Level 2 mortality	14	100,000	0.0005	Off	12,7	7,4	Off	0.40	100
Level 3 mortality	14	100,000	0.0005	Off	12,7	8,5	Off	0.40	100
Level 4 mortality	14	100,000	0.0005	Off	12,7	10,6	Off	0.40	100
Gulf coast, one generation (Figs. 9, 11, 16)									
Base Case	14	70,000	0.0005	Off	12,7	0,0	Off	1.20	100
Level 1 Mortality	14	70,000	0.0005	Off	12,7	5,3	Off	1.20	100
Level 2 Mortality	14	70,000	0.0005	Off	12,7	7,4	Off	1.20	100
Level 3 Mortality	14	70,000	0.0005	Off	12,7	8,5	Off	1.20	100
Level 4 Mortality	14	70,000	0.0005	Off	12,7	10,6	Off	1.20	100
Gulf Coast Two-Generation Figures 13, 16									
Base case	14	30,000	0.0005	Off	12,7	0,0	Off	1.20	100
Level 1 mortality	14	30,000	0.0005	Off	12,7	5,3	Off	1.20	100
Level 2 mortality	14	30,000	0.0005	Off	12,7	7,4	Off	1.20	100
Level 3 mortality	14	30,000	0.0005	Off	12,7	8,5	Off	1.20	100
Level 4 mortality	14	30,000	0.0005	Off	12,7	10,6	Off	1.20	100
Increased variation in fecundity (Fig. 12)									
Base case	14	60,000	0.0005	On	12,7	0,0	Off	0.40	100
Level 1 mortality	14	60,000	0.0005	On	12,7	5,3	Off	0.40	100
Level 2 mortality	14	60,000	0.0005	On	12,7	7,4	Off	0.40	100
Level 3 mortality	14	60,000	0.0005	On	12,7	8,5	Off	0.40	100
Level 4 mortality	14	60,000	0.0005	On	12,7	10,6	Off	0.40	100
Decreased fraction spawning (Fig. 12)									
Base case	14	120,000	0.00005	Off	12,7	0,0	Off	0.40	100
Level 1 mortality	14	120,000	0.00005	Off	12,7	5,3	Off	0.40	100
Level 2 mortality	14	120,000	0.00005	Off	12,7	7,4	Off	0.40	100
Level 3 mortality	14	120,000	0.00005	Off	12,7	8,5	Off	0.40	100

TABLE 2.
continued

Simulation Series	No. of Disease-Resistant Alleles	Carrying Capacity Scale (Carry Capacity)	Fraction Parents (FracParents)	Variation in FracParents	Mortality Parameters				
					AvgAgeMort AvgSpreadMort	dAvgAgeMort dAvgSpreadMort	Offspring Variance	Bertalanffy-k	Bertalanffy-L_∞
Level 4 mortality	14	120,000	0.00005	Off	12,7	10,5	Off	0.40	100
Increased fraction spawning (Fig. 13)									
Base Case	14	80,000	0.005	Off	12,7	0,0	Off	0.40	100
Level 1 Mortality	14	80,000	0.005	Off	12,7	5,3	Off	0.40	100
Level 2 Mortality	14	80,000	0.005	Off	12,7	7,4	Off	0.40	100
Level 3 Mortality	14	80,000	0.005	Off	12,7	8,5	Off	0.40	100
Level 4 Mortality	14	80,000	0.005	Off	12,7	10,6	Off	0.40	100
Increased variation in spawning (Fig. 12)									
Base case	14	100,000	0.0005	Off	12,7	0,0	On	0.40	100
Level 1 mortality	14	100,000	0.0005	Off	12,7	5,3	On	0.40	100
Level 2 mortality	14	100,000	0.0005	Off	12,7	7,4	On	0.40	100
Level 3 mortality	14	100,000	0.0005	Off	12,7	8,5	On	0.40	100
Level 4 mortality	14	100,000	0.0005	Off	12,7	10,6	On	0.40	100
Increased population abundance (Figs. 13, 17)									
Base case	14	900,000	0.005	Off	12,7	0,0	Off	0.40	100
Level 1 mortality	14	900,000	0.005	Off	12,7	5,3	Off	0.40	100
Level 2 mortality	14	900,000	0.005	Off	12,7	7,4	Off	0.40	100
Level 3 mortality	14	900,000	0.005	Off	12,7	8,5	Off	0.40	100
Level 4 mortality	14	900,000	0.005	Off	12,7	10,6	Off	0.40	100
Seven-allele case (Fig. 14)									
Base case	7(C1L1,C4L1, C7L1L4,C9L1L3, C10L1)	100,000	0.0005	Off	12,7	0,0	Off	0.40	100
Level 1 mortality	7(C1L1,C4L1, C7L1L4,C9L1L3, C10L1)	100,000	0.0005	Off	12,7	5,3	Off	0.40	100
Level 2 mortality	7(C1L1,C4L1, C7L1L4,C9L1L3, C10L1)	100,000	0.0005	Off	12,7	7,4	Off	0.40	100
Level 3 mortality	7(C1L1,C4L1, C7L1L4,C9L1L3, C10L1)	100,000	0.0005	Off	12,7	8,5	Off	0.40	100
Level 4 mortality	7(C1L1,C4L1, C7L1L4,C9L1L3, C10L1)	100,000	0.0005	Off	12,7	10,6	Off	0.40	100

TABLE 2.
continued

| Simulation Series | No. of Disease-Resistant Alleles | Carrying Capacity Scale (CarryCapacity) | Fraction Parents (FracParents) | Variation in FracParents | Mortality Parameters | | Offspring Variance | Bertalanffy-k | Bertalanffy-L∞ |
					AvgAgeMort AvgSpreadMort	dAvgAgeMort dAvgSpreadMort			
Two-allele case (Fig. 14)									
Base case	2(C1L1,C7L1)	100,000	0.0005	Off	12,7	0,0	Off	0.40	100
Level 1 mortality	2(C1L1,C7L1)	100,000	0.0005	Off	12,7	5,3	Off	0.40	100
Level 2 mortality	2(C1L1,C7L1)	100,000	0.0005	Off	12,7	7,4	Off	0.40	100
Level 3 mortality	2(C1L1,C7L1)	100,000	0.0005	Off	12,7	8,5	Off	0.40	100
Level 4 mortality	2(C1L1,C7L1)	100,000	0.0005	Off	12,7	10,6	Off	0.40	100

population achieves a modal fitness of 0.4 200 generations after disease onset, with most of the population falling between 0.3 and 0.55 (Fig. 6). In each case, population mortality rate declined commensurately (Fig. 7). For the highest mortality level (level 4), with an initial mortality rate of ~40% per year, however, population fitness reached an asymptote at a level lower than observed for the 2 intermediate initial mortality rates (levels 2 and 3, Fig. 7) (Fig. 9). Perusal of allele trajectories shows that some alleles conferring disease resistance were lost from the population through drift during the initial epizootic, when population abundance strongly declined, and their loss thwarts the return to historically low mortality rates through selection by limiting the number of loci undergoing selection for disease resistance (Fig. 10, Table 3).

The Influence of Oyster Growth Rate

Oyster growth rate varies with latitude as a function of the degree and duration of warm temperatures. Comparison of the analogous set of simulations for the moderately more rapid growth rates of Chesapeake Bay (Fig. 1) shows that increasing disease pressure results in increasing disease resistance in both cases (Fig. 9). Moreover, in both cases, at the highest level of disease pressure, the increase in disease resistance is truncated in later years by the unfortunate loss of rare alleles during the initial epizootic. The Delaware Bay and Chesapeake Bay cases differ in minor details; however, minor changes in growth rate and maximum size (W_∞, Eq 6) result in limited differences in the outcome of selection after disease onset.

In comparison, the much higher growth rates characteristic of the Gulf of Mexico result in substantially improved levels of disease resistance, most evident at the highest disease pressure (level 4, Fig. 9). This differential results from reduced allele loss through drift in the Gulf simulation where oyster abundance remains substantially higher at a given mortality rate (Fig. 11). The higher growth rate increases fecundity and recruitment at a given mortality rate, and the higher abundance thus maintained provides increased opportunity for alleles conferring disease resistance to increase in frequency through selection, rather than decrease in frequency through drift (Table 3).

The Influence of Variability in Fecundity or Recruitment

Considerable variability can occur in egg quantity and egg quality for females of a given size (e.g., Davis & Chanley 1955, Gallager & Mann 1986, Mann et al. 1994). Variations in egg quality influence larval survival (e.g., Thompson & Harrison 1992, Powell et al. 2002, Powell et al. 2011b). Increased egg quality and enhanced egg production both result in increased recruitment per female. Except at the highest level of mortality, varying egg production by a factor of 2 had little influence on the trajectory of increasing population fitness over time (Fig. 12). The improvement noted at the highest mortality level (Offspring Variation, level 4, Fig. 12) occurred because fewer alleles conferring disease resistance were lost through drift (Table 3). Thus, increased variability in the quantity or quality of eggs produced had little impact on the development of disease resistance as long as population dynamics minimized allele loss through drift. When drift endangered retention of rare alleles, increased recruitment in some years increased the probability that they would be retained in the population.
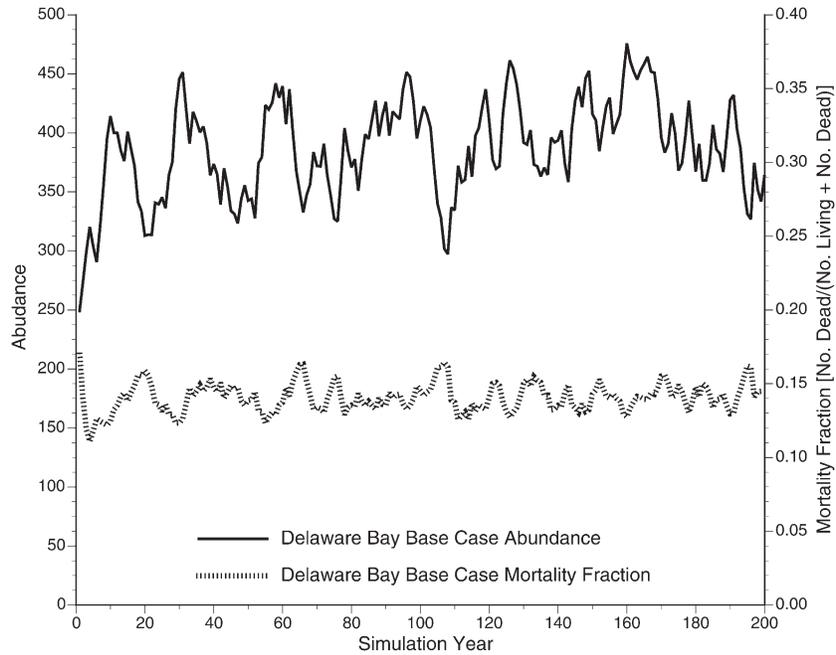
Figure 3. The time series of abundance and mortality rate, represented as the fraction dying each year, for the Delaware Bay base case (Table 2).

The Influence of Variations in Effective Population Size

Previous simulations assumed a constant fraction of the stock successfully spawning, consistent with measures of effective population size in oyster populations (Hedgecock et al. 1992, Hedgecock 1994). Increased variability in the fraction of the population spawning impacted the outcome little when disease pressure was moderate (Fecundity Variance, level 2, Fig. 12), but resulted in enhanced disease resistance after 200 generations when disease pressure was high (level 4, Fig. 12). Fewer alleles conferring disease resistance were lost through drift when year-to-year variation occurred in the fraction of the population spawning successfully (Table 3).
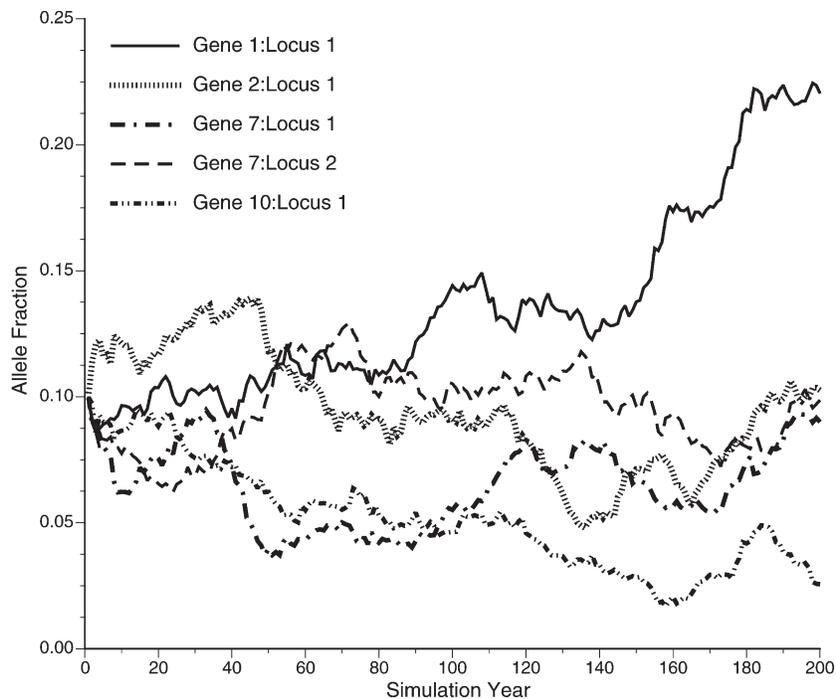

Figure 4. Trajectories for 5 representative alleles conferring disease resistance for the Delaware Bay base case (Table 2). Dermo disease was not present; thus, these alleles drifted over time as neutral alleles. Initial allelic frequency was near 10%.
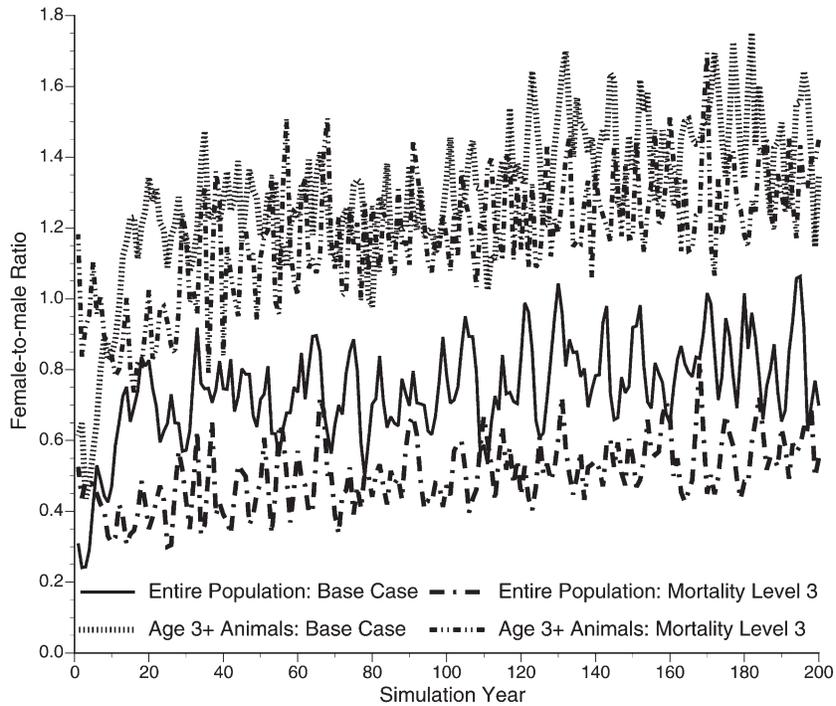
Figure 5. Female-to-male ratio for the entire population and for animals 3 y or more of age for the Delaware Bay base case and for a case influenced by an increment of mortality to level 3 (about 25% per year).

A factor of 10 reduction in the fraction of the stock reproducing successfully each year reduced significantly the ability of the stock to develop resistance to the disease (Fig. 12). A more limited cadre of parents each year results in increased drift. More of the alleles conferring disease resistance were lost before the advantages of selection could be realized (Table 3). A factor of 10 increase in the fraction of the stock reproducing successfully enhanced significantly the ability of the stock to
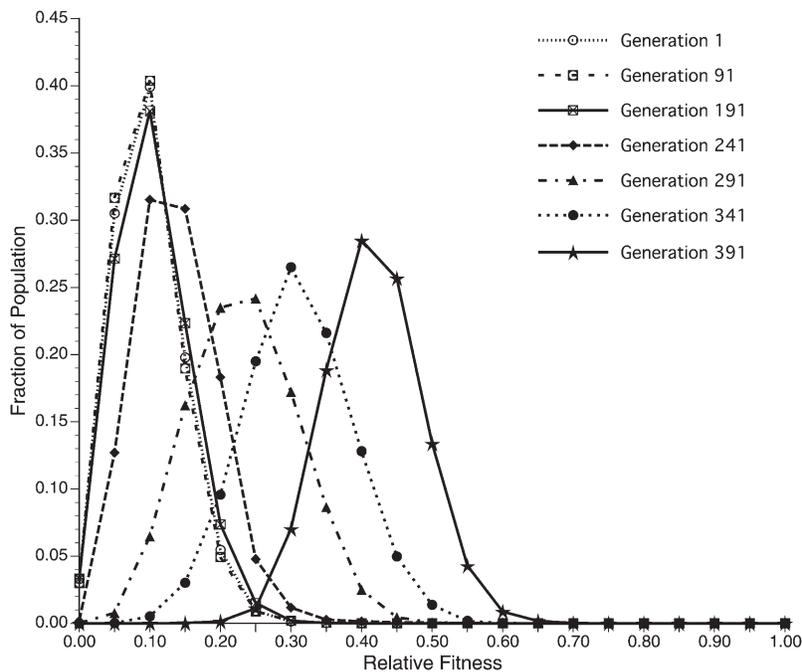

Figure 6. The frequency of adult fitness over a suite of selected generations for the Delaware Bay base case. In this simulation, Dermo disease entered the population at generation 201 with a disease intensity set at level 3 (Fig. 8). The trajectory for adult fitness improvement is shown in Figure 9. The distribution of adult fitness values for the naïve population is shown for generations 1, 91, and 191. During this time, alleles conferring disease resistance were allowed to drift without selective advantage for 200 generations. The resulting frequency shifts by drift only modestly in comparison with generations greater than 200.
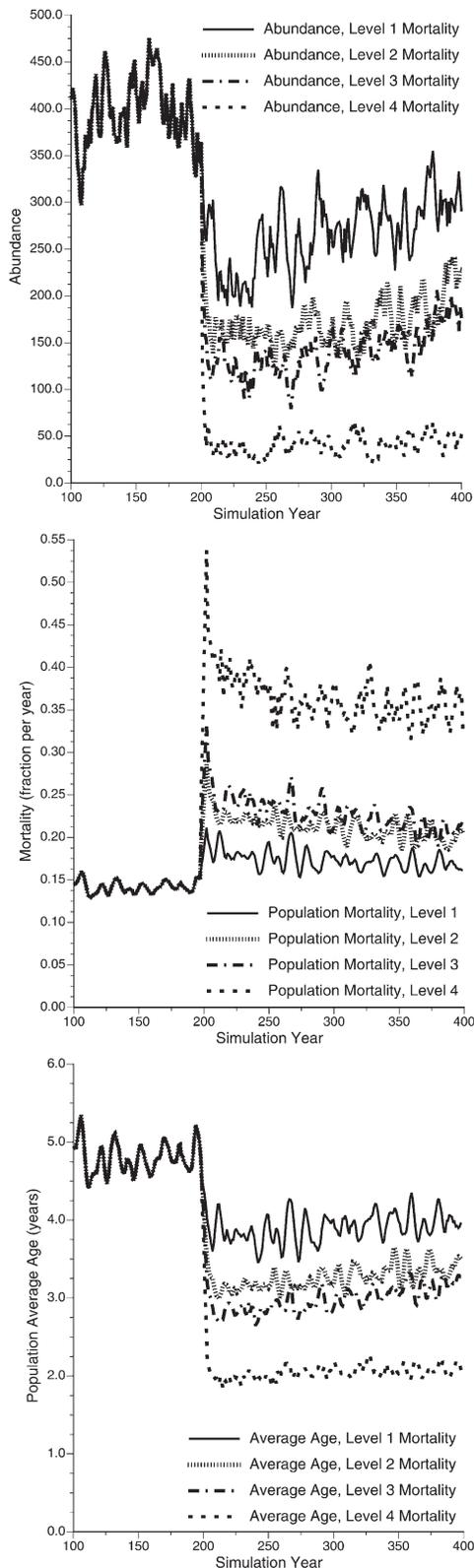
Figure 7. Comparison of population abundance (top), population mortality rate (middle), and population average age (bottom) after the onset of disease at generation 201 for the 4 degrees of pathogen virulence and commensurate increases in natural mortality rate. Abundance and average age slowly increase over the last 200 generations in some cases because the population develops a degree of disease resistance and, thus, survival increases.

develop resistance to the disease (Fig. 12). The effect was most pronounced at the highest disease pressure (level 4, Fig. 12). A greater cadre of parents resulted in decreased drift. More of the alleles conferring disease resistance were retained in the population so that the advantages of selection could be realized (Table 3).

The Influence of Oyster Abundance

A factor of 10 increase in oyster abundance varies the outcome of the two lowest levels of disease pressure very little (Fig. 12). However, disease resistance develops more completely with higher population abundance when the disease intensity is high (level 4, Fig. 12). Higher abundance reduces the loss of alleles conferring disease resistance by drift, thus permitting selection to favor more of the inherent capacity of the animal to resist the disease.

The Influence of Increased Spawning Frequency

In some areas of the Gulf of Mexico, growth rates are sufficiently fast that two generations per year may occur, yet most of the mortality from Dermo disease remains concentrated in the late summer and early fall. Parameterization of these simulations takes into account the likelihood that the second generation, having individuals of smaller size, is also likely to escape some mortality during its first late summer of life as a result of late-season infection limiting the time for proliferation. Two generations per year moderately decrease the rate of development of disease resistance at all mortality levels (compare the 1-generation case in Fig. 12 with Fig. 13). Two generations per year permit alleles conferring vulnerability to be passed from one generation to the next before selection occurs. Thus, selection acts less efficiently and the per-year rate of development of disease resistance is appreciably slowed.

The Influence of Genetic Contribution

Most simulations were run with 14 loci having alleles conferring disease resistance. However, the degree of importance of these loci in establishing the phenotype enhancing survival significantly is unknown. Some smaller fraction may be of primary importance. We examined two of many possible cases, one in which the number of important loci was halved and one in which only 2 loci played a preeminent role (Table 2). Note that particular outcomes of the 2-loci case are strongly dependent on the specific loci chosen from the suite in Table 1. Choosing genotypes showing underdominance, for example, would very likely produce strongly divergent results. Reported simulations are for loci characterized by relatively classic additive dominance (Tables 1 and 2).

Reducing the number of alleles conferring disease resistance by half, to 7, accelerated substantially the rate of acquisition of disease resistance, and this effect was enhanced at the higher disease intensities (e.g., level 4, Fig. 14). Reducing the number of alleles to 2 further enhanced this effect, regardless of comparison with the 14-loci or 7-loci case (Fig. 14). For the 2-loci case, simulated oyster populations become completely or nearly completely disease resistant at the three highest disease pressures, with the outcome being achieved within 100 generations at the highest disease pressure (level 4, Fig. 14). In both the 2-loci and 7-loci cases, one of the primary outcomes was a reduction in the likelihood that the alleles conferring disease resistance
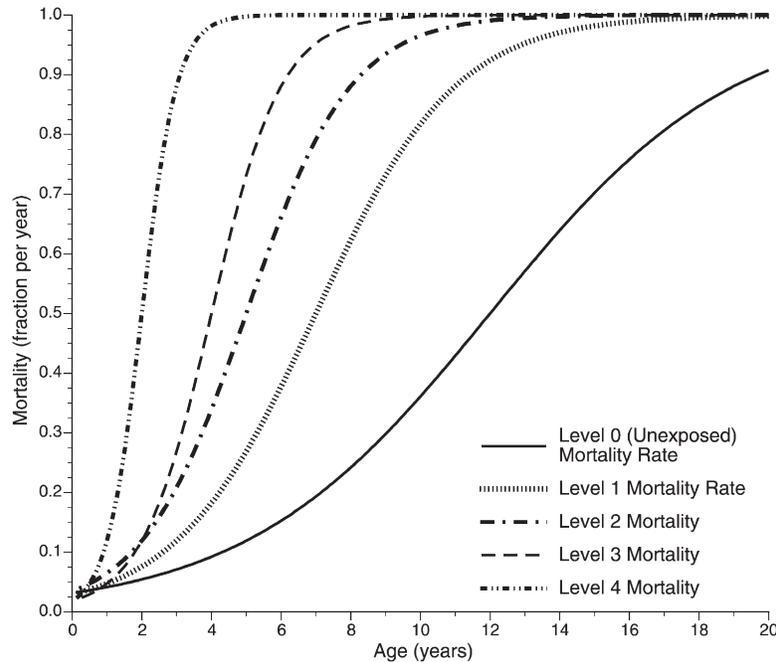
Figure 8. The range of age-dependent mortality rates used in simulations described in Table 2. Level 0 represents the predisease mortality rate for oyster populations in the Mid-Atlantic region. Levels 2 and 3 are consistent with whole-stock Dermo mortality rates observed in Delaware Bay, as well as the mortality rate observed on the medium-mortality beds in Delaware Bay and for the third epizootic on the high-mortality beds of Delaware Bay. Level 4 is a representative mortality rate for the Gulf of Mexico and for the 2 initial epizootics in the high-mortality region of Delaware Bay. Bed allocation to bed groups is defined in Powell et al. (2008) (see also Fig. 15).

would be lost by drift (Table 3). For the 2-loci case, one or both of the alleles conferring disease resistance became fixed in the population at the three highest disease pressures, with both loci having frequencies for the allele conferring disease resistance exceeding 99%.

<center>DISCUSSION</center>

Perspective

Perhaps the paramount obstacle to the investigation of the development of disease resistance in marine populations is the inability to deduce the vulnerability of the naïve population to the disease. Often, the disease agent has been present for some time prior to initial characterization, so that the population available to the investigator has already survived at least the initial phases of epizootic disease. Few cases are so fully documented as the case for MSX disease outbreaks that began during the mid-1950s (e.g., Andrews 1968, Farley 1975, Ford & Haskin 1982; see also Burreson 1991, Burreson et al. 2000, Burreson & Ford 2004). *P. marinus* has a particularly abstruse time history. Over much of its original range, many (and possibly untold numbers of) generations likely passed between its initial onset and the first series of investigations during the late 1940s to early 1950s. A compounding difficulty is the absence or limited availability of time series describing the host population dynamics, particularly the mortality rate prior to, during, and after onset of the disease. Few long-term time series exist (e.g., Elston et al. 1987, Powell et al. 2008, Mann et al. 2009b), and the data are extremely limited for *P. marinus.* Delaware Bay presents an opportunity in this context because

detailed time series data exist prior to Dermo becoming a significant source of mortality in the bay, and this time series has carried through to the present day. Furthermore, the population was almost certainly truly naïve circa 1989 to 1990 when *P. marinus* first became epizootic in Delaware Bay (Ford 1996). Last, adequate data exist to deduce the mortality rate prior to any significant influence of disease and during times of insignificant disease pressure, as good time series data exist back to 1953 (Powell et al. 2008).

Some impediments exist to modeling the development of disease resistance in *C. virginica* populations after the onset of disease. Chief among them is the likelihood that larvae from naïve populations will continually insert susceptible alleles into the population so that the assumption that recruits are derived from the local population is somewhat to substantively invalid (but see Dekshenieks et al. 2000, Powell et al. 2003, Powell et al. 2009a). This impediment poses a constraint that limits reconstruction of genetic response to the onset of disease even when adequate time series data on population dynamics are available, as the development of disease resistance will likely be slowed to some poorly defined extent by down-estuary insertion of alleles from susceptible larvae. Our study is not immune to this constraint; the rate of development of disease resistance in our simulations is likely biased high accordingly. The remaining impediments are more easily overcome, or at least addressed. Chief among these is the specification of the population dynamics of the naïve population. Time series analyses from Delaware Bay (e.g., Powell et al. 2009a), corroborated in the main for the James River in Chesapeake Bay (Mann et al. 2009b), identify a predisease mortality rate for the older adult population of about 10–15% per year. This is consistent with
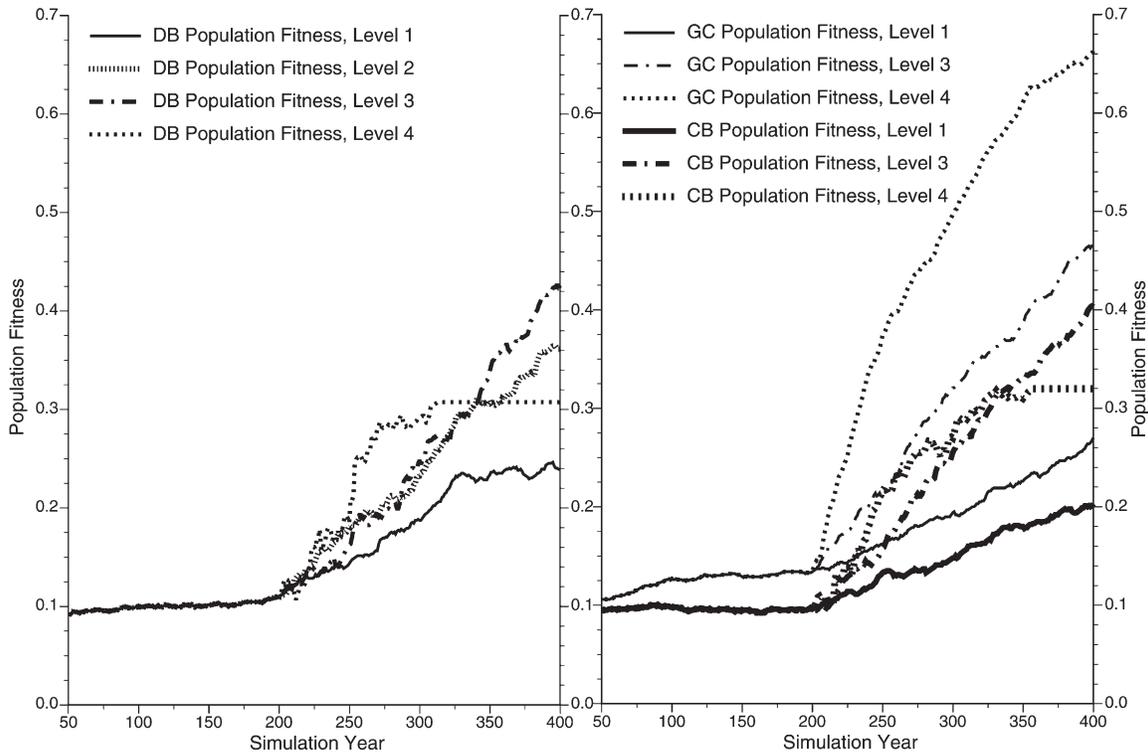
Figure 9. Population fitnesses for the Delaware Bay case (DB), the Chesapeake Bay case (CB), and the Gulf coast (GC) for various levels of initial pathogen virulence and commensurate population mortality (Table 2). The naïve, unexposed case is shown for generations 50–200. The 4 levels of disease pressure begin in generation 201. Fitness ranges from 0–1. Note that the y-axis scale reaches only 0.7.

inferences about the life span of *Crassostrea virginica*, which very likely is on the order of 10–20 y.[4] We have used a mortality function that includes a moderate increase of mortality rate with age (Fig. 2). Insufficient information is available for

oysters regarding the age dependency of mortality prior to the onset of disease; however, the relationship is consistent with inferences for *M. mercenaria* (Kraeuter et al. 2008) and with the tendency for longer lived animals to have a "plateau of adult
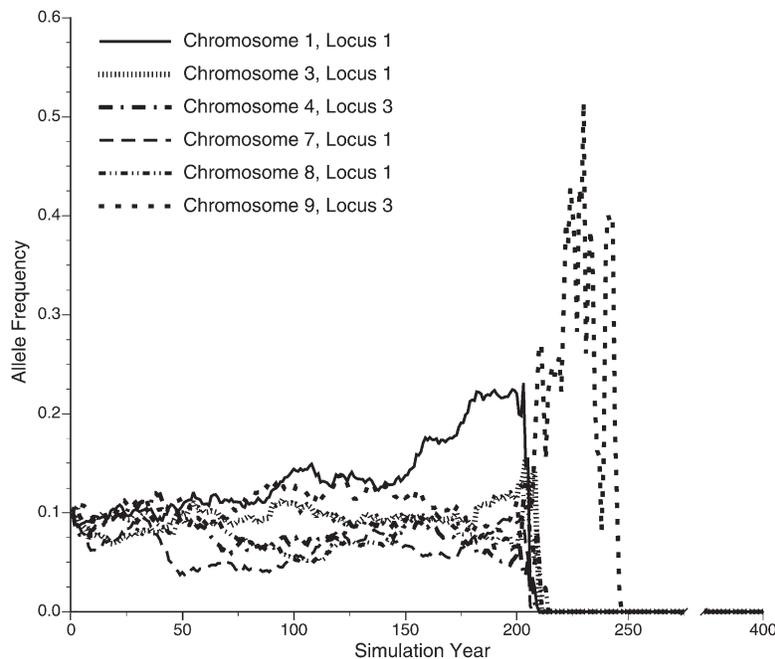

Figure 10. Trajectories of allelic frequencies for alleles conferring disease resistance that were lost through drift in the Delaware Bay case with high disease pressure (level 4). Exposure to disease in the naïve population commenced in generation 201. The depicted alleles were lost between generations 201 and 250.

TABLE 3.

The number of loci in which alleles conferring disease resistance were lost through drift or fixed over 200 generations (400 for the Gulf coast case of 2 generations per year) through selection for disease resistance.

Simulation Series	Mortality Level	No. Lost	No. Fixed
Delaware Bay	1	1	0
	2	2	0
	3	5	0
	4	10	4
Chesapeake Bay	1	0	0
	2	2	0
	3	3	0
	4	10	4
Gulf Coast, 1 generation	1	0	0
	2	1	0
	3	2	0
	4	2	0
Gulf Coast, 2 generations	1	0	0
	2	0	0
	3	0	0
	4	0	0
Increased variance in fecundity	1	3	0
	2	5	0
	3	5	1
	4	8	5
Reduced fecundity	1	11	0
	2	11	2
	3	12	1
	4	12	2
Increased fecundity	1	0	0
	2	0	0
	3	0	0
	4	0	0
Increased population abundance	1	0	0
	2	0	0
	3	0	0
	4	0	0
Increased variance in spawning	1	7	0
	2	6	0
	3	7	2
	4	6	3
Seven-loci case	1	1	0
	2	1	0
	3	2	0
	4	1	1
Two-Loci case	1	0	0
	2	0	1
	3	0	1
	4	0	2

Note that all simulation series are based on 14 total alleles except for the 7-loci and 2-loci cases.

vigor" in their survivorship curves (e.g., Schmidt & Warme 1969, Stanton et al. 1981, Vetter 1987). The inference of mortality rate from the finding of animals of oldest age remains a thorny proposition (Hoenig 1983, Hewitt & Hoenig 2005). Nevertheless, simulations of average age from our implementation of mortality at age, age distribution estimated from von Bertalanffy curves (e.g., Kraeuter et al. 2007), and mortality rates inferred from time series data (Powell et al. 2009a) dovetail sufficiently to provide some confidence in the parameterization.

The remaining descriptors of the population dynamics pertinent to this modeling exercises are better known. These include the growth rate (Kraeuter et al. 2007), the influence of size on functional sex (Kennedy 1983), population abundance (Powell et al. 2009a), and effective population size (Guo, unpubl. data). Simulations of the naïve population without disease returned results consistent with time series observations and expectations from published sources, suggesting that the population dynamics of the species is adequately characterized.

An additional set of obstacles is posed by the need to model the population dynamics of the naïve population at the onset of the disease. Chief among these is the initial epizootic mortality rate. Here, time series data have proved invaluable (Fig. 15). The initial epizootics in Delaware Bay produced mortality rates for the adult populations of 30–50% per year. The epidemiology of the disease is relatively well-known and has been modeled (e.g., Hofmann et al. 1995, Powell et al. 1996, Ragone Calvo et al. 2001). Comparison with the demographics observed in populations at the onset of Dermo disease suggests again that the population dynamics of the species in its most vulnerable state has been described adequately.

The investigation of the development of disease resistance also requires specification of the number and nature of alleles conferring resistance. Here, some information is also available. Guo (unpubl. data) identified 14 loci with alleles associated with lower mortality in conditions where Dermo challenge was high. Sufficient information is available to apportion a relative degree of influence to the more resistant homozygote, the heterozygote, and the more sensitive homozygote. The phenotypic expression of these alleles, however, is unknown, so that the relative importance of each locus in determining the outcome of selection is undefined. We assume equivalency but not additivity nor epistasis (e.g., Grosholz 1994, Nath et al. 2008, see also Sokolova et al. 2006, Hallander & Waldmann 2007). We also assume that alleles conferring disease resistance are neutrally selected in populations unaffected by *P. marinus*. Many studies have identified negative effects of alleles conferring disease resistance in naïve, undiseased populations (Cotter et al. 2004, Feng & Costillo-Chavez 2006, Duffy & Forde 2009, Zbinden et al. 2008, Hasu et al. 2009).

The appropriateness of these assumptions can only be addressed by comparison of simulations with observed development of disease resistance; a verification that, to some extent, may be possible. Figure 15 documents the time series trends in mortality for selected components of the Delaware Bay oyster stock. The time series is only 20 y long following the 1989/1990 onset of Dermo disease as a contributing influence to mortality in Delaware Bay, and this length of time is troublesomely short. Moreover, the dramatic modulation of epizootic intensity of Dermo by temperature, salinity, and the history of temperature and salinity change (e.g., Soniat & Gauthier 1989, Paynter & Burreson 1991, Burreson & Ragone Calvo 1996, Powell et al. 1996, Soniat et al. 1998, Ford & Smolowitz 2007) restrict simple inferences about disease resistance from time series information. Nevertheless, two aspects of the Delaware Bay time series are noteworthy. The first is the drop in the epizootic mortality rate for the down-estuary regions (the high-mortality and very-high-mortality beds) from about 30–50% per year from 1994 to 2003 to about 25% per year in the epizootic ongoing during 2009 and 2010 (except for the offshore high-mortality beds). The second is the more constant value for the mid-estuary (medium-mortality) population at about 20–25% per year. One interpretation of these trends is that the environmental conditions during the late
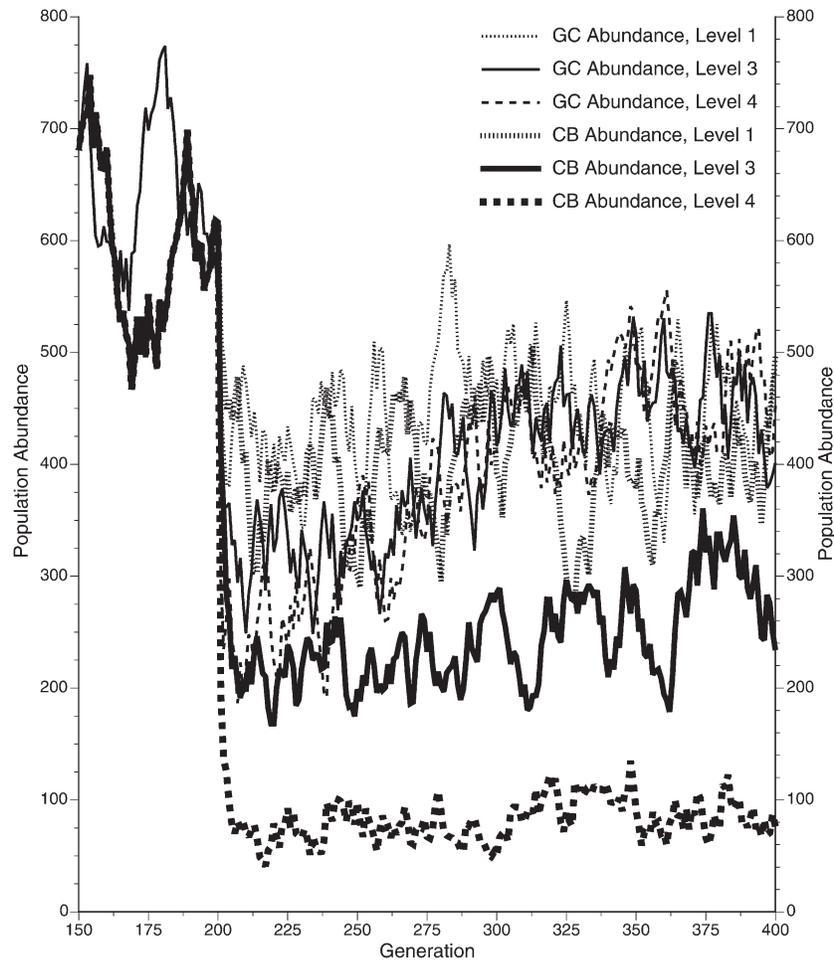
Figure 11. Comparison between population abundance in populations exposed to 3 disease pressures for oysters parameterized for Chesapeake Bay (CB) and the Gulf coast (GC; Table 2). The naïve, unexposed case is shown for generations 150–200. The 3 levels of disease exposure begin in generation 201. Population fitness trajectories are shown in Figure 9.

2000s remained conducive to epizootic development, and this maintained an equivalency of effect up-estuary, where selection is less effective; however, the vulnerability of the down-estuary populations to epizootic mortality is muted during the last epizootic, when the highest selection pressure had existed prior to that time. In a later section, we compare this time series with model simulations.

Response of the Naïve Population to Disease Onset

Simulated populations were exposed to 4 levels of mortality covering the range in mortality observed in Delaware Bay during the 1990s (Figs. 7 and 8). In each case, disease resistance increased over time (Fig. 9). Up to a point, higher mortality rates resulted in more rapid development of disease resistance. Commensurately, the mortality rate declined, as the opposing trends must co-occur. However, at the highest disease pressure (40%-per-year mortality), the expected outcome did not materialize, as the development of disease resistance was short-circuited by the loss of alleles through drift, which restricted the genetic ambit of the population's response.

During the initial epizootic, mortality rate increases rapidly. Commensurate with this is a decline in population abundance.

The population dynamics of the species is inadequate to this population decline fully, and, indeed, the demise of oyster populations with Dermo disease is not unexpected (Southworth & Mann 2004, Powell et al. 2008). In any period of declining abundance, the loss of alleles by drift is a risk. By definition, alleles contributing to disease resistance normally must be rare initially (e.g., Galvani & Slatkin 2004, Harding et al. 2005, Duffy & Forde 2009) and therefore more prone to loss (Wilhoit 1991). In the case of a simulated Delaware Bay population exposed to an incremental increase in mortality rate to about 40% per year, the influence of drift strongly limits the outcome, but this is true only if the number of successful parents is initially relatively low. It is the scale of the change in mortality rate that exerts the primary influence on the rate of development of resistance to the disease.

Modulation of Response of the Naïve Population

The population dynamics is an important modulator of the outcome of disease challenge. Certain aspects minimize loss of alleles by drift. Initially large population size is an effective protectant. Factors that facilitate the ability of the population to maintain abundance at increased mortality are another,
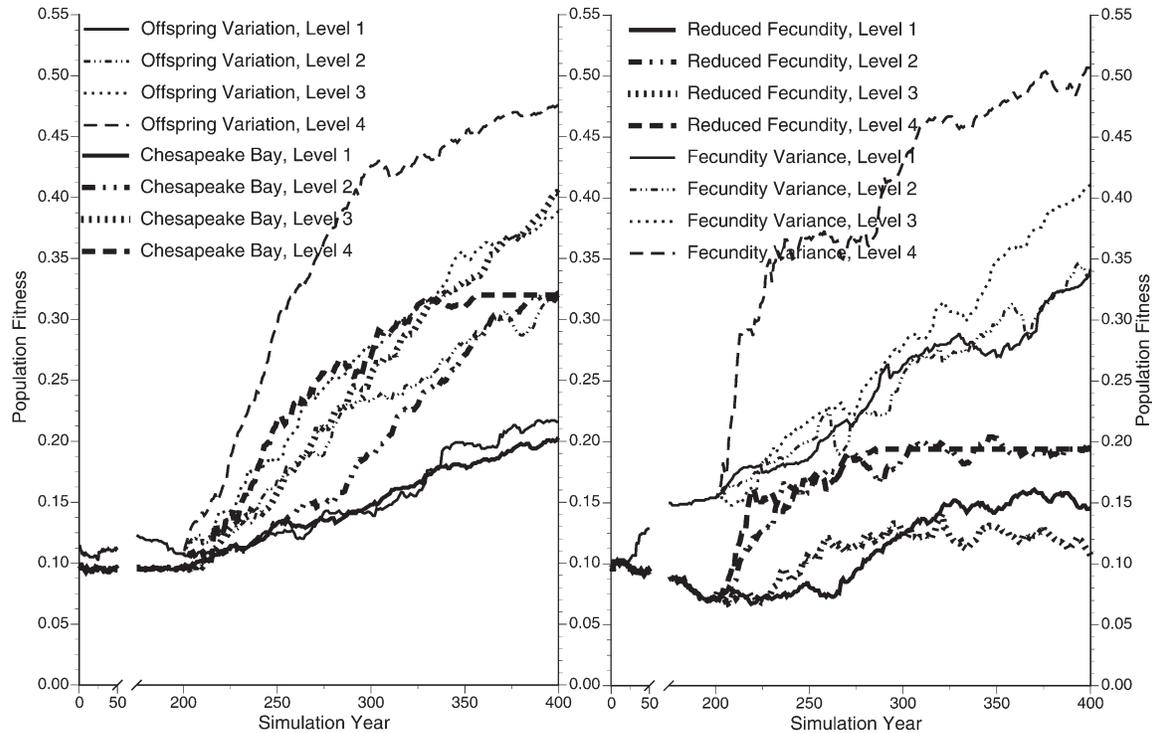
Figure 12. Comparison between population fitness for 4 disease pressures for oysters parameterized for Chesapeake Bay with variation in the number of offspring produced per mating pair (Offspring Variation) with a variable proportion of the population mating (Fecundity Variance), and with reduced fecundity resulting from smaller effective population size (Reduced Fecundity) relative to the Chesapeake Bay case without these effects (Table 2). The naïve, unexposed case is shown for generations 1–200. The 4 levels of disease exposure begin in generation 201. Population fitness ranges from 0–1. Note that the y-axis scale only reaches 0.55.

including faster growth rates and higher population fecundity either by a larger fraction of the population being reproductively successful or, to a lesser degree, increased variability in individual spawning potential. All of these permit the population to withstand a greater increment in mortality rate without the loss of rare alleles, thus facilitating the response of the population to disease challenge.

The Rate of Development of Disease Resistance

The aforementioned aspects of population dynamics contribute significantly to the rate of development of disease resistance. However, the simulations as a whole show that a significant population response occurs at its most rapid rate on decadal to vicennial timescales, with a half century being the more likely time span. Improvement in disease resistance comes slowly with respect to the patience of human observation, and ponderously slow in the context of the demands of fisheries management and ecological restoration (Mann 2000, Brumbaugh et al. 2006, Mann & Powell 2007, Beck et al. 2009).

Although a number of processes modulate what apparently may be a ploddingly protracted affair, the primary restraint on the rate of development of disease resistance, on which these modulators build, is the number of loci potentially conferring disease resistance (see also Hallander & Waldmann 2007). Lacking information to the contrary, each of the 14 potential loci was weighted equally in the valuation of adult fitness. Thus, each locus can contribute only in small measure to the outcome. Because the alleles are rare initially, few naïve animals will have

more than 1 or 2 (Fig. 6); thus, the range of predisposition to disease will be restricted, and the response of the naïve population limited. Simulations restricting the development of disease resistance to a few controlling alleles consistently predict much more rapid development of disease resistance, although the degree is strongly dependent on the specific loci chosen. That such a rapid development in disease resistance is not observed in the Delaware or Chesapeake Bays offers the strongest evidence against the presence of a few governing alleles.

The Case of the Gulf of Mexico

In extensive areas of the Gulf of Mexico, temperatures rarely drop below 10°C in the winter and often rise above 30°C in the summer, whereas salinities frequently exceed 15‰ for much of the time. (e.g., Copeland & Hoese 1966, Hofmann et al. 1994, Soniat et al. 1998, Gullian-Klanian et al. 2008). Rates of proliferation of Dermo *in vivo* must be near maximal in these regions (Soniat 1985, Fisher et al. 1992, Powell et al. 1996). Nevertheless, recorded mortality rates are not unduly different from those observed in more northern climes, although they may average higher (e.g., Mackin & Hopkins 1962, Mackin & Sparks 1962). The more rapid growth rates of Gulf coast oysters minimize the decline in abundance that necessarily follows a significant increment in mortality rate, thereby insulating the population from collapse during epizootic times (Fig. 16). The degree of disease resistance is only moderately increased, if at all, however, by the more rapid rate of growth (e.g., Brown et al. 2005a, Brown et al. 2005b, Encomio et al. 2005). In some regions, growth rates and
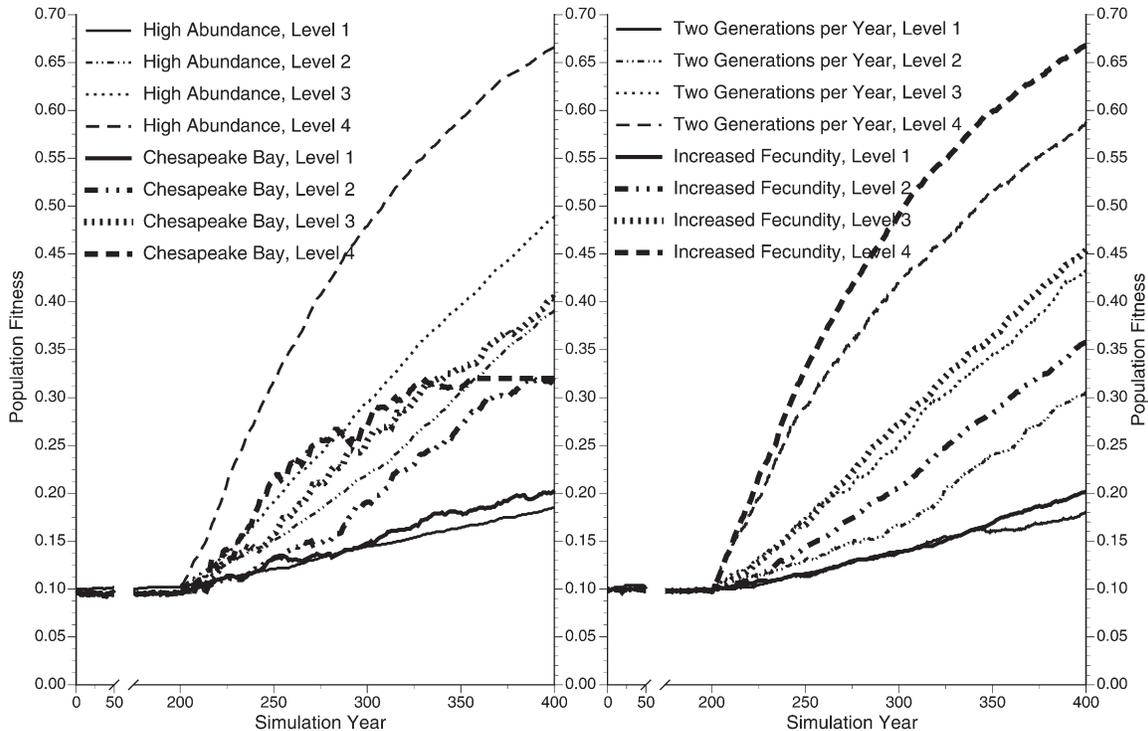
Figure 13. Comparison between population fitness for 4 disease pressures for oysters parameterized for Chesapeake Bay, but with increased population abundance generated by increased carrying capacity (High Abundance) and increased fecundity produced by an increase in the number of successful mating pairs (Increased Fecundity), relative to Chesapeake Bay without these effects (Table 2). Also included is the case of a Gulf of Mexico population with 2 generations per year. The naïve, unexposed case is shown for simulation years 0–200. The 4 levels of disease exposure begin in year 201. Population fitness ranges from 0–1. Note that the y-axis scale only reaches 0.7.

maturity are sufficiently rapid that two generations per year might occur. Multiple generations per year may further insulate the population from a reduction in abundance that should accompany an increment in mortality; but here, too, the rate of development of disease resistance is little changed if not slowed. The mortality rate drives selection and, thus, the rate of increase in disease resistance and the mortality rate are independent of abundance and other abetting processes maintaining abundance (discounting the possible contribution of density to transmission (Andrews 1988, White et al. 1998, Ford 1992, Gray et al. 2009)).

Rationale for the Apparent Absence of Disease Resistance

Epizootics by Dermo routinely produce mortality rates on the order of 20–30% of the adult stock, and sometimes higher. Mortality rates in the Gulf of Mexico often exceed 50% per year (Owen 1953, Mackin 1959, Mackin & Hopkins 1962, Mackin & Sparks 1962). On the onset of Dermo, mortality rate in Delaware Bay was initially upward of 40% in a stock that numbered 300 million–500 million animals in the high-mortality reach of the estuary (Powell et al. 2008). This is an increment in mortality of about 15–25% yearly. Arguably, abundance in this region was sufficient to minimize the loss of alleles by drift, thus permitting the full range of genetic response. The population response would appear to be a lessoning of mortality rate to about 25% over 1 to 2 decades (Fig. 15). This is consistent with simulations (Fig. 17). Figure 17 shows a case in which the initial mortality rate approached 50% per year. The time trend shows a decline in

mortality rate to about 30% per year, somewhat faster than observed in Delaware Bay (Fig. 15), and a further decline to about 25% per year 25 years after disease onset, somewhat slower than observed in Delaware Bay (Fig. 15). However, the simulation does not include insertion of susceptible alleles from larvae originating up-estuary early during the epizootic that would have maintained the mortality rate after disease onset higher than simulated. Overall, the time trends in Figure 17 support the inference that model parameterization provides simulated outcomes consistent with observation, that disease resistance is a process dependent on many loci, and that the rate of development of disease resistance will slow markedly when the population mortality rate declines from its initially high level.

So, the interdependence of mortality rate and selection cannot be denied its obfuscatory role. As the mortality rate declines with increased disease resistance, the rate of improvement in disease resistance must likewise decline. Rates of development of disease resistance at a 22% mortality per year are little influenced by variations in population dynamics (Fig. 18) relative to a higher mortality rate of 40% per year (Fig. 19). The modulatory effects of abundance, growth rate, fecundity, and so forth, are no longer critical to the outcome, and the rate of development of further disease resistance becomes increasingly slow. Incremental improvement occurs on half-century timescales (Fig. 17). To the observer, increments in disease resistance have ceased.

The disheartening profundity is that a mortality rate of 25% per year, yielding a rate of selection profoundly slow, is still very high. In northern climes, significant decrements in abundance
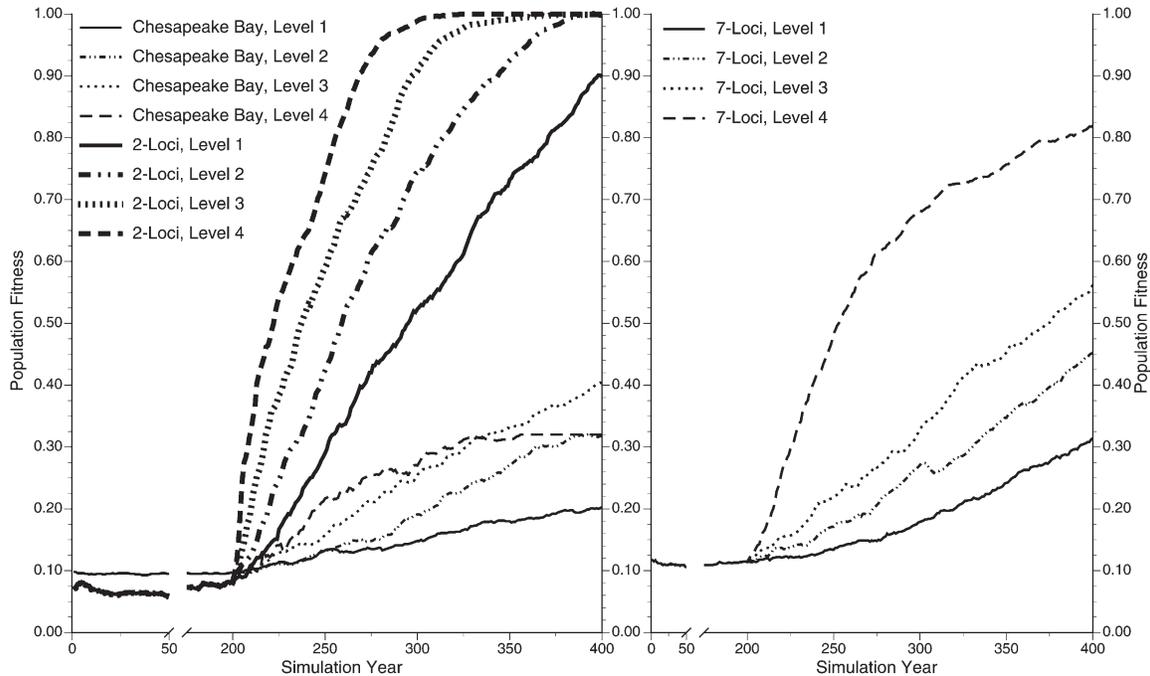
Figure 14. Comparison between population fitness for 4 disease pressures for oysters parameterized for Chesapeake Bay with 2 (2-Loci), 7 (7-Loci), or 14 (Chesapeake Bay) loci contributing to disease resistance (Table 2). The naïve, unexposed case is shown for generations 0–200. The 4 levels of disease pressure begin in generation 201. Population fitness ranges from 0–1.

will occur. Evidence from fisheries data sets and retrospectives suggests that oysters cannot withstand a constant removal at this scale without compromising population integrity noticeably (e.g., Rothschild et al. 1994, Powell et al. 2008, Powell et al.

2009b). *C. virginica* evolved at natural mortality rates on the order of half this level. Sustaining the reef itself requires high abundance (Powell & Klinck 2007, Mann et al. 2009a, Powell et al. submitted a). The ambit of response in reproduction to
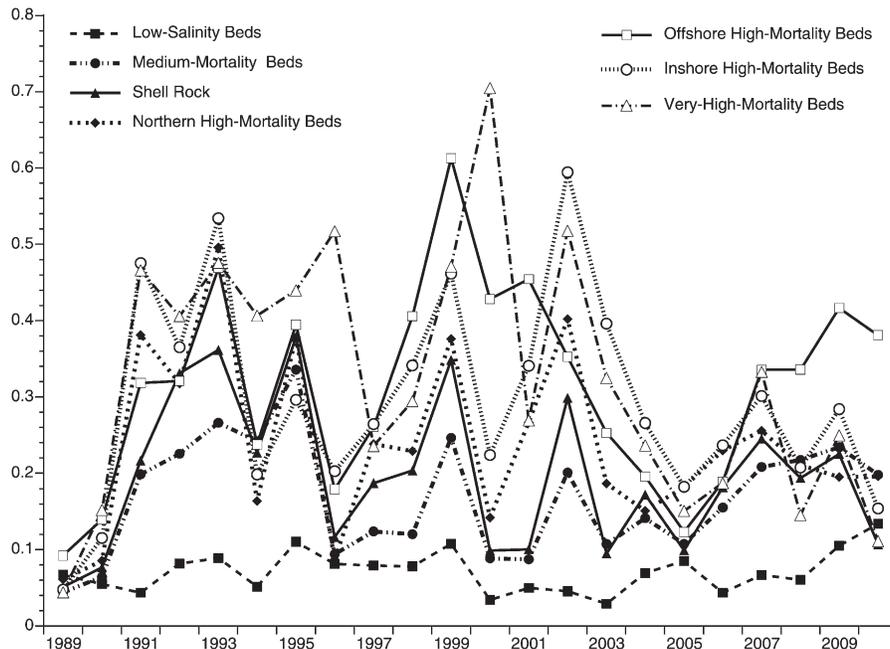

Figure 15. Trends in box-count mortality for a series of bay regions in Delaware Bay during the Dermo era, 1989 to 2010. Bed locations are shown in Figure 1 of Powell et al. (2009a). The method of calculation is provided in Powell et al. (2008, 2009a). Bay regions are defined by the following bed groupings: low salinity (Fishing Creek, Liston Range, Hope Creek, Arnolds, Upper Arnolds, Round Island), medium mortality (Upper Middle, Middle, Sea Breeze, Cohansey, Ship John, Shell Rock), northern high mortality (Bennies Sand, Nantuxent Point), offshore high mortality (Bennies, New Beds), inshore high mortality (Hog Shoal, Strawberry, Vexton, Beadons, Hawk's Nest), and very high mortality (Egg Island, Ledge). The low-salinity beds are not significantly impacted by Dermo disease. Epizootics, including the one ongoing during 2009 and 2010, have occurred 3 times since 1989 in the remaining bed regions.
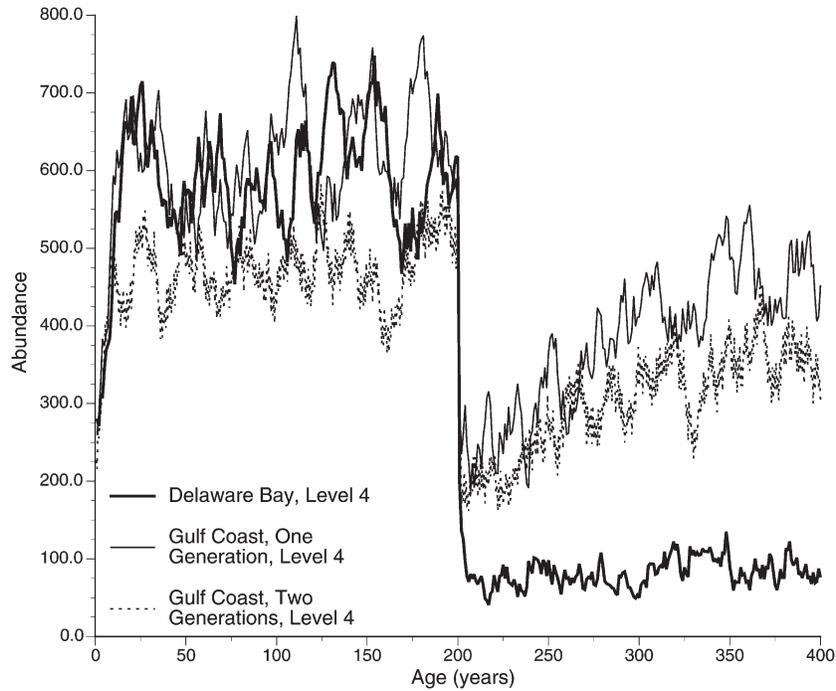
Figure 16. Comparison between population abundance after onset of Dermo disease in year 201 for slow (Delaware Bay) and fast (Gulf Coast) growth rates and for the Gulf coast case with 2, rather than 1, generations per year.

offset mortality is not large (Powell et al. 2009a). So, a mortality rate that sorely limits the development of disease resistance still strains grievously the species' ability to maintain a vibrant population dynamics necessary to its long-term survival. Is it for this reason that oysters and Dermo have been so intertwined in the internecine battle for so long?

CONCLUSIONS

It is the manager's fate to deal with the quasi-perpetual challenge of a natural mortality rate in *C. virginica* populations that is nearly too high to withstand a commercial harvest and possibly too high to sustain the reef structure itself in regions
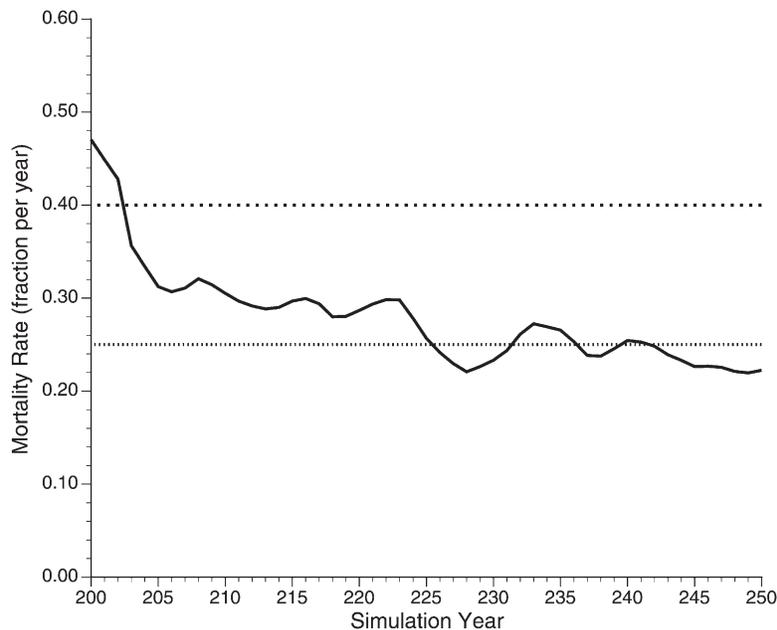

Figure 17. Trends in population mortality rate for the case of high disease pressure (level 4) and a high population abundance (Table 2, Fig. 13) for the 50-y period immediately after the onset of disease. Dotted lines record the approximate mortality rate at disease onset for disease pressures at level 4 (0.4) and level 3 (0.25).
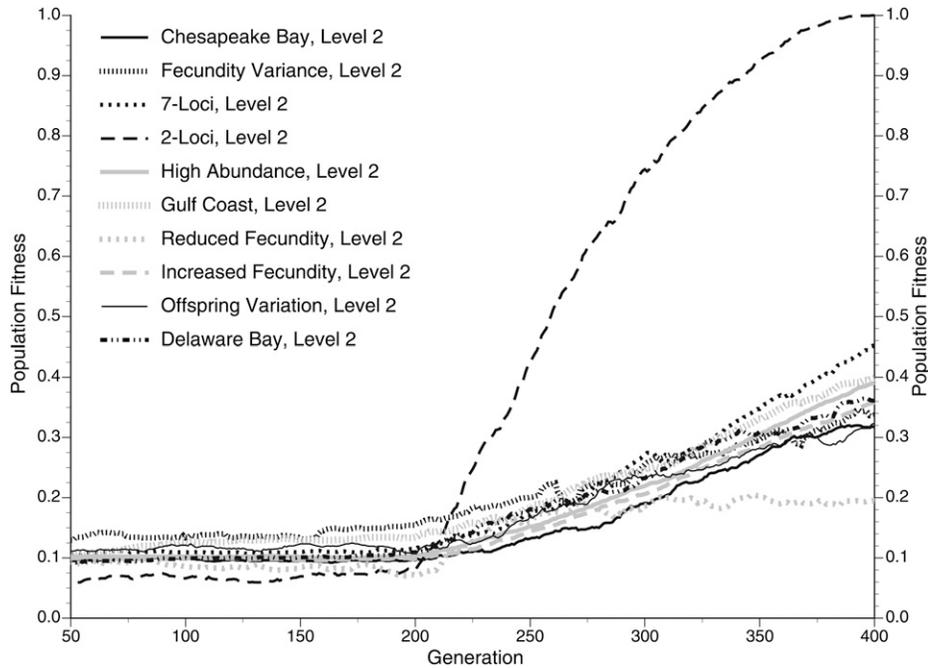
Figure 18. Comparison between a range of cases in which Dermo disease reached epizootic mortality rates of about 22% per year at its onset in generation 201. Legend titles refer back to earlier figures (Figs. 9, 12, 13, and 14).

susceptible to epizootic Dermo disease (e.g., Mann & Powell 2007). The simulations provided here support the most pessimistic of long-term predictions. An internecine equilibrium perhaps does not exist. Barring insertion of susceptible alleles from up-estuary sources, slow progress toward further disease resistance may yet be achieved, assuming of course that Dermo is inordinately incapable of responding geneti-

cally to the challenge of selection. But only a limited infusion of susceptible lavae might be sufficient to offset any selective advantage realized from an epizootic mortality rate of 20–25%. Thus, very likely, a quasi-equilibrium exists between the genetics of the oyster and the disease agent *P. marinus* that is quasi-permanent on any tractable scale of years afforded the responsive manager.
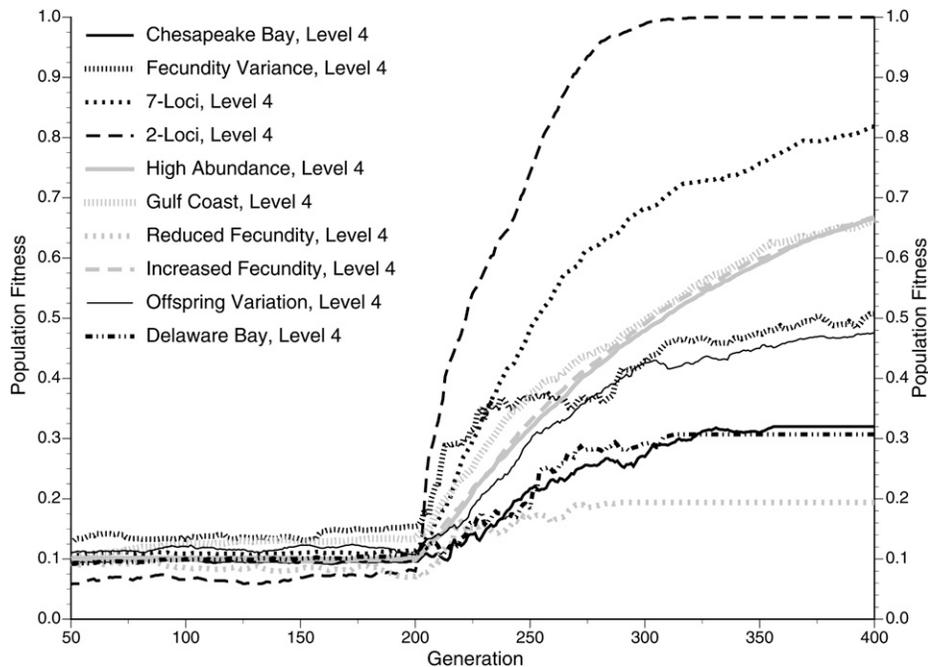

Figure 19. Comparison between a range of cases in which Dermo disease reached epizootic mortality rates of about 40% per year at its onset in generation 201. Legend titles refer back to earlier figures (Figs. 9, 12, 13, and 14).

Of course, what would be useful would be to remove selectively the susceptible individuals. A fishery removing 15% of the stock annually, if targeting the individuals predisposed to disease, would substantively augment the natural process of selection. Alas, currently the recognition of such animals is not feasible, even if it were ever possible. Much of the recent literature has focused on adaptive management consistent with the demands of the disease (e.g., Andrews & Ray 1988, Krantz & Jordan 1996, Mann & Powell 2007). Refinement of this option should be the imperative in sustaining populations wherein epizootic mortalities routinely occur.

ACKNOWLEDGMENTS

NOTES

1. Throughout, the term "mortality rate" applies to the fraction dying per year. Values given are not true rates; rather, they are equivalent to $1 - e^{-mt}$ in the equation $N_t = N_{\circ}e^{-mt}$, where m is measured in units per year and t is 1 y.

2. Model parameters are shown in `typewriter` font whereas variables are shown in *italics* font.

3. Larval fitness, an available option in DyPoGEn (Powell et al. 2011b), was not invoked in the set of simulations presented here, based on the assumption that Dermo disease does not influence larval mortality significantly.

4. Galtsoff (1964) maintained *C. virginica* for at least 9 years, so *Crassostrea* likely can live for a decade or longer, considerably beyond the conservative estimates provided by Comfort (1957) and Custer and Doms (1990), but consistent with estimates for fossil species (Kirby 2000), and recent estimates reported in Berrigan et al. (1991) (but see Harding et al. (2008) and inferences from Kraeuter et al. (2007)).

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