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Daphne M. Munroe

Eric N. Powell

Susan E. Ford

Eileen E. Hofmann
Old Dominion University, ehofmann@odu.edu

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

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Outcomes of asymmetric selection pressure and larval dispersal on evolution of disease resistance: a metapopulation modeling study with oysters

Daphne M. Munroe[1,*], Eric N. Powell[2], Susan E. Ford[1], Eileen E. Hofmann[3], John M. Klinck[3]

[1]Haskin Shellfish Research Laboratory, Rutgers University, 6959 Miller Ave., Port Norris, NJ 08349, USA
[2]Gulf Coast Research Laboratory, University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, MS 39564, USA
[3]Center for Coastal Physical Oceanography, Department of Ocean, Earth and Atmospheric Sciences, 4111 Monarch Way, 3rd Floor, Old Dominion University, Norfolk, VA 23529, USA

ABSTRACT: Marine diseases are a strong selective force that can have important economic and ecological consequences. Larval dispersal patterns, selective mortality and individual growth rates can modulate metapopulation responses to disease pressure. Here, we use a modeling framework that includes distinct populations, connected via larval transport, with varying disease selection pressure and connectivity to examine how these dynamics enhance or inhibit the evolution of disease resistance in metapopulations. Our system, oysters and MSX disease, is one in which disease resistance is highly and demonstrably heritable. Simulations show that under conditions of population isolation (i.e. local retention of larvae) and strong disease selection, populations rapidly evolve genetic disease resistance. Varying the patterns of larval dispersal alone doubles the time for evolution of disease resistance. Spatially varying disease in the absence of larval dispersal leaves some populations unable to respond to the disease, whereas adding larval dispersal slows the response of populations under strong selection and speeds the response in populations under low selection when fitness is based on relatively limited genetic structure ('juvenile fitness' in our simulations). Under spatially variable disease pressure, larval dispersal generates a fourfold greater variance in fitness outcomes across the dispersal patterns tested. In a metapopulation, populations experiencing lower selection pressure will tend to slow the development of other, more heavily selected populations. This suggests that conservation efforts aimed at improving overall metapopulation resistance in the face of marine diseases should target those populations under modest or high disease pressure, rather than protecting those experiencing low selective pressure.

KEY WORDS: Larval dispersal · Metapopulation dynamics · Connectivity · Disease · Oyster · Resistance · Structured population model · Genetic adaptation · Evolution

INTRODUCTION

Marine diseases are a strong selective force in the world's oceans, and can have significant and long-lasting economic and ecological consequences (Burge et al. 2014, Lafferty et al. 2015). Oyster diseases including MSX (Ford & Tripp 1996, Ford & Bushek 2012), dermo (Ragone Calvo et al. 2003, Bushek et al. 2012), OsHV-1µVar (=oyster herpesvirus) infection (Segarra et al. 2010, Paul-Pont et al. 2014) and *Roseovarius* (=juvenile) oyster disease (Davis & Barber 1999, Maloy et al. 2007) have caused high mortality in

*Corresponding author: dmunroe@hsrl.rutgers.edu

wild-harvested and aquacultured populations, which has translated into major economic and ecological losses (Mann et al. 2009, Lafferty et al. 2015). Examples of catastrophic disease mortality can also be found in crustacean fisheries (Shields 2012, Lafferty et al. 2015) as well as in other taxa such as abalone (Friedman et al. 2014, Lafferty et al. 2015), echinoderms (Scheibling & Hennigar 1997) and corals (Patterson et al. 2002, Kim & Harvell 2004), where massive die-offs have been associated with disruption of ecological functioning (Altizer et al. 2003, Miner et al. 2006). The economic and ecological consequences of disease in these open (dispersive) populations illustrates that a greater understanding of the mechanisms and rates of possible development of genetic resistance to disease at a metapopulation level would improve management and mitigation of losses.

The rate at which evolution of resistance occurs is controlled jointly by the strength of selection for survival (Kingsolver et al. 2001), the heritability of resistance (Fisher 1930) and the degree of genetic connectivity between a given population and others under various selection pressures (Sanford & Kelly 2011). Spatially variable selective pressure within a metapopulation may drive genetic divergence among subpopulations experiencing different levels of selection (Feng & Castillo-Chavez 2006, Dégremont et al. 2010). Alternatively, in cases where a selection (disease) refuge exists spatially (Hofmann et al. 2009, Bushek et al. 2012) or temporally (Harding et al. 2005, Powell et al. 2012), development of genetic resistance in the metapopulation could be inhibited (Ford et al. 2012) through continual input of unselected genotypes from refuge areas. This genetic connectivity among subpopulations via larval dispersal creates a homogenizing influence that tends to mix selected and unselected genotypes and slow the influence of local selection pressure (Sanford & Kelly 2011). Thus, we may predict that species with high rates of dispersal should display lower spatial genetic structure and relatively slow evolution of resistance. In practice, this has not been demonstrated as clearly as would be expected (Weersing & Toonen 2009, Selkoe & Toonen 2011); in fact, the only clear pattern that exists regarding pelagic larval duration and genetic structure is the null pattern that species with direct development (i.e. no larval dispersal) have high spatial genetic structure (Weersing & Toonen 2009, Kelly & Palumbi 2010). Larval dispersal of varying pelagic duration creates genetic mixing; however, the specific relationship between larval duration and evolution of disease resistance within spatially structured metapopulations remains unclear.

Metapopulations consist of any number of open subpopulations connected by larval dispersal, and have local selective pressures that are heterogeneous in time and space (Kritzer & Sale 2004). This variability has the potential to create uncertainty in metapopulation response to selection. Observation of, and experimentation with, these processes of selection pressure and genetic response on an ecologically relevant scale are challenging if not impossible to fully execute. One hindrance for experimentation is that direct observation of larval dispersal presents a number of challenges (Piggott et al. 2008, White et al. 2010). Increasingly, connectivity matrices generated from biophysical models are being used to predict larval dispersal in marine metapopulations (North et al. 2008, Haase et al. 2012, Narváez et al. 2012a,b). These connectivity patterns have been used to successfully predict population genetic structure in coral reef metapopulations, allowing direct comparisons between simulated and empirical genetic patterns (Galindo et al. 2006, Kool et al. 2011, Foster et al. 2012); however, these studies used neutral alleles and thus failed to include the effect of selection in their calculations. The combined influence of spatially varying selective pressure and larval dispersal (connectivity) is more complex, but has important consequences for adaptation and evolution (Sanford & Kelly 2011).

For more than half a century, eastern oysters *Crassostrea virginica* have been affected by MSX disease, caused by the protozoan pathogen *Haplosporidium nelsoni*. Eastern oysters have shown the ability to rapidly develop resistance to disease and mortality caused by *H. nelsoni* in both 'common garden' field-challenge experiments (Haskin & Ford 1979, Ragone Calvo et al. 2003) and in wild populations (Ford et al. 2009, 2012). In the Delaware estuary, a large portion of the oyster population is managed as a state fishery (Powell et al. 2011a) and annual quantitative assessments have provided a detailed account of population abundance and disease prevalence from 1953 until the present day (Ford & Haskin 1982, Powell et al. 2008). *H. nelsoni* is intolerant of low salinity and therefore the strong salinity gradient that exists in the Delaware estuary generates a corresponding disease gradient (Ford & Bushek 2012, Powell et al. 2012) such that oyster populations in the upper estuary (low salinity) experience lower disease pressure, whilst lower estuary populations in higher salinity experience higher disease pressure. The salinity gradient in the estuary creates a corresponding gradient in oyster growth, such that growth is faster in higher salinity (Kraeuter et al. 2007). It is as yet uncertain

how these environmentally-driven spatial gradients in disease pressure and oyster demographics, in combination with variable larval dispersal (Narváez et al. 2012a) influence the evolution of disease resistance.

Here, we investigate the hypothesis that spatially varying disease selection, local demography and larval dispersal influences metapopulation genetic-connectivity dynamics and the evolution of disease resistance using a framework that includes an individual-based model that simulates an eastern oyster metapopulation. We parameterized the model using the comprehensive long-term data from oyster fishery stock assessments in the Delaware estuary (Powell et al. 2011a) and historical data on the oyster stock response to MSX disease pressure (see Hofmann & Ford 2012 and other papers in the same volume for an overview). The modeling framework includes distinct populations that can be manipulated by varying levels of selective disease pressure and connectivity via larval transport.

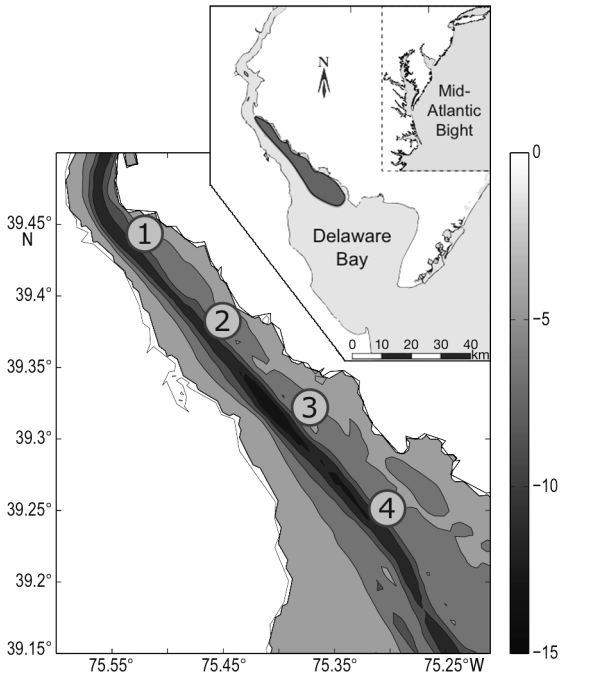

Fig. 1. Locations and depths (m) of eastern oyster *Crassostrea virginica* populations in Delaware estuary used in model simulations. Inset shows location of fished beds in Delaware estuary, and the Delaware estuary on the Atlantic Coast of the USA. Map modified from Munroe et al. (2013c)

MATERIALS AND METHODS

The model

The Dynamic Population Genetics Engine (DyPoGEn) (Munroe et al. 2012, 2013a,b, Powell et al. 2011b,c) is a numerical model that simulates the genetic structure and population dynamics of a metapopulation. We parameterized the model to simulate a metapopulation containing 4 connected populations of eastern oysters *Crassostrea virginica* in the Delaware estuary (Fig. 1). In the model, each simulated population is composed of multiple cohorts of oysters whose populations interact via larval dispersal. Larvae (offspring) are created annually from parent pairs via independent assortment of parental genotypes to simulate meiosis and random egg fertilization. The larvae produced in each population can remain within the source population (local retention, sensu Hogan et al. 2012) or disperse to any of the other populations, in which the conditions for growth and survival can differ. No larvae are lost from the system, and all are constrained to settle within 1 of the 4 populations. The general characteristics of each population are given in Table 1.

DyPoGEn has 3 basic components: (1) a post-settlement population dynamics sub-model that contains parameterizations for growth, mortality and reproduction; (2) a larval sub-model that contains parameterizations for larval mortality, larval exchange and early juvenile survival and (3) a gene sub-model that describes each individual in terms of its genetic structure to which differential fitness and survival

Table 1. Characteristics for each of the 4 eastern oyster *Crassostrea virginica* populations used in the metapopulation simulations, based on recently observed dynamics in the fished oyster stock in Delaware estuary (2000 to 2010). ND: no data

	Population			
	1	2	3	4
Abundance (millions of oysters)[a]	492	395	868	197
Average adult natural mortality rate[a]	8%	10%	16%	26%
Natural juvenile mortality rate[a]	ND[b]	8%	23%	47%
von Bertalanffy growth parameters (k / L_∞)[c]	ND[b]	0.175/110	0.26/125	0.23/140

[a]From Powell et al. (2011a); [b]ND: L_∞ estimated from stock assessment data[a]; juvenile mortality and k assumed equal to Population 2; [c]From Kraeuter et al. (2007): L_∞ in mm, k in yr^{-1}

probabilities can be applied to individual genotypes. Additional details of the single population model structure and formulation (on which our metapopulation model is based) are given in Powell et al. (2011b,c). The model processes, including specifications for growth, reproduction and mortality, have been described previously (see Munroe et al. 2012, 2013a,b), and population abundances in the model were maintained high enough to minimize the influence of genetic drift. In this study, we include a genotype–phenotype interface that interprets individual genotypes in terms of fitness, which then influences the post-settlement sub-model. This permits the feedback between genotype and phenotype that allows for selection. For brevity, we describe only those novel components of the model that were previously undescribed, and the mechanisms by which the previously described model processes are influenced by the genotype–phenotype interface.

Post-settlement population dynamics

MSX disease is thought to cause oyster mortality in 2 ways (Ford et al. 1988). The first is by high and rapid mortality of naïve individuals, and the second is a result of prolonged (chronic) disease in individuals that have experienced selection but are still carrying the parasite (Ford 1985, Ford & Haskin 1987, Ford et al. 1988, 1999). For the purposes of the present model, this difference is imposed on populations such that juveniles are considered naïve and therefore experience high, rapid mortality, whereas adults (having survived initial mortality episodes) experience lower, chronic exposure mortality. Thus, the probability of mortality of an individual oyster (P_{Mort}) is calculated as the combined probability of juvenile mortality (P_{JuvMort}) and adult mortality ($P_{\mathrm{AdultMort}}$):

$$P_{\mathrm{Mort}} = P_{\mathrm{JuvMort}} + P_{\mathrm{AdultMort}} \quad (1)$$

where P_{JuvMort} is calculated as:

$$P_{\mathrm{JuvMort}} = 0.3 + (1 - \mathrm{FitFacJuv}) \times d\mathrm{JuvMort}^{-0.973 \times \mathrm{Age}} \quad (2)$$

and $P_{\mathrm{AdultMort}}$ depends on the age of the animal (Age, in years) as follows:

$$P_{\mathrm{AdultMort}} = 0.5\left\{1 + \tanh\left(\frac{Age - [18 - ((1 - \mathrm{FitFacAdult}) \times d\mathrm{AdultMort})]}{9 - ((1 - \mathrm{FitFacAdult}) \times d\mathrm{AdultSpreadMort})}\right)\right\} \quad (3)$$

The probability of mortality is modulated by the individual's age and fitness (FitFac). For young oysters, FitFacJuv generates an increasing probability of mortality relative to the genetic disease resistance of an individual at a given age as *d*JuvMort (which varies from 0 under conditions of low disease pressure to 2.2 in high disease pressure; Fig. 2A); however, as age increases, the probability of mortality decreases (Fig. 2A). In older oysters, FitFacAdult specifies a probability range of mortality depending on the degree of genetic disease resistance as *d*AdultMort (varies from 0 in low disease pressure to 17.8 in high disease pressure) and *d*AdultSpreadMort (varies from 0 in low disease pressure to 6 in high disease pressure). Likewise, for oysters older than approximately 3 yr, as age increases, the probability of mortality increases (Fig. 2A).

Genetic structure and fitness

Definition of fitness

Fitness is often defined as the number of reproducing offspring that a parent produces (Charnov et al. 2007). Only a small fraction of an oyster population spawns successfully, and many recruits fail to spawn successfully before they die. Whether a recruit will reproduce is controlled by its growth rate, which controls lifetime spawning potential by influencing size-at-age (e.g. Galvani & Slatkin 2004) and the age-dependent mortality rate. Size-at-age in the model is assigned using a von Bertalanffy relationship (Kraeuter et al. 2007). In this study, age-dependent mortality is influenced by selection. For simplicity, we use modifiers to the term 'fitness' to refer to 3 subsets of this overall process. The term 'juvenile fitness' refers to the genetic complement of any oyster with an age <3 that influences the probability of death, whereas 'adult fitness' refers to the genetic factors that influence the probability of death at age 3 or greater. The term 'population fitness' refers to the arithmetic average of the fitness values for the individuals in the population.

Most models of genetically based disease resistance rely on 1-locus (e.g. MacKenzie & Bishop 1999, Abell et al. 2005) or 2-loci (e.g. Galvani & Slatkin 2004) configurations. DyPoGEn permits simulations using many loci. In this formulation, we can arbitrarily assign a designation of *A* for the allele conferring disease resistance and *B* for the alternative allele; therefore an individual that is *AA* at a given loci is homozygous resistant. Ximing Guo, from the Haskin Shellfish Research Laboratory at Rutgers University, identified 15 loci with alleles that may confer some degree of resistance to mortality from MSX disease (X. Guo unpubl. data). These loci were identified

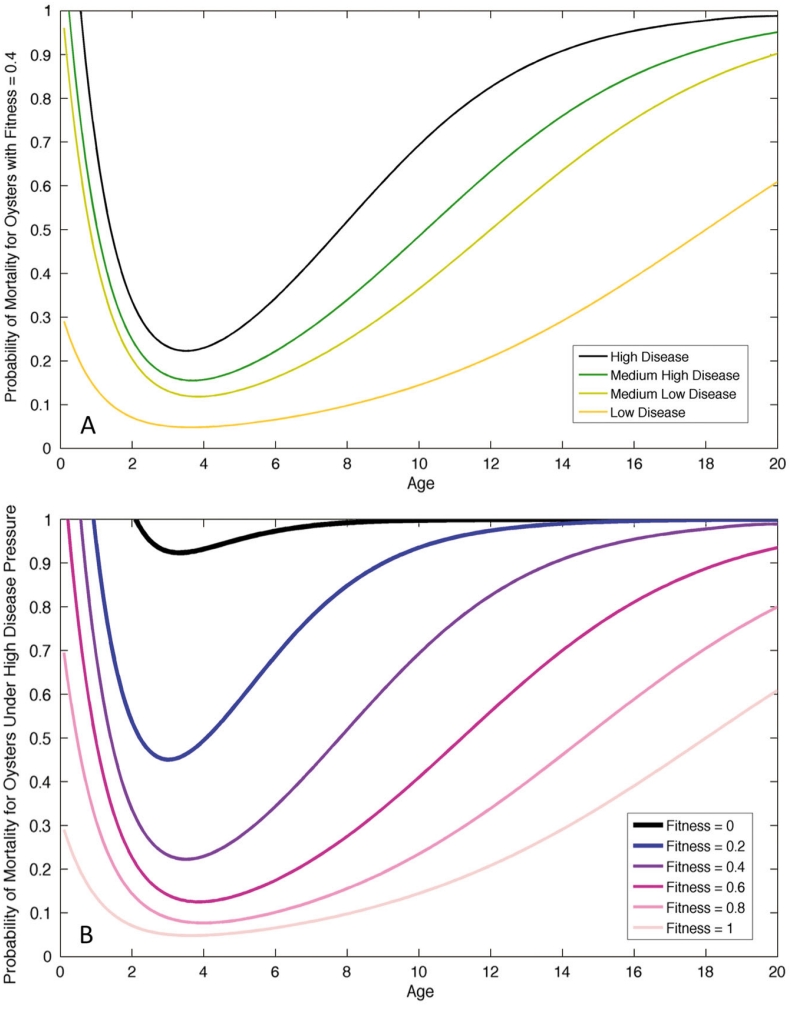

Fig. 2. Probability of mortality for an individual eastern oyster *Crassostrea virginica* as the oyster ages, showing how the probability changes (A) holding fitness constant at 0.4 but varying disease pressure, and (B) with age of the oyster, holding disease constant (high) but varying fitness

as having significant shifts in genotype frequency within families after being exposed to MSX disease-caused mortality. Some of these alleles may confer a greater increment in survival than others; however, the data currently available are insufficient to provide more than a ranking of selection pressure associated with each allele. Which loci influence juvenile mortality and which modulate adult mortality is also unknown. Based on evidence from multiple selection trials using laboratory-produced cohorts, Haskin & Ford (1979) suggested that a single locus, randomly distributed within the population could be responsible for evolution of MSX disease resistance. Using data obtained from previous disease selection experiments, which demonstrated strong selection during the first (juvenile) year of exposure of naïve stocks (Haskin & Ford 1979, Ford & Haskin 1987), we determined that the observed selection for MSX disease resistance in juveniles could not be simulated appropriately unless selection in juveniles was influenced by only a single, highly influential locus; consequently, we assigned the locus with the strongest selection (i.e. the largest differential between *AA* and *AB* genotypes) to juvenile fitness (Table 2). The remaining 14 loci were assigned to alleles associated with adult fitness, consistent with Guo's observations of the number of loci involved. Further support for the assignment of multiple loci to adult fitness is found in the observation of slower development of disease resistance in later generations of selection (Haskin & Ford 1979). The decreasing rate of disease resistance development is most easily explained by a multiple locus selection process (e.g. Powell et al. 2012). For the purposes of this study, the importance of this information is to establish (1) that selection pressure for survival through early life stages of a naïve stock is strong, (2) that a large number of loci may be involved in the adult selection process and (3) that these loci are distributed among the majority of the chromosomes (Table 2).

Genotype–phenotype interface

The genetic structure of each oyster is defined by 10 pairs of chromosomes (Wang et al. 2005); for computational convenience, we assigned 4 genes to each chromosome and 2 alleles per gene. Thus, each animal is specified by 40 loci, with the genotypes permitted at each locus being *AA*, *AB* and *BB*. To initiate each simulation, an initial population is created with a defined genetic structure. Alleles on loci not involved in disease resistance are assigned to be *A* or *B* randomly, and act as neutral alleles. A single locus is used to confer disease resistance which results from early exposure of naïve animals (here termed juvenile fitness) and 14 loci are used to specify adult fitness. Generally, alleles conferring disease resistance are present at low frequency in an uninfected population; that is, these alleles are not favored by selection in the absence of disease (e.g. Detilleux

Table 2. Relative eastern oyster *Crassostrea virginica* allele fitness values and chromosome locations for each of the 15 loci associated with MSX disease resistance. Each is referenced to the assumed maximum fitness of 1 for the *AA* genotype. Relative fitness values are based on unpublished data provided by X. Guo (Haskin Shellfish Research Laboratory, Rutgers University)

Locus	Chromosome	Life stage	Relative fitness		
			AA	*AB*	*BB*
1	2	Adult	1	0.726	0.504
2	3	Adult	1	0.556	0.162
3	4	Adult	1	0.554	0.390
4	4	Adult	1	0.561	0.325
5	5	Adult	1	0.952	0.423
6	6	Adult	1	0.419	0.131
7	6	Adult	1	0.496	0.249
8	7	Adult	1	0.424	0.152
9	7	Adult	1	0.412	0.294
10	8	Adult	1	0.566	0.089
11	9	Juvenile	1	0.385	0.179
12	9	Adult	1	0.531	0.439
13	9	Adult	1	0.969	0.395
14	10	Adult	1	0.469	0.260
15	10	Adult	1	0.622	0.389

2005, Powell et al. 2012). Since the actual allele frequencies in the naïve population are unknown, we initially implemented both juvenile and adult fitness at a 1:9 ratio of *A*:*B* alleles. Verification against observations (see 'Results') supports this choice for adults; however, the 1:9 allele frequency failed to provide an adequate response for the juvenile trait. Thus, we initiated the juvenile fitness allele frequency assuming Hardy-Weinberg equilibrium (50:50 ratio) based on the work of Haskin & Ford (1979). The etiological agent of MSX disease, *Haplosporidium nelsoni*, was introduced; that is, MSX is not a naturally occurring disease in *C. virginica* (Burreson et al. 2000), and thus there was no prior selection for or against the allele conferring juvenile resistance in the population.

In these simulations, the 15 loci are assigned an allele fitness as described in Table 2, based on the designation of *A* for the allele conferring disease resistance and *B* for the alternative allele. 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 currently be assigned with any degree of confidence. The locus and chromosome to which each gene is assigned are reported in Table 2.

Juvenile fitness for an individual oyster is determined by the fitness value that corresponds to its genotype at locus 11 (Table 2), whereas adult fitness is the average of the 14 values corresponding to its genotype from each of the loci conferring adult disease resistance (Table 2). 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* spp. (Hedgecock et al. 1995, 1996). In some cases, the simple average of the maximum or minimum values of allele fitness for the designated loci may define a range narrower than 0 to 1, inclusive. From Table 2, for example, an animal with a *BB* genotype at the juvenile locus has a juvenile fitness of 0.179, whereas an animal with genotype *BB* at all 14 adult loci would have an adult fitness value of 0.292. Therefore, to scale the genotypes on a range from 0 to 1, the minimal and maximal fitnesss values obtained for both the juvenile locus, and the adult fitness obtained from the 14 loci are standardized to values of 0 and 1; any genotype falling between 0 and 1 is standardized within the 0-to-1 continuum by interpolation. Both the final juvenile and adult fitness values for each animal, then, have an easily quantifiable distinction between the least and most fit animals; a value between 0 and 1, inclusive.

Hosts might evolve in response to a pathogen such as *H. nelsoni* in 1 of 3 ways: decreased susceptibility (the inability to become infected), increased resistance (control of pathogen proliferation once infected), or increased tolerance (lack of disease despite infection; Ford 1988, Boots & Bowers 1999). For our purposes, fitness is used as a summary response; that is, we do not try to represent the 3 ways that the evolution of response to disease might occur. In the model, selection simply operates by controlling variation in the probability of death at a given age based on an individual's value of fitness (either FitFacJuv or FitFacAdult, depending on the age of the animal), as specified in Eqs. (1) & (2).

Simulations

Simulations in this study include a single-population selection trial case (with which the genotype/phenotype response of a series of cohorts was tuned) plus a range of metapopulation simulations using 4 populations and covering a range of disease pressures, larval connectivity patterns and local demo-

graphic conditions. All simulations initiate with a fully developed oyster population structure (a normal range of size, sex and age frequencies as described in Powell et al. 2011a) in each population, and were run for 100 yr.

I. Selection trial case

The selection trial case simulates a single cohort of oysters, and is designed to reflect the population responses that resulted from a series of controlled laboratory selection experiments which generated a relatively rapid and repeatable evolution of resistance to MSX disease-caused mortality in the oysters of Delaware Bay (Haskin & Ford 1979). In these laboratory-based trials, parental stock was taken from wild oyster populations in the estuary. These parents were bred, and the offspring (F_1) were put in cages in the estuary where they were exposed for 3 yr to relatively high natural levels of MSX disease. The surviving offspring were then returned to the laboratory and bred to generate the second generation (F_2). The F_2 generation was again exposed to naturally high disease levels in cages in the estuary for 3 yr before returning the survivors to the laboratory for breeding again to generate the next generation (F_3). This selection experiment was performed multiple times by researchers at the Haskin Shellfish Research Laboratory over more than 2 decades with relatively consistent responses, such that survival increased (on average) with each generation (Haskin & Ford 1979). Results of those repeated selection experiments are summarized by Haskin & Ford (1979), and average mortality curves from these experiments are used to tune the selection pressure of disease in the model by altering the way that mortality depends on FitFac and the relative fitness of alleles. Tuning is accomplished by a single cohort model run, in which that cohort is exposed to high rates of disease selection, and oysters are allowed to reproduce only once in their lifetime (at 3 yr of age). This allowed direct comparison of the observed survival curves with simulated survival of each cohort to tune selection pressure and oyster mortality in the model, ensuring model selection pressure reflected the observed responses to MSX disease of initially naïve populations. Pathological examinations revealed that *H. nelsoni* was present, and heavy, in oysters during that time and that MSX disease was the major cause of mortality; other oyster diseases (including dermo) were not major sources of mortality during that period (Ford & Haskin 1987).

II. Metapopulation simulations: varying disease, larval and demographic conditions

The following sections describe the ways that disease pressure, larval connectivity and local demographic conditions are varied in simulations. All metapopulation scenarios include 4 spatially distinct populations of oysters, connected to one another by larval dispersal. In the simulated metapopulation, each population has distinct characteristics of disease pressure, larval export and local demography (i.e. growth rate and carrying capacity), thereby allowing the model to represent spatial gradients in these characteristics. Each combination of population-level settings for these categories (disease pressure, larval dispersal and local demography) is fully crossed with the others, resulting in a total of 24 metapopulation simulations (Fig. 3).

(a) Disease pressure. Two disease conditions are simulated, both of which employ constant disease pressure over time. In one condition, disease pressure is high and constant throughout all populations, thereby simulating a situation without spatial dynamics in disease pressure. In the other condition, disease pressure varies along an estuarine gradient where disease is high in Population 4 (high salinity) and grades to very low in Population 1 (low salinity) (Fig. 2A).

(b) Larval dispersal. Larval connectivity matrices specifying larval dispersal in the larval sub-model include 6 distinct patterns, 5 of which are hypothetical and 1 of which represents a Delaware estuarine-based pattern obtained from connectivity matrices calculated by Narváez et al. (2012a,b) using the Regional Ocean Modeling System (ROMS; Haidvogel et al. 2000). The hypothetical patterns of dispersal are described in greater detail below and are shown in Fig. 4 (also see Munroe et al. 2012, 2013a,b for description of the larval sub-model and behavior of neutral alleles in a metapopulation under various dispersal patterns).

For any given larval dispersal pattern, the same dispersal matrix is used in each simulated year. The contrived patterns of dispersal include one in which no connectivity occurs among populations (i.e. all larvae are locally retained) and all larvae self-recruit. A second contrived larval dispersal pattern has larvae dispersed evenly among all populations (homogenous full mixing). In the downbay larval cascade (directional dispersal) case, larvae do not move 'upbay' from their birth population but instead most larvae disperse to the adjacent population downbay. The inverse (an upbay cascade) is also used. The

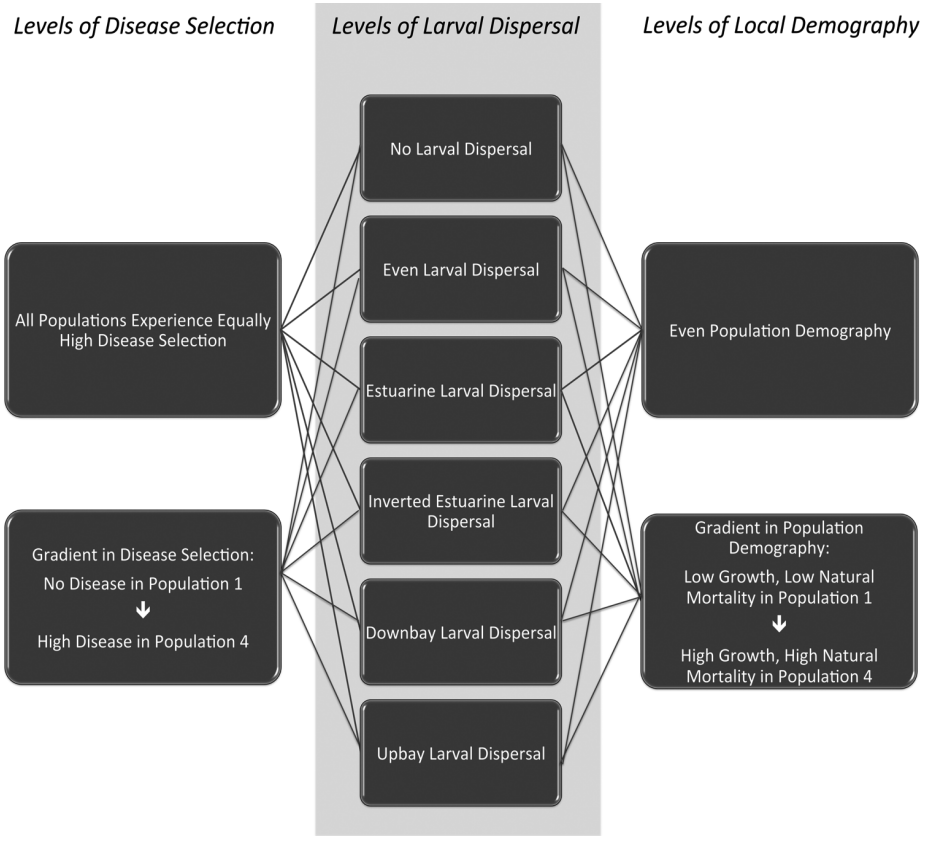

Fig. 3. All combinations included in the simulations reported in this study. Left column: levels of disease selection; center column: levels of eastern oyster *Crassostrea virginica* larval dispersal; right column: levels of local demographics. All combinations of levels are simulated, as represented by the lines connecting the levels

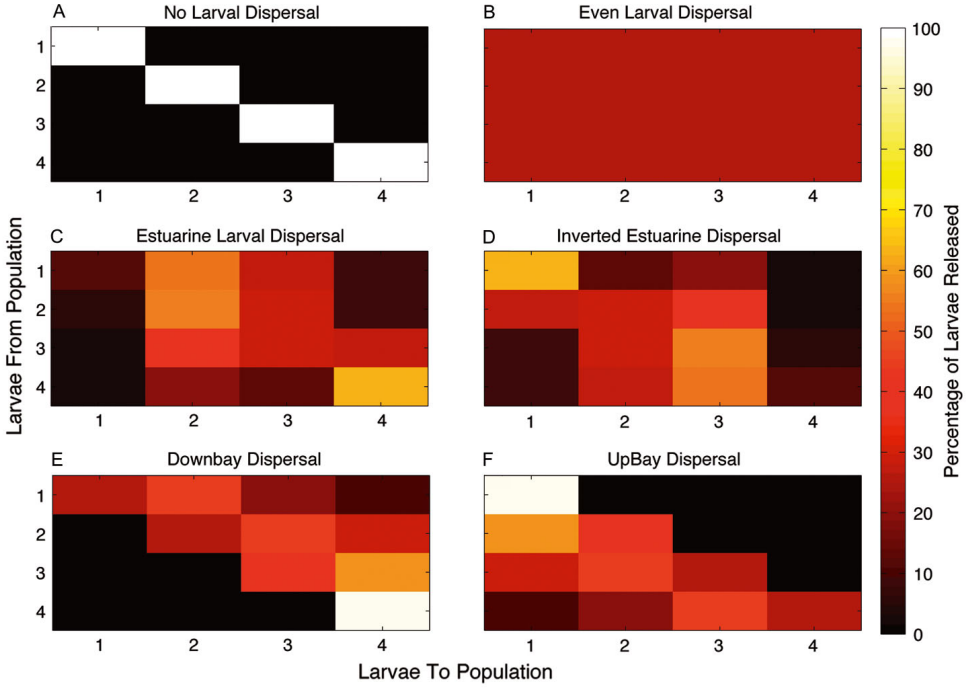

Fig. 4. Dispersal matrices for the 6 eastern oyster *Crassostrea virginica* larval dispersal patterns used in simulations: (A) local retention, (B) even dispersal, (C) estuarine dispersal, (D) inverted estuarine dispersal, (E) downbay cascade and (F) upbay cascade. Color corresponds to the number of larvae dispersed from the population listed on the *y*-axis to the population on the *x*-axis

more realistic larval dispersal condition—the estuarine larval dispersal—is based on transfer probabilities from Lagrangian larval tracking simulations as previously discussed. In a final contrived dispersal pattern, this estuarine pattern is inverted.

(c) Population demography. Two patterns of population demography are simulated: in both, local population dynamics remain constant for the duration of the 100 yr simulation (i.e. local growth rate and carrying capacity does not change over time). In one pattern of demography, all 4 populations are set to have the same conditions of local growth rate and carrying capacity, thereby simulating a case without spatial variation in local demographics. The contrasting pattern varies growth rate and carrying capacity among populations such that growth rate and carrying capacity are low in Population 1 (low salinity), grading to high in Population 4 (high salinity). This pattern agrees well with the observed conditions along the estuarine gradient in Delaware Bay that occurred during the period 2000 to 2010 (Kraeuter et al. 2007, Powell et al. 2008, 2009).

Analysis

To assess the relative influence of changes in levels of model population processes among the simulations, we use analysis of variance (ANOVA) to rank the population processes (disease pattern, larval dispersal and demographics) for each simulation output in each population (Ginot et al. 2006). The change in both juvenile and adult fitness from time zero to 25, 40, 60 and 100 yr for each population and each simulation is used as a summary metric representing the genetic response of the population over time. Change in fitness was the response variable in each ANOVA that included first-order interactions only, where the dependent variables were the levels of population parameters in each simulation. Specifically, the levels of dependent variables were: whether a gradient in selection pressure was present (2 levels), the various larval connectivity patterns (6 levels), whether a gradient in local growth and carrying capacity was specified (demographics: 2 levels), and which population was being observed (4 levels). ANOVA was used here simply as a means to rank the relative influence of changes in the population parameters of interest, rather than as a direct test of significance. For each ANOVA, variables that are significant ($p < 0.05$) are ranked from highest to lowest according to the associated *F*-statistic.

RESULTS

Comparison of simulated and observed selection trials

Oyster fitness in the single cohort selection trial case responded rapidly to disease exposure. A simulation using the genotype fitness values shown in Table 2 and high disease pressure modifiers in the mortality equations (Eqs. 1 & 2) generated an initial unselected cohort of oysters that experience very high mortality early in life when exposed to disease. This high rate of juvenile mortality in unselected oysters agrees well with the average response of naïve populations observed during multiple selection experiments performed at the Haskin Shellfish Research Laboratory (Haskin & Ford 1979, our Fig. 5). Subsequent generations of the simulated selection trial (generations 2 and 4) show greatly reduced early mortality compared to the unselected cohort, reflecting a rapid phenotypic response to selection pressure, although not as rapid as the average response from selection experiments. The same is true for mortality rates in later life stages; between simulated selection trial generation 2 and 4, a large reduction in the cumulative mortality is observed 3 and 4 yr after exposure. The rate at which older oysters die (the slope of the lines in Fig. 5) increases slightly from one cohort to the next. Again, this increase is not as pronounced as was observed for average experimental cohorts (Haskin & Ford 1979).

Metapopulations

Influence of varied disease pressure

When all populations experience homogeneously high disease selection pressure, the entire metapopulation steadily and relatively rapidly evolves higher fitness (shown by solid lines in Fig. 6). Juvenile fitness doubles from about 0.4 to 0.8 in all populations in 30 yr, whilst adult fitness doubles from about 0.05 to 0.1 in one-third of the time (10 yr). The differences in the initial fitness values between juvenile and adult fitness are due to the different initial allele frequencies and varied fitness weighting of different loci (Table 2). When spatially heterogeneous disease pressure exists across the metapopulation (dotted lines in Fig. 6), a much slower fitness response is observed. Spatially varying disease pressure among populations generates a doubling of juvenile fitness in about 100 yr, whilst adult fitness barely increases over the same duration.

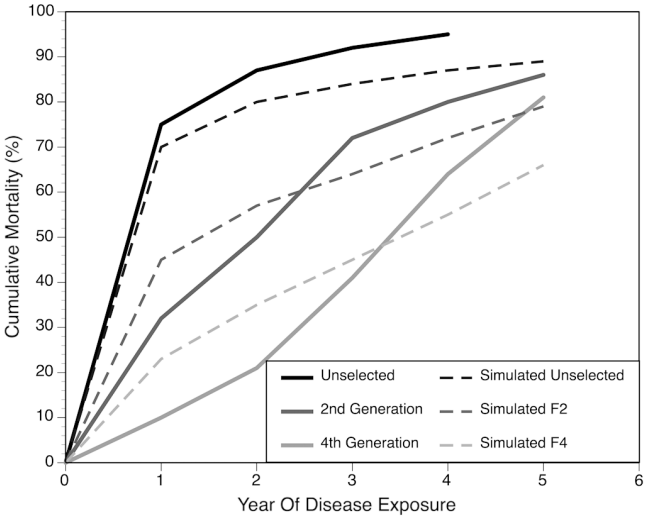

Fig. 5. Cumulative mortality curves from multiple eastern oyster *Crassostrea virginica* disease selection experiments (data from Haskin & Ford 1979, their Fig. 2) shown with solid lines. Cumulative mortality for simulated 'selection trial' oysters shown with dashed lines

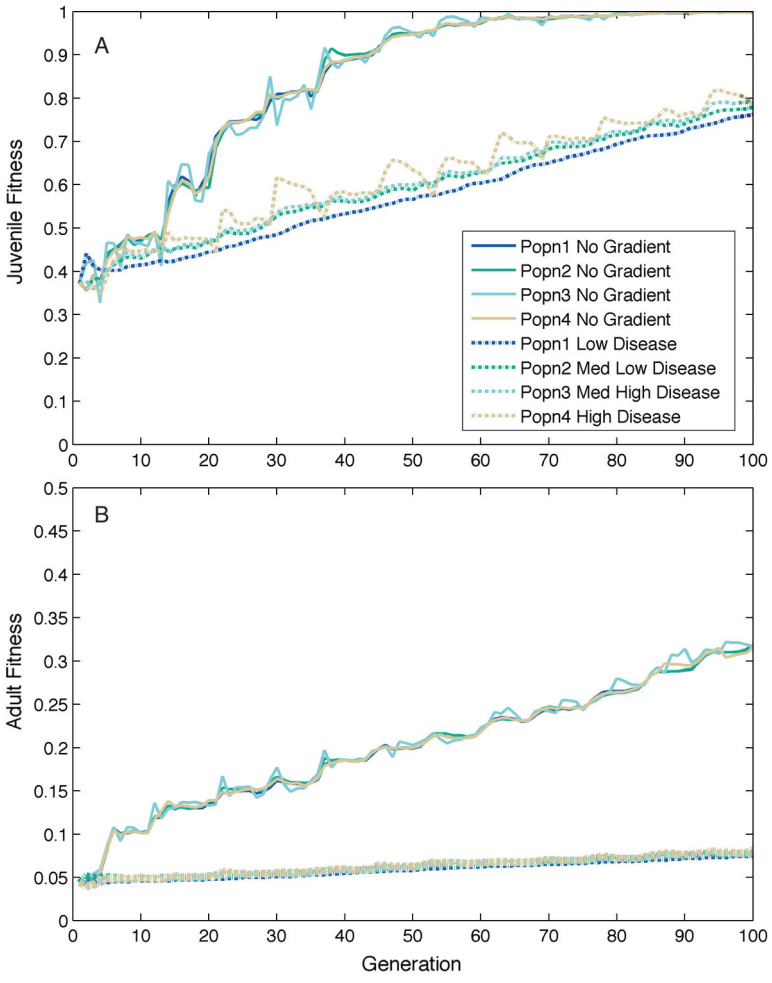

Fig. 6. (A) Juvenile and (B) adult fitness over time for each of the 4 eastern oyster *Crassostrea virginica* populations from simulations with homogeneous high disease pressure (solid lines) and with a gradient in disease pressure (heterogeneous case; dotted lines). Simulations plotted here with both homogeneous and heterogeneous disease pressure have even larval dispersal and homogeneous population demographics

Influence of varied larval dispersal

All 4 populations evolve a rapid increase in juvenile fitness and a constant increase in adult fitness when no larval dispersal occurs, with disease pressure homogeneously high and demographic conditions constant (heavy black line in Fig. 7). In the simulation with no larval dispersal, all 4 populations demonstrate identical patterns in evolution of fitness, verifying that the disease and demography parameterizations in each of the 4 isolated populations are influencing phenotypes and genotypes consistently. Therefore, allowing larval dispersal to vary while holding disease pressure and demography constant means that dispersal is the only character (of the simulations shown with the thinner lines in Fig. 7) that generates a change in the fitness response of each population. Allowing any pattern of larval dispersal tends to slow the juvenile fitness response of the population. Under conditions of no larval dispersal, the populations double juvenile fitness in approximately 15 yr, whereas allowing larval dispersal slows the doubling to approximately 25 yr. The response of adult fitness does not show the same pattern. Instead, allowing larval dispersal tends to generate a relatively constant and matching increase in adult fitness relative to the no larval dispersal case. One might expect that the directional larval dispersal patterns (upbay and downbay dispersal) should be differentially beneficial for populations at either end of the baywide metapopulation; however, this is not observed. The upbay and downbay dispersal patterns generate juvenile fitness responses that are more similar to each other than to those generated by the remaining dispersal patterns in both upbay (Population 1) and downbay (Population 4) populations.

Influence of varied population demographics

When population demographics are equal among all populations in the metapopulation and all experience high disease pressure, all 4 populations steadily evolve higher fitness (shown by solid lines in Fig. 8). Both juvenile and adult fitness double, from about 0.4 to 0.8, and 0.05 to 0.1 respectively, in all populations in approximately 30 yr. Metapopulation conditions in which all else is held constant except spatially heterogeneous demography among populations (dotted lines in Fig. 8) show a similar response of fitness in each population compared to the homogenous demography case.

ANOVA ranks

The influence of changes in disease pressure (homogeneous to heterogeneous) ranked higher than changes in other variables throughout the duration of

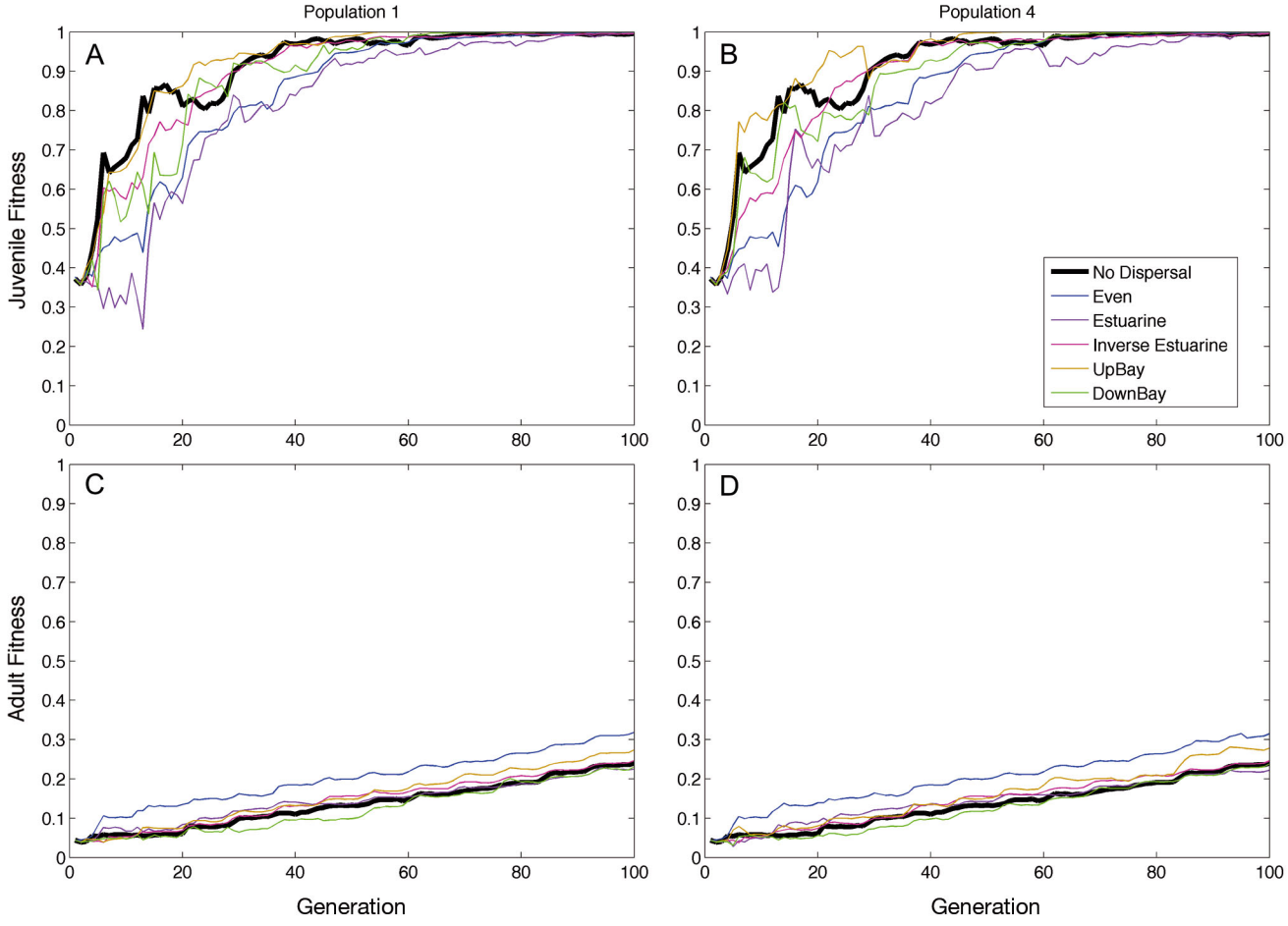

Fig. 7. (A,B) Juvenile and (C,D) adult fitness over time for the most 'upbay' eastern oyster *Crassostrea virginica* population (Population 1; A,C) and the most 'downbay' population (Population 4; B,D) under conditions of varying larval dispersal. Lines in each plot are from simulations wherein only larval dispersal varies and disease and demography are held constant among populations (homogeneous high disease and homogeneous demography). Heavy black line shows the case of no larval dispersal; remaining lines show each of the larval dispersal patterns used (see Fig. 3)

the simulation for both juvenile and adult fitness (Fig. 9). Varying larval dispersal also ranked consistently high, especially for fitness responses in the first 40 yr (generations). The interaction of selection and larval dispersal also ranked high for both juvenile and adult fitness, whereas the interaction of demographics and larval dispersal ranked relatively high when considering change in adult fitness only (Fig. 9). Under conditions of heterogeneous disease pressure, larval dispersal tends to speed the increase in fitness in unselected (low disease) populations (Fig. 10A,C), and slow the increase in fitness in heavily selected (high disease) populations (Fig. 10B,D). Larval dispersal patterns that are the most evenly mixed lead to the most rapid evolution of fitness of all populations under conditions of heterogeneous disease pressure (Fig. 10).

DISCUSSION

In any ecosystem, a number of factors play a role in the dynamics of population genotype, including supply-side factors such as larval dispersal, and post-settlement factors such as selection and growth rate. The model described here provides a way to consider these factors together and to assess their relative importance to selection for disease resistance in a metapopulation over time. Neutral alleles can be maintained homogeneously in a metapopulation with very little larval dispersal (Munroe et al. 2012); however, if a diversifying selection is present in one part of a metapopulation, we would expect that the selected allele will change in frequency over time (Dégremont et al. 2010). The simulations performed here show that pattern, with alleles conferring a

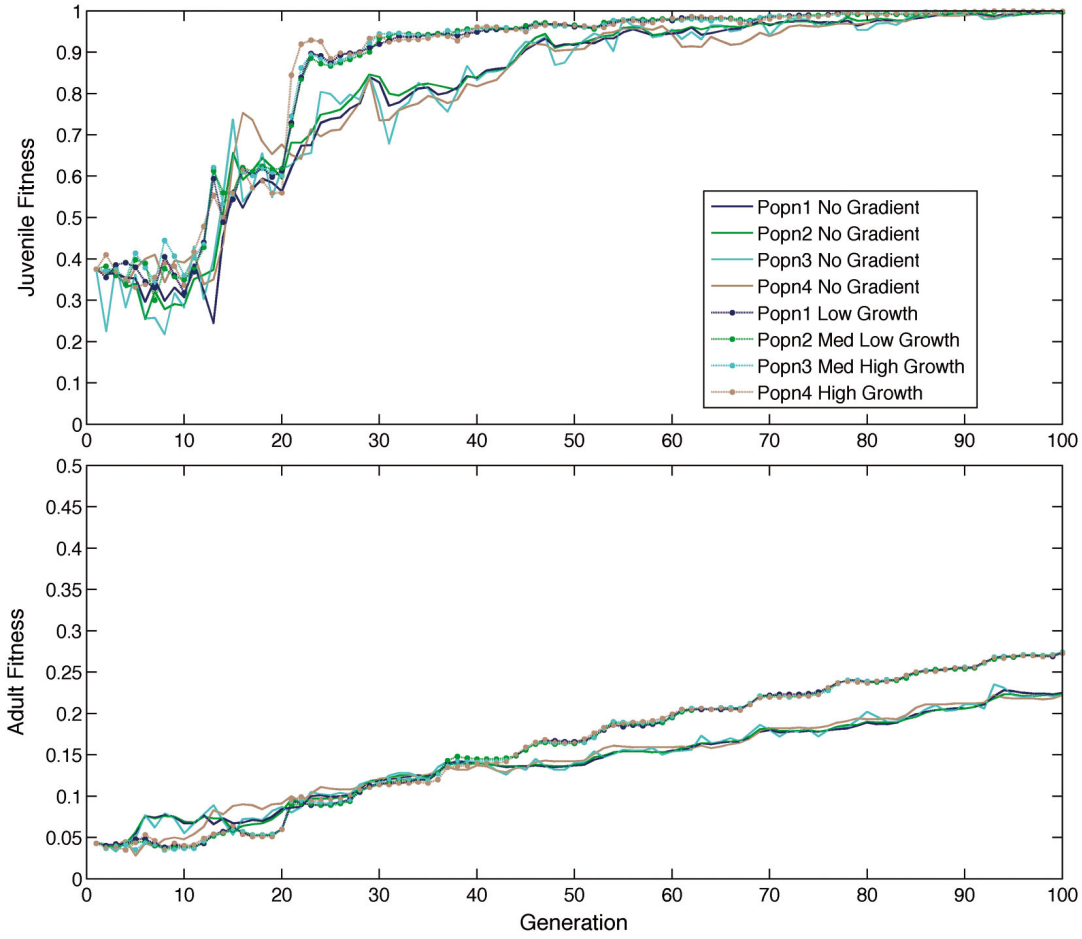

Fig. 8. (A) Juvenile and (B) adult fitness over time for each of the 4 eastern oyster *Crassostrea virginica* populations from simulations with homogeneous demographic conditions (solid lines) and an estuarine gradient in demographic conditions (heterogeneous case; dotted lines). Lines in each plot are from simulations wherein only conditions of demography change; both homogeneous and heterogeneous demographic simulations use estuarine larval dispersal and homogeneous high disease pressure

selective advantage increasing in frequency over time, and the alleles not linked to fitness (neutral alleles) remaining at about 50:50 (data not shown) in the metapopulation over time.

Selection trial simulations

Cumulative mortality within a single selection trial cohort matched results obtained empirically from repeated selection trials. We tuned fitness and selection associated with the allele parameters in the model to obtain simulation results that reflected these selection experiments; we argue that this tuning is appropriate for 2 reasons. First, these simulations are meant to follow the metapopulation response to disease exposure of a naïve metapopulation, and the laboratory-based experiments were initiated using naïve oysters (those that had not previously been exposed to MSX disease; Haskin & Ford 1979). Second, observations made on wild populations during the initial 3 yr of the MSX epizootic in Delaware Bay documented mortality rates in wild oyster spat and recruits that follow the mortality of sequential generations shown in Fig. 5 (Haskin & Ford 1979), suggesting that the cohort response demonstrated in the experimental observations was also evident in wild naïve populations. The allele parameters that generate a simulation reflective of the observed oyster mortality curves made during those repeated selection experiments is one in which the allele that is strongly selected against in early life (the first 2 yr) is initiated at 50% frequency in the naïve population. This initial frequency distribution allows for strong selection before reproduction and supports the suggestion of Haskin & Ford (1979,

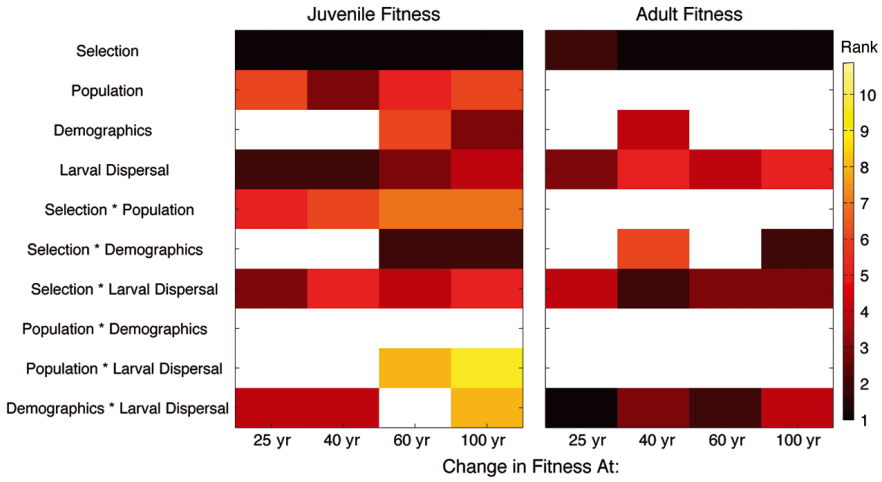

Fig. 9. Ranks of each factor and first-order interactions (*y*-axis) at 4 time points along each simulation (*x*-axis) for juvenile (left panel) and adult (right panel) eastern oyster *Crassostrea virginica* fitness. Darker colors: factors with a stronger influence on the change in fitness at a given time (i.e. higher ranks); white squares: non-significant factors. Ranks are calculated based on ANOVA with change in fitness as the response variable

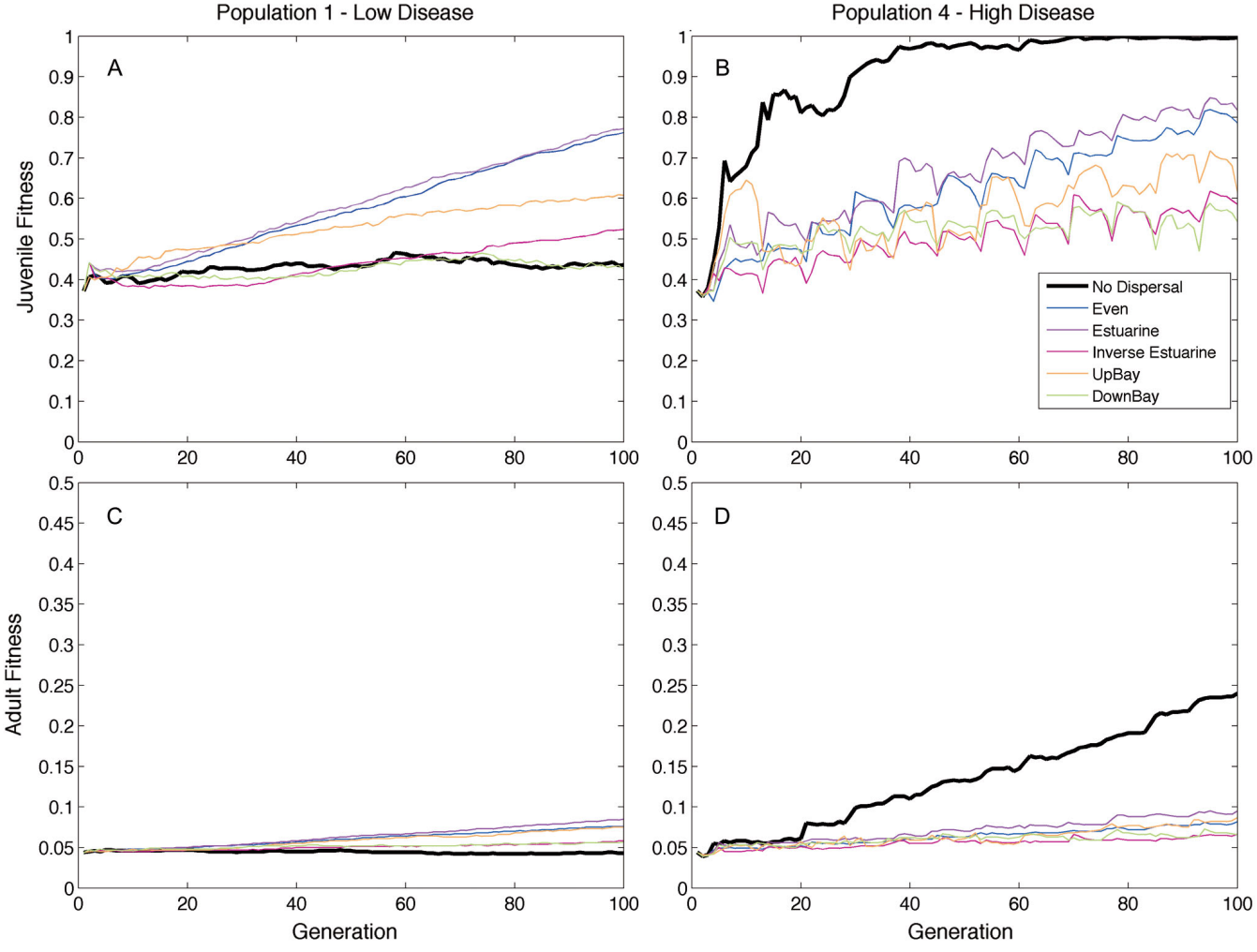

Fig. 10. (A,B) Juvenile and (C,D) adult eastern oyster *Crassostrea virginica* fitness over time for Populations 1 and 4 under conditions of varying larval dispersal and spatially varying disease pressure. Lines in each plot are from simulations wherein only larval dispersal varies and disease and demography conditions are held constant. Disease conditions follow a gradient from low disease upbay (Population 1) to high disease downbay (Population 4). See Fig. 7 legend for further description

p. 61) that 'a population of oysters that has never been exposed to MSX contains a random distribution of those genes which will determine its capacity to deal with disease'. The full complement of 15 alleles associated with resistance to mortality due to MSX disease allowed for a rapid and high early mortality in highly susceptible individuals, followed by a slower further response in individuals that survived the early exposure (Fig. 2). This condition agrees with observations made by Ford et al. (1988, p. 41), who hypothesized a 'rapid early mortality in unselected stocks… induced by a toxin and later mortality associated with loss of condition and impaired physiological functioning'. Thus, our model performance agrees with the most robust datasets available that document the response of naïve *Crassostrea virginica* to MSX disease.

Metapopulation simulations

These simulations show varied genotypic and phenotypic responses of a metapopulation to disease selection pressure under spatially varying conditions of disease pressure and larval dispersal. Biophysical larval dispersal models have proven useful in predicting the dynamics of population genetics through time. Galindo et al. (2006) used biophysically-based larval connectivity matrices to simulate the development of population genetics over time in a Caribbean broadcast spawning coral. Likewise, Kool et al. (2011) used a larval dispersal model with a genetic matrix model to predict coral population genetic structure over time. Munroe et al. (2012, 2013a,b) used an individual-based metapopulation genetics model with biophysically estimated larval dispersal patterns to simulate genetic connectivity of estuarine oysters. All of these studies were able to simulate spatial genetic population structure for sessile marine invertebrate populations; however, all used neutral allele conditions and failed to include the influence of spatially heterogeneous selection pressure. Our results provide a unique integration of the role of supply (larval dispersal) and post-settlement (selection and growth) processes on genetic dynamics in a marine system.

Spatially varying disease pressure

Spatial variability in selection pressure has the greatest influence on the overall change in fitness of a metapopulation (Fig. 9). In simulations where disease pressure is changed from homogeneously high to spatially varying, the ability of the metapopulation to respond to disease is slowed considerably by the inclusion of populations with lower disease pressure (Fig. 6). Larval dispersal of unselected genotypes from populations in low disease conditions restricts (slows) development of fitness in the remaining populations, including those under high selection pressure. When selection pressure is controlled (modulated) by the environment, as is the case for oysters in the Delaware estuary where a dominant upbay/downbay gradient in salinity controls the disease pressure (Haskin & Ford 1982) and larval dispersal allows all populations to be connected (Narváez et al. 2012a), the environmental gradient will tend to maintain unselected alleles in the metapopulation and decrease the capacity for the metapopulation to evolve resistance to the disease.

The results of our simulations support the suggestion by Ford et al. (2012) that the presence of disease refuges (Hofmann et al. 2009) within a metapopulation could slow or prevent that metapopulation from developing resistance to disease. Munroe et al. (2013b) raised similar concerns in considering the appropriate application of Marine Protected Areas (likewise for oyster sanctuaries, Rodney & Paynter 2006 and no-take reserves, Mroch et al. 2012) as a component of oyster restoration plans. The limited success of disease-tolerant oyster strains introduced into the wild emphasizes the importance of understanding the relationship of spatial variations in selection, dispersal and demography (Hare et al. 2006). In our simulations, conditions of consistently high disease selection pressure generate a relatively rapid increase in overall metapopulation fitness, particularly for the case of no larval dispersal. This rapid response is consistent with observations made in Delaware Bay after an extensive selective mortality event in 1985/1986 that resulted from a prolonged period of drought. The low river flow conditions during the drought increased salinity in the upper reaches of the estuary, thereby allowing elevated prevalence of MSX disease for all portions of the metapopulation including areas previously considered disease refuges (Ford & Bushek 2012, Ford et al. 2012). The result of this extensive and strong selection event was a relatively disease-resistant metapopulation in Delaware Bay, despite the continued presence of the disease agent, *Haplosporidium nelsoni*. In contrast to the general success of larval survival during dispersal predicted by Lagrangian particle simulations for 1985/1986 (Narváez et al. 2012b), our results suggest that for such a rapid response to

occur, these drought years may have also been times of reduced larval survival and dispersal and/or relatively high larval retention locally. However, Narváez et al. (2012b) also demonstrated that the timing of oyster spawning relative to the tidal cycle can have an impact on larval survival and the amount of local retention, with neap tides generating more local retention relative to spring tides; a prediction that agrees with patterns observed in spawning and retention of clam larvae (Carriker 1961, Chícharo & Chícharo 2001). Therefore, elevated local retention in 1985/1986 may have been a result of a large cohort from a successful spawn occurring during neap tides.

We can contrast the rapid development of MSX disease resistance in the Delaware estuary metapopulation in the mid-1980s to the slower development of resistance to the same disease in the Chesapeake Bay. Oyster populations in the Chesapeake tend to be found in higher abundances in portions of the estuary where lower salinity protects them from MSX disease (Carnegie & Burreson 2011). These populations, existing under lower disease pressure, exhibit lower disease resistance (Carnegie & Burreson 2011), and are connected through larval dispersal with other, more heavily selected populations in the estuary (North et al. 2008). In agreement with Mann et al. (1991), our simulations show that connectivity among populations under varying disease selection pressure retards overall development of disease resistance. Another factor that has failed to produce a similar metapopulation level response in oyster disease resistance in Chesapeake Bay is drought. The Chesapeake metapopulation has a much more complex larval connectivity pattern because of the numbers of tributaries and resulting hydrodynamics (North et al. 2008) than the basically funnel-shaped Delaware estuary. This more complex metapopulation structure may mean that events such as drought may have a lower chance of generating rapid evolutionary events such as that observed in the Delaware estuary. It should be noted that recent evidence shows some disease resistance may be developing in wild populations in the Chesapeake Bay (e.g. Wreck Shoal in the James River; Carnegie & Burreson 2011).

In our simulations, spatially varying disease pressure slows, but does not completely halt, the evolution of increased fitness in populations. Considering that initial MSX mortality began in 1959 in Chesapeake Bay (Andrews 1964), our simulations show that after 50 yr of exposure, fitness could increase by approximately 40 to 50% under spatially varying disease conditions (Fig. 6). Carnegie & Burreson (2011) also noted the presence of a gradient in resistance coincident with the gradient in disease pressure; this is also predicted in our model where, in a given year, the population under the highest disease pressure has a slightly higher juvenile fitness than the population under low pressure (Fig. 6). An important outcome of these simulations, in contrast to other population dynamics models that consider source–sink dynamics but do not consider spatially varied selection pressure within the metapopulation (Lipcius et al. 2008), is the suggestion that populations in areas of higher disease pressure (e.g. higher salinity populations) should be protected when the goals of protection are to facilitate development of disease resistance. In a fully connected metapopulation, regardless of the prevailing larval dispersal pattern, populations experiencing lower selection pressure will tend to slow the development of other more heavily selected populations. This suggests that efforts intended to protect specific populations (e.g. sanctuaries) would be best targeted at those populations under modest or high disease pressure to allow those populations to thrive and possibly begin to supply a selected (disease-resistant) genetic contribution to the overall metapopulation. This strategy agrees with that suggested for certain Chesapeake Bay oyster populations (Carnegie & Burreson 2011).

Larval dispersal

During selection trial exposure experiments that used oysters from locations around the estuary and exposed them to heavy disease, all responded to the disease consistently, regardless of the disease pressure they had experienced locally (Haskin & Ford 1979). This consistency among populations regardless of local disease pressure agrees with the fitness response that our simulated populations experience when larval dispersal among populations occurs (Figs. 6 & 8) and further supports that these populations are well connected through larval dispersal.

Development of disease resistance in metapopulations has been examined in other species of broadcast-spawning molluscs. Black abalone is a well-studied example in which populations have demonstrated variable responses to disease pressure (Friedman et al. 2014), with some populations failing to develop resistance to disease. The severity of abalone infection and mortality due to withering syndrome is tightly linked to temperature (Ben-Horin et al. 2013); therefore, environmental heterogeneity in temperature could result in spatially varying disease

pressure among populations. Larval dispersal from populations experiencing lower disease pressure could explain the slower-than-expected rates of development of disease resistance in some populations.

Selection is the driver of genetic change whilst larval dispersal has a homogenizing influence (Sanford & Kelly 2011). In general, this was shown in our simulations. Any connectivity among differentially selected populations tended to slow the fitness response of heavily selected populations, and speed the response of less selected populations. As such, we could expect that the most evenly dispersed larval pattern should generate the most homogenizing, or slowest rate of selection. This was true for our larval dispersal simulations, where the longest time to double juvenile fitness was seen in the 'even' and 'estuarine' larval mixing simulations (those simulations where all populations exchange larvae evenly; Fig. 7A,B) and 'even' and 'estuarine' larval mixing generated the most intermediate (average) rate of fitness change when a gradient in disease pressure was present (Fig. 10A,B). Similarly, we might expect that the 2 cascading larval dispersal patterns (upbay, downbay) should show opposite results when applied to a metapopulation with a spatial gradient in disease pressure, such that a downbay larval cascade should allow for greater influence of unselected genotypes (slower fitness response) because disease selection in the upbay population is low, and an upbay larval cascade should allow for greater influence of selected genotypes (faster fitness response) because disease selection downbay is high. The downbay cascade showed this pattern, and the upbay cascade initially showed a rapid evolution of fitness, but fitness declined around year 12, possibly due to limited larval supply from downbay because the downbay population abundance became constrained by limited recruits and high mortality (Fig. 10).

IMPLICATIONS

The importance of understanding the ecological processes that facilitate or hinder evolution of disease resistance is elevated by the possibility that disease outbreaks are increasing in frequency and severity due to climate change and other anthropogenic factors (Harvell et al. 1999, 2002, Lafferty et al. 2004, Burge et al. 2014). Our rapidly increasing detection capacity and more robust monitoring networks have confounded our ability to distinguish the artefacts of increased intensity of observation from true trends of increasing outbreaks (Lafferty et al. 2004). Nonetheless, compelling examples of well-documented diseases in marine molluscs, sponges, mammals, and even marine diseases in humans show that increasing water temperature and other anthropological stressors including pollutants and ocean acidification have the capacity to affect the severity and frequency of marine disease outbreaks (Lafferty 2009, Burge et al. 2014). In addition to changes in severity, climate change has the potential to influence the spread of marine diseases. Northward progression of MSX disease outbreaks since it was first observed in the Delaware estuary in 1957 (Haskin et al. 1966) is a classic example that has been linked with increasing temperature (Hofmann et al. 2001). The northward progression of the disease is associated with epizootic oyster mortality events over the 30 yr following initial detection that have caused massive economic burdens to fishing communities along the east coast of the United States (Burreson & Ford 2004). As disease intensity changes and species' ranges (both host and disease) extend with changing climate, we must consider the importance of understanding the mechanisms and rates of response in newly exposed populations and their capacity to develop resistance.

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