



# Laboratory transmission studies of QPX disease in the hard clam: Interactions between different host strains and pathogen isolates

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## ABSTRACT

Quahog Parasite Unknown (QPX) disease is attributed with hard clam mortalities from Atlantic Canada down to Virginia. Prior field investigations discovered that resiliency to QPX infection varied considerably among different clam stocks. QPX descriptions and pathologic severity have also varied among field surveys and diagnostic reports, raising the question of whether there is more than one strain of the parasite throughout its known geographic range. In this study we used a recently developed experimental transmission methodology to test the hypothesis that genotypic variability in the host and/or in the pathogen was responsible for differences in the severity of QPX infections. Inoculation methods were applied in a trial that utilized three QPX isolates, geographically or morphologically distinct, and naïve juvenile clams obtained from culture facilities in Massachusetts (MA), New York (NY), Virginia (VA), and Florida (FL). Trends in prevalence and disease severity were significantly associated with seed origin and QPX isolate. Results show clams from FL and VA to be noticeably more susceptible toward QPX infection than clams from MA or NY. QPX isolated from infected NY clams appeared more virulent than the QPX isolated from infected MA clams. Hard clam stock susceptibility differences are important considerations for aquaculture applications in the field, it is also important to be aware that different QPX strains represent different potential threats of disease.

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## 1. Introduction

Quahog Parasite Unknown (QPX) is a thraustochytrid (Maas et al., 1999; Ragan et al., 2000; Stokes et al., 2002; Qian et al., 2007) that infects wild and cultured hard clams, *Mercenaria mercenaria*, from the Gulf of St. Lawrence down to Virginia (Ragone Calvo et al., 1998; MacCallum and McGladdery, 2000). Most of what is known about this infectious disease is from a few diagnostic reports and field studies. Mortality events were often associated with cultured clams, spurring initial field investigations to look at grow-out plots with known QPX activity and it was found that Northeastern clam stocks were more resilient to QPX infection than clam stocks imported from South-eastern states (Ford et al., 2002b; Ragone Calvo and Burrenson, 2002; Ragone Calvo et al., 2007). In their field study, Ragone Calvo et al. (2007) deployed 5 different strains of clams at sites in New Jersey and Virginia, both known to harbor QPX, and demonstrated that Florida and South Carolina clam seeds were more sensitive to QPX than seeds from Massachusetts or New Jersey. They concluded that a clam genotype may be predisposed toward infection by QPX.

The possibility that more than one strain of the parasite exists throughout its known geographic range has been proposed by prior reports noting differences in disease presentation, diagnostic descrip-

tions, and even suggested differences in parasite pathogenicity (Ragone Calvo et al., 1998; Smolowitz et al., 1998; Dove et al., 2004). Smolowitz et al. (1998) suggested that differences in histological staining between the QPX from clams in Massachusetts (MA) and the QPX from clams in Canada “may indicate that a slightly different labyrinthomorphid organism may be responsible for the MA-QPX infections”. QPX positive clams in Provincetown (Massachusetts) had a high mantle infection up to 91% (Smolowitz et al., 1998). High mantle infection (62.8%) was also observed from the Raritan Bay (New York) mortality event but the intensity of those infections were generally more severe and diffuse, including substantial visceral infections (57.1%), equating to a higher parasite numbers and biomass than strictly mantle infections (Dove et al., 2004). If disease intensity can be taken to implicate pathogenicity, as Ragone Calvo et al. (1998) suggested, then NY QPX could be even more pathogenic than MA-QPX, especially considering the Raritan Bay outbreak has been the only incident of QPX associated mortalities in a wild population published since the first report over 40 years ago.

Since QPX is regularly detected in apparently healthy clams from field surveys (Ragone Calvo et al., 1998; MacCallum and McGladdery, 2000), the consensus among researchers is that QPX is probably an opportunistic facultative parasite, adapting to parasitism when favorable, causing disease and mortality only in certain groups that may be disadvantaged in some way (Ford et al., 2002b; Ragone Calvo and Burrenson, 2002). Ford et al. (2002b) speculated that the disadvantage may derive “perhaps from an unfavorable (clam)

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genotype–environment interaction”. Evaluation of results from field trials that use hard clams from varied geographic sources are limited because the clam stocks are inherently different in regards to tolerance to prevailing environmental conditions in the transplant site.

This study had two objectives. First, to confirm field observations made by other researchers showing variability among different clam strains (i.e. clams from Massachusetts (MA), Virginia (VA), and Florida (FL)) with regard to QPX susceptibility using laboratory (controlled) experiments that exclude variables related to natural field conditions. An additional seed group from New York (NY), which no trials have used before, was also tested. The second objective was to investigate the pathogenicity of different QPX isolates obtained from infected clams collected from two Northeastern states. We employed our recently developed experimental challenge method (Dahl and Allam, 2007) to expose four clam strains to three different QPX isolates. The underlying hypothesis we wanted to test was that disease occurrence and intensity could be the result of an unfavorable genotype interaction of both the parasite and host.

## 2. Materials and methods

### 2.1. Clams

Naïve seed clams (*M. mercenaria*, *notata* variety) of approximately 1 year of age were acquired from commercial hatcheries that operate in one of the following states; MA, NY, VA, and FL. Seeds were placed in recirculating tanks filled with filtered and ultraviolet treated seawater and allowed to acclimate. They were fed daily using commercial live concentrated phytoplankton (DT's Live Phytoplankton, [www.dtplankton.com](http://www.dtplankton.com)). Seawater (30 psu and 20–21 °C) was filtered using biological filter cartridges containing activated carbon and was continuously oxygenated to saturation.

### 2.2. QPX

Three QPX isolates were used in this challenge. Two isolates (NY0313808BC7 and NY0314220AC5) were established from two infected clams collected from 2 field sites in New York (Qian et al., 2007). QPX isolation and culturing was performed as described by Kleinschuster et al. (1998) and sub-culturing was performed weekly for 10 months before use in the challenge experiment. The third isolate, originally isolated from Massachusetts hard clams in 1997 (Kleinschuster et al., 1998), was purchased from ATCC (Number 50749) as a cryopreserved sample, thawed and maintained in culture the same way as the new isolates for about 3 months before the challenge experiment. All QPX isolate cultures were propagated in Minimal Essential Medium Eagle (MEM, Sigma M06440) according to methods described by Kleinschuster et al. (1998).

### 2.3. Challenge experiments

Experimental transmission of QPX disease was performed by injecting cultured parasite cells into the pericardial cavity of naïve clams as described previously (Dahl and Allam, 2007). This reliably reproducible technique induces QPX disease symptoms and associated clam mortalities in a few months (Dahl and Allam, 2007). An exponentially growing culture of each isolate was diluted with sterile MEM to obtain about  $2 \times 10^5$  QPX cells mL<sup>-1</sup>. Naïve clams from each seed type were separately injected with New York isolates NY0313808BC7 (strains subsequently designated as NY-QPX1) or NY0314220AC5 (NY-QPX2), or the Massachusetts isolate (MA-QPX). The volume of the inoculum applied to each seed type varied proportionally to average weight of that clam seed type (Table 1). All injections were applied adjacent to the shell hinge at the ligature crease in between the valves, aimed internally for the apex of the

**Table 1**

Weight of 100 individuals (g) from each experimental seed type and volume (μl) of inoculate injected to each clam

Seed type	Weight (g/100 seeds)	Inoculate (μl/clam)
MA	94.2	30
NY	62.8	20
VA	156.8	40
FL	109.9	30

MA, NY, VA and FL: seeds from Massachusetts, New York, Virginia and Florida, respectively.

visceral mass, into the pericardial cavity. Control injections were performed with equivalent amounts of sterile MEM. Each injection treatment was administered to 120 seed clams of a single type, subdivided into 2 replicates of 60 clams each. A total of 1920 clams were injected (including MEM-injected controls). Replicates were separately incubated in rectangular tanks containing 35 L of UV treated seawater (30 psu and 20–21 °C). They were fed daily using DT's Live Phytoplankton. Air stones connected to a compressor were used in a 'corner' style filter housing (Lee's ® Aquarium & Pet Products, Triple-flow medium corner filter) in each tank, with biological filtration substrate. Mortality of seed clams in all individual tanks was monitored on a daily basis. Dead (gapers) and moribund (extremely slow reaction to physical stimuli) clams were removed, recorded on a time log and placed in Formalin (10%, buffered) to fix tissue for histology. The experiment was conducted for a total duration of 27 weeks. In addition to an initial histopathological screening before the beginning of the experiment (T-0); seed clams were sampled for histology 15 and 27 weeks after challenge. Twenty clams were sampled from each replicate at each time interval, although actual number of seed sampled may have been less due to loss of individuals from mortality (see Results Section 3.2).

### 2.4. Histopathology

Sampled seed clams were placed in a solution that simultaneously initiated fixation and decalcification (Protocol® Decalcifying Solution A then B; Fisher Scientific). Once fully decalcified, the clam samples were transferred to formalin (10%, buffered) for preservation until dissection. A transverse slice of tissue roughly between 3 and 5 mm in thickness through the central region of the meat was made in an attempt to include visceral organs, as well as gill and mantle. Effort was taken to include tissue from the base of the siphon, where infection seems to be initiated (Smolowitz et al., 2001). Tissue sections were placed in histo-cassettes, embedded in paraffin, sectioned (5 to 6 μm in thickness), and mounted on histology slides. Stained (Harris's hematoxylin for 2 min and Eosin Y for 1 min) slides were examined by light microscopy for presence of QPX. When QPX cells were discovered, the tissue(s) infected and the infection intensity was recorded as described in Ragone Calvo et al. (1998). This classification system ranked intensity based on the number of QPX cells present on the histological section as: rare (<10 QPX cells on the section), light (11–100), moderate (101–1000) and heavy (>1000).

### 2.5. Statistical analyses

Mortality and QPX prevalence data were analyzed for significant differences according to clam seed type (geographic origin) or to the QPX isolate inoculated. Counts of QPX-infected and uninfected individuals from each histological diagnosis sample were arranged in a 2-way, Row by Column (R×C), contingency table and tested for independence of variables by means of the G test through BIOMstat (Statistical Analysis for Biologists, Version 3.3, Applied Biostatistics, Inc.). The first variable was classes of seed type, for one set of tests, and classes of QPX isolate for another set. The second variable for

both sets of tests was infection status, with one class for infected and one class for uninfected. Counts in a table were pooled from two replicate samples of the same time period (e.g. 15 weeks, 27 weeks, or Moribund). William's correction for  $G$  was determined to obtain a better approximation to the Chi-square distribution (Sokal and Rohlf, 1995). The frequency analysis program additionally carried out unplanned tests of all subsets of rows and columns in the  $R \times C$  contingency table by Gabriel's simultaneous test procedure which finds all maximal non-significant sets of rows and columns (i.e. a set that becomes significantly heterogeneous if any other row or column is added). BIOMstat was also used to perform Log-linear analysis of 3-way tables for the three variables (clam seed type, QPX isolate, and QPX infection). This analysis fits a succession of log-linear models (in which a model term has been removed, representing an interaction or variable) to frequency data in a three-way contingency table and calculates a probability of the data resulting from the model without that term. These test results can be used to determine the simplest model to fit the associations (interactions) among the 3 variables.

Mortality data, consisting of time of death (i.e. day of experiment) for individual clams, were compared by survival analysis through SigmaStat for Windows Version 3.10 (Systat Software, Inc). Kaplan-Meier survival analysis was employed, which includes both failures (death) and censored values. Censored values, from the expression 'censored from observation', mean the data has been lost from view of the study. Censored values occurred from the removal of clams at set points in time for histological diagnosis. Though a death did not occur, this information is useful since the clam survived up until the time it left the study. A survival curve results from Kaplan-Meier survival analysis distribution. A LogRank test was then performed to determine whether survival curves are significantly different. The Holm-Sidak test was used for multiple comparison procedures to determine exactly which pairs of curves are different and applies a sequential adjustment of critical values that compensates for the number of comparison tests. All results were considered significant at an overall level of  $\alpha \leq 0.05$ .

### 3. Results

#### 3.1. Mortality

##### 3.1.1. Massachusetts seed clams

Most of the mortality was moderate (<30%) and not significantly greater than the control except for the NY-QPX2 treatment (Fig. 1a). Cumulative mortality plot for NY-QPX2 rose steeply, reaching 56%, and was significantly different from the three other treatment plots ( $p < 0.001$  for each pairwise, LogRank Holm-Sidak test).

##### 3.1.2. New York seed clams

The NY-QPX1 mortality curve had the most rapid ascent, reaching over 50%, before decelerating around 2 months after challenge (Fig. 1b), and was significantly different from all other curves ( $p < 0.017$ , LogRank Holm-Sidak test). Mortality levels for NY-QPX2 and MA-QPX were similar, but levels for NY-QPX2 were significantly greater than the control ( $p = 0.01$ , LogRank Holm-Sidak test), whereas mortality levels caused by MA-QPX were not.

##### 3.1.3. Virginia seed clams

Inoculate MA-QPX had the highest total mortality at 50%, followed by NY-QPX1 (41%) and NY-QPX2 (37%) (Fig. 1c). Significant differences were detected between MA-QPX and NY-QPX1 mortality plots ( $p = 0.017$ , LogRank Holm-Sidak test). Control clams endured substantially lower mortality (<20% total) than all challenged seeds ( $p < 0.001$ , LogRank Holm-Sidak test).

##### 3.1.4. Florida seed clams

The highest mortalities (~60%) were observed in clams submitted to the NY-QPX1 and NY-QPX2 treatments (Fig. 1d). The mortality for MA-QPX followed the control injection mortality very closely reaching about half of the total mortality of the other two challenge batches at just over 30%. The contrasting sets of mortality curves were significantly different from each other ( $p < 0.001$ , LogRank Holm-Sidak test).

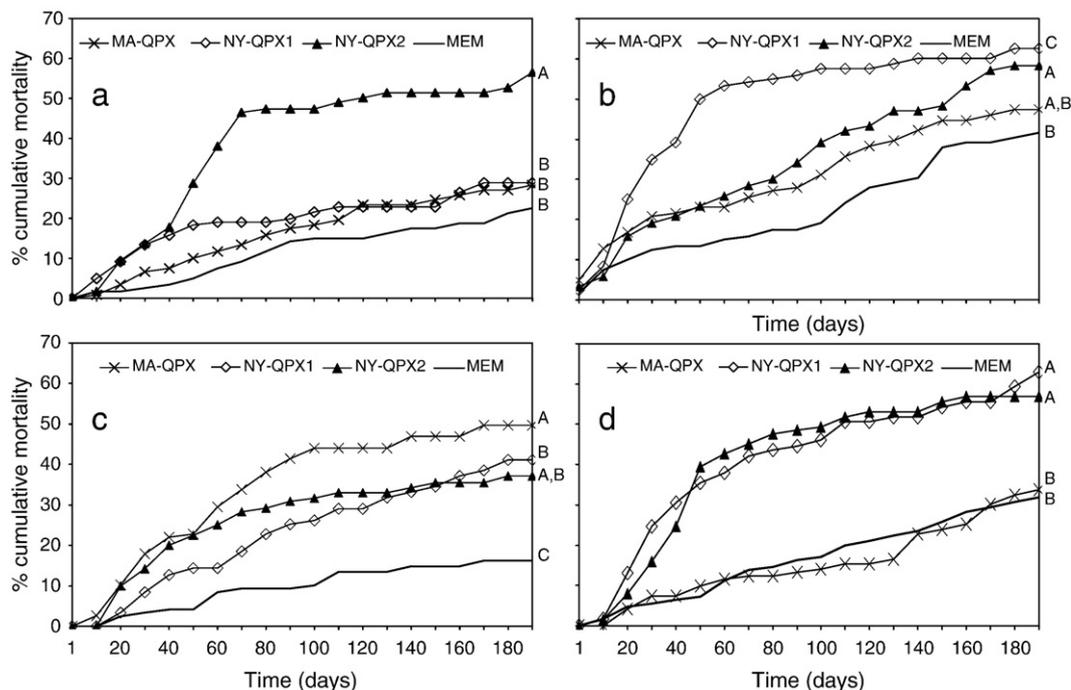


Fig. 1. Cumulative mortality plots for seeds from Massachusetts (a), New York (b), Virginia (c) and Florida (d) following challenge with three different isolates of QPX (MA-QPX, NY-QPX1, NY-QPX2). Control clams were injected with sterile culture media (MEM). Pair-wise Holm-Sidak comparisons showed that the curves labeled with the same capital letter were similar; while curves with different capital letters (e.g. A, B, or C) were significantly different from each other.

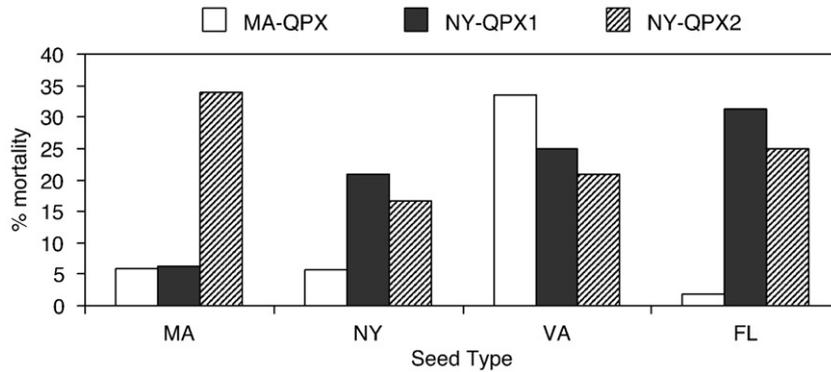


Fig. 2. Cumulative mortality obtained at the end of the experiment in QPX-challenged clams reduced by the amount of control mortality for each seed group. Same data and legend as in Fig. 1.

3.1.5. Cumulative total mortality

Adjusted total mortalities were calculated as the relative mortality in each seed type following challenge by different QPX isolates, corrected (by subtraction) for the mortality observed in control (MEM-injected) clams (Fig. 2). This calculation made the assumption that mortality in QPX-challenged clams includes clams that died for reasons other than QPX infection and subsequent disease (e.g. “natural” mortality possibly related to wounding during the injection, husbandry practices, etc.). This extrapolation was not intended to offer a precise level of mortality solely caused by QPX, but was performed to get a better impression of the mortality trends that could be attributed only to the parasite. Results showed that, most often, the mortality from the NY-QPX1 isolate was slightly greater than from the NY-QPX2 isolate, except for the MA seed. The mortality from the MA-QPX isolate was typically the lowest, except for the VA seed group, which had relatively high mortality from all of the isolates.

3.2. QPX prevalence and intensity

3.2.1. Seeds inoculated with MA-QPX

Infected individuals were rare for the first and second samplings and none were observed in the NY seed (Fig. 3a). The VA seed displayed the most prominent infections, increasing from 7.5% at 15 weeks to more than double at 27 weeks (16.7%). Moribund clams displayed the highest infection for each seed type; of which NY being the least and VA the greatest. None of the tests of independence for seed type and infection were significant. The highest infection intensities in the 15 weeks and 27 weeks samples were found in the VA seed and ranked light. The MA seed had 1 infected clam of rare intensity for the 15 weeks sample. The FL seed had 1 infected clam ranked rare for both the 15 and 27 weeks samples. The majority of VA moribund clams were of rare intensity with a few light infections (Fig. 4a). The MA moribund seed displayed some light (40%) infections as well as rare (60%). Only 1 moribund NY seed clam was diagnosed with QPX, and was ranked as rare.

3.2.2. Seeds inoculated with NY-QPX1

During the first sampling, prevalence ranged between 21% for the NY seed and 45% for the FL seed (Fig. 3b). Prevalence decreased during the second sampling and ranged from 0 (NY) to 39% (FL). QPX prevalence among moribund clams was high for all seed types; ranging from a value of 45% for MA to nearly 55% for NY. QPX prevalence frequencies were significantly different enough in the second sampling to indicate that clam seed types and QPX infections were associated ( $p=0.034$ , G test). Particularly high QPX prevalence in FL seed clams was the major source of the significance difference (Gabriel's simultaneous test procedure). All of the seed types had a proportional increase of light infections from the 15 weeks to 27 weeks sampling, with the exception of the NY seed because infection was not observed in that second sample. The MA seed had the least intense infections among moribund clams (Fig. 4b). FL and

VA both had moderate cases (7–10%) at the first sampling, and again in their moribund samples. The dying FL clams had the most intense infections of all the samples for the NY-QPX1 injection.

3.2.3. Seeds inoculated with NY-QPX2

Fifteen weeks following challenge, prevalences ranged from 12% for the MA seed to 37% for the VA seed (Fig. 3c). Prevalences decreased during the second sampling and ranged from 0 (MA) to 24% (VA). Infection was observed in at least 25% or more of the moribund clam samples from all seed types. The greatest prevalences among all samples were consistently observed in the VA seed. QPX prevalences in the

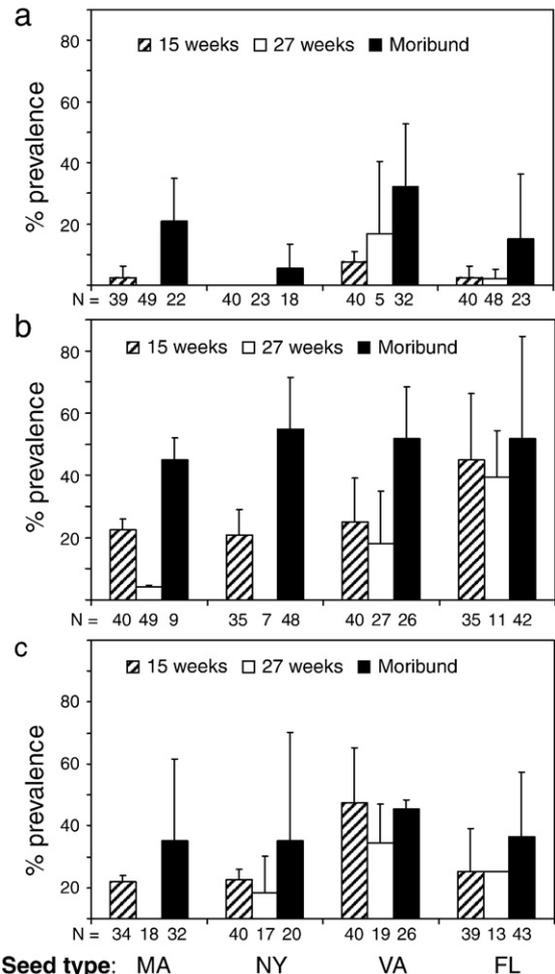


Fig. 3. QPX prevalence in clams inoculated with different QPX isolates. a: MA-QPX, b: NY-QPX1, c: NY-QPX2. The number of clams (N) is given for each group.

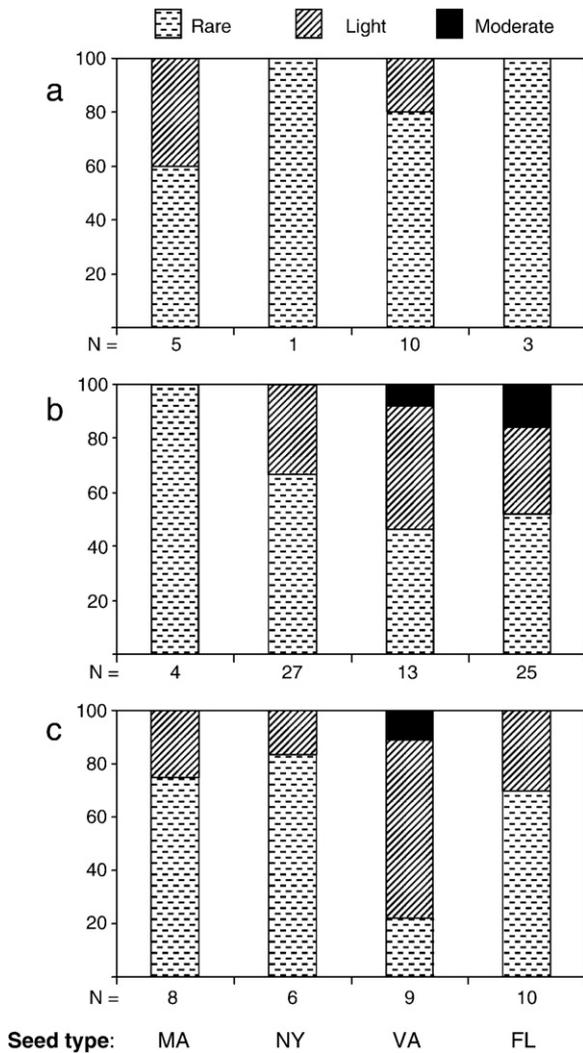


Fig. 4. QPX disease intensity in moribund clams sampled throughout the experiment following inoculation with different QPX isolates. a: MA-QPX, b: NY-QPX1, c: NY-QPX2.

15 weeks samples were significantly different enough to indicate that the clam seed type and QPX infection frequencies are associated ( $p=0.018$ ,  $G$  test). The major source of the significance difference was from the particularly high prevalence in the VA seed samples (Gabriel's simultaneous test procedure). Infected MA seed consisted of a moderate case and the rest rare in the first sampling. No infection was detected during the second MA sampling (of 18 processed clams). The infections for FL and NY seeds were similar; some light and the rest rare for the first sample and light cases equaled the number of rare cases in the second sample. The VA moribund seed infections were the most intense (Fig. 4c), with a majority (67%) of light cases and a case in the moderate category.

### 3.3. Contingency table analysis of QPX prevalence data

#### 3.3.1. Log-linear analysis of 3-way tables

Interactions among the variables account for the resultant prevalence data frequencies (Table 2). Simultaneous three factor (clam seed type, QPX isolate and QPX infection) interactions were not apparent. Among the counts of infected and uninfected clams, there were significant associations between the clam seed type and QPX isolate variables for the 27 weeks and moribund samples. According to the QPX isolate, there were significant associations between the clam seed type and QPX infection variables for the 15 and 27 weeks samples. Within the clam seed types, there were significant associations of QPX isolate and QPX infection for all

three samples. Counts of infected and uninfected clams were not independent of the clam seed type, a single 'factor' (variable), for the 27 weeks and moribund samples. All three samples were significant for the QPX isolate variable.

#### 3.3.2. Two-way table analysis

This test was performed to indicate which class of QPX isolate is influencing the significant difference among infection frequency results according to each seed type. The first samples (15 weeks) of the MA, NY, and VA seed had significantly different QPX infection frequencies ( $p \leq 0.02$ ), primarily due to a lack of infection from the MA-QPX isolate treatment. The NY moribund seed samples ( $p=0.0002$ ) and the 27 weeks FL seed sample ( $p=0.0107$ ) had significantly different QPX infection frequencies also, due to lack of infection from the MA-QPX isolate. Significant frequencies for the 15 weeks ( $p=3.46 \cdot 10^{-5}$ ) and moribund ( $p=9.49 \cdot 10^{-5}$ ) FL seed samples were due to very high infection prevalences from NY-QPX1 isolate treatment.

## 4. Discussion

High mortality and high infection prevalence were frequently correlated within each experimental batch. There may have been mortality caused by factors other than QPX disease, such as physical insult from the injection, as seed clams injected with sterile MEM suffered mortalities of ~15–40%. A clam size related trend is apparent in the control mortality range since the NY clams had the smallest average weight (Table 1) and the highest (40%) control mortality, while the VA clams had the largest average weight and the least (15%) control mortality. Prevalence from the 15 weeks sample to the 27 weeks sample usually decreased within the same seed/treatment. High mortality rates frequently leveled off after the first histology sample. Individual clams with advanced infections could have died before the second sampling. The prevalences of the moribund samples were higher than the prevalences for the 15 weeks and 27 weeks samples for almost every seed and inoculate combination, correlating QPX disease and mortalities.

QPX disease trends can be categorized by the seed type, or by the isolate, used for inoculation. Overall, NY-QPX1 displayed the highest pathogenicity, followed by NY-QPX2 and finally MA-QPX. The MA seed were the most resilient to the challenge, followed by NY seed and finally FL and VA seed. The VA seed was susceptible to infection from all three isolates while the FL seed represented the general range of pathogenicity inflicted by each QPX isolate; low for MA-QPX, moderate for NY-QPX2, and high for NY-QPX1 treatment.

Hard clams used in aquaculture may be highly variable in susceptibility toward QPX infection according to broodstock origin, with clams originating from states farther north on the East coast being more resistant to QPX than stock originating more southern

Table 2

Output table of probability values from models tested through Log-linear analysis of 3-way tables using the  $G$  statistic

	15 weeks	27 weeks	Moribund
<i>Interaction term</i>			
Simultaneous 3 factor	0.11	0.23	0.33
Clam seed type and QPX isolate	0.45	$2.52 \cdot 10^{-9}$ *	$6.04 \cdot 10^{-5}$ *
Clam seed type and QPX infection	$0.0185$ *	$0.0126$ *	0.51
QPX isolate and QPX infection	$2.28 \cdot 10^{-8}$ *	$0.0216$ *	$4.25 \cdot 10^{-6}$ *
<i>Factor term</i>			
Clam seed type	0.13	$4.13 \cdot 10^{-11}$ *	$0.0001$ *
QPX isolate	$1.75 \cdot 10^{-6}$ *	$6.54 \cdot 10^{-11}$ *	$1.66 \cdot 10^{-10}$ *
QPX infection	$5.60 \cdot 10^{-9}$ *	$0.0006$ *	$1.12 \cdot 10^{-5}$ *
Complete independence	$5.20 \cdot 10^{-7}$ *	$3.71 \cdot 10^{-12}$ *	$9.75 \cdot 10^{-10}$ *

Factors terms are the variables as follows: Clam seed Type (4 classes), QPX isolate (3 classes), QPX infection (2 classes). Significant values are denoted by an \*.

states (Ford et al., 2002b; Ragone Calvo and Burreson, 2002, Ragone Calvo et al., 2007). Our study confirmed this and demonstrated that VA and FL seeds are significantly more susceptible to the infection than MA or NY seeds. Ford et al. (2002b) and Ragone Calvo et al. (2007) argued that southern stocks are genetically selected for life in warm environments and that deploying such poorly acclimated stocks in northern locations will submit clams to stressful winter temperatures, leading to lower metabolic rates and therefore a poorer defense against the parasite. Our study tends to discredit this scenario. For instance, clams were held in the lab at temperatures observed in northern states only during summer, and allowed to acclimate for at least 3 weeks before the trial began. All of the clams were injected into tissue, bypassing pallial defense mechanisms. Essentially all of the clams were at a similar disadvantage, and yet there were clear differences in prevalence among the four seed types. Acclimation being the source of difference in susceptibility is improbable for these laboratory culture injection challenges.

Genotypic based differences in resistance to disease have been documented for valuable aquacultured bivalve species including for oysters infected with protistan parasites such as *Perkinsus marinus* (Encomio et al., 2005), *Haplosporidium nelsoni* (Barber et al., 1991), and *Bonamia ostrea* (Naciri-Graven et al., 1998). Variations in the selection strategies practiced by hatcheries will favor different traits in their offspring and is a likely source of physiological differences among clam seed. Metabolic compensation for energetic burdens posed by the *H. nelsoni* helped explain tolerance aspects of MSX resistant oysters (Barber et al., 1991). Among oysters selected for MSX resistance, it was suggested that the metabolic adjustment to parasitism could involve many genes allowing for physiologically superior resistance (Barber et al., 1991).

The fact that clams originating from southern states have shown less resistance to QPX infection than clams from the northern states could relate to the distribution of QPX itself. QPX was first documented in maritime Canada and since then it has not been documented any farther south than Virginia, despite notable clam aquaculture activities along the coast down to Florida. Even in Virginia it is uncommon. These field observations may be related to parasite's inability to grow at high temperatures as optimal growth of cultured QPX occurs between 20 and 23 °C (depending on each isolate) and higher temperatures significantly reduces parasite's survival (Buggé et al., 2005). Thus, the apparent lack of QPX in southern coastal environments means clams in that region may have never been exposed to the parasite historically, and consequently not subjected to population selection pressure caused by mortality from the disease. Investigations regarding interactions between oysters and protistan parasites linked oyster resistance to the duration parental populations had been exposed to each parasite. For example, Bushek and Allen (1996a) showed that populations exposed to *P. marinus* for >40 years (i.e. from the Gulf of Mexico) developed some resistance toward the parasite and concluded that mortality caused by *P. marinus* represents a strong selective force on natural oyster populations. The first cases of noticeable hard clam mortalities attributed to QPX were reported in 1959 in Canada (Drinnan and Henderson, 1963) and in the 1970s in the Northeastern United States (Ford et al., 2002b). Thus, northern clams are much more likely to have been exposed to QPX, possibly >40 years. Mortalities from QPX disease could impart selection within a clam population for traits of resistance in successive generations of survivors and at some point broodstock could have been chosen from such a population. Southern clam populations may be susceptible to QPX because of lack of previous exposure to this parasite. The seed clams from MA were the most resilient of the trial. Coastal Massachusetts has some of the longest known ongoing QPX infections.

Varied presentations of QPX disease from hard clam mortality reports suggested there may be different strains of QPX, but evidence as to differences in virulence was circumstantial. Deliberate QPX

culture injections presented here provided explicit evidence of variance in pathogenic insult caused by different QPX isolates. Ragone Calvo et al. (1998) noted that QPX disease in VA clams was characterized by lower prevalence and mortality as compared to disease episodes in MA, and they suggested that the MA-QPX may be more pathogenic. Following this rationale the infections reported from NY were more severe and diffuse than previously documented suggesting that NY QPX could be even more pathogenic than the MA "type" of the parasite (Dove et al., 2004). The results from this inoculation trial indicate exactly that and show that the MA-QPX isolate was not nearly as pathogenic as the two NY QPX isolates used. Differences observed in the pathogenicity of various isolates could arguably be attributed to differences in the duration of culture maintenance. The results of Ford et al. (2002a) showed that *P. marinus* maintained in cultures, as opposed to freshly obtained isolates, can lose virulence toward oysters. However, the MA-QPX culture (isolated first) was maintained by the ATCC in a cryopreserved form. Moreover, the two NY isolates were subcultured weekly for 10 months before inoculation. Thus, none of the isolates could be considered 'fresh', all three were laboratory maintained cultures. Even within the NY isolates, which have been maintained the same way and for the same duration, culture NY-QPX1 was overall more virulent. The presence of different strains displaying various pathogenicities has already been reported in other protists infecting bivalves. For example, field and laboratory observations of *P. marinus* indicate races exist that vary in virulence and/or environmental tolerance (Bushek and Allen, 1996b). Geographically distinct isolates of *P. marinus* had different levels of virulence, which lead researchers to suspect that differences observed had a genetic basis (Bushek and Allen, 1996a). Investigation of the geographic distribution of *P. marinus* confirmed that previously documented differences in virulence were consistent with genotypic differences (Reece et al., 2001). Reece et al. (2001) found that different regions (US Atlantic and Gulf of Mexico coasts) possessed unique assemblages of genetic strains. The theory that various QPX strains exist is further supported from results of trials performed *in vitro* with QPX laboratory isolates. QPX cultures were grown with manipulated environmental conditions (e.g. temperature) and parasite proliferation was compared. Isolates were found to have significant differences in optimal growth parameters (Buggé et al., 2005). Interestingly, the two New York QPX isolates, which were initially discriminated based on morphological differences (Qian et al., 2007) and were shown here to display different levels of virulence toward clams, were isolated from clams collected from the same embayment. But since these isolates were established from two different clams, it is unclear whether a clam can be infected with more than one single strain of QPX or not. Co-infection of oysters with multiple strains of *P. marinus* have already been demonstrated (Reece et al., 2001) and a similar scenario might be present in clams infected with QPX but such speculations require more in-depth specific investigations.

This study generated data concerning infectivity of different QPX strains and susceptibility of various seed clam strains, which advances the basic understanding of this host/parasite disease system. Similar laboratory based exposures of oyster populations to *P. marinus* found that oyster populations possess different abilities to inhibit infection and isolates of *P. marinus* possess different levels of infectivity (Bushek and Allen, 1996a). Significant mitigating factors of QPX disease are clam type and QPX strain. Interpretation of the 3-way Log-linear analysis results reveals that QPX prevalence is correlated to the type of clam inoculated and a strong and consistent influence is attributed to the isolate of QPX that was injected into the clam. In Bushek and Allen (1996a), "relative infection intensities among oyster populations remained more or less constant across parasite isolates and vice versa", there was lack of significant interaction between host populations and parasite isolates, indicating resistance and virulence were general and not 'race-specific'. Based on QPX disease prevalence and intensity obtained in this study, the interaction of parasite (QPX)

and host (hard clam), which significantly alters the severity of infection, also appears to be generalized and not unique strain specific interactions. In oysters, *P. marinus* infection intensity varied dependent on the combination of host population and the race of the parasite (Bushek and Allen, 1996a,b). The two NY QPX strains (more virulent) combined with either the VA or FL clam seed (more susceptible) were the most severe combinations. A significant source of fluctuation in disease development is due to innate qualities of the parasite itself. Results of this study advocate clam population selection processes, through historical parasite exposure, as a source of resiliency for a given clam stock which encourages particular consideration for local brood stock selection. When *Ostrea edulis* stocks of different geographic origins were compared for *Bonamia ostreae* susceptibility, oysters from autochthonous origins performed significantly better, regarding growth and mortality (da Silva et al., 2005). Within hard clam culture practices it is most certainly important to be considerate of the clam stock source. In a larger perspective of hard clam resource management it is also as important to be aware of different QPX strains and the potential threat of QPX infections is considerably determined by the virulent capabilities of that strain.

In conclusion, our laboratory study demonstrates that disease severity is related to the QPX isolates involved, as well as host genetics. It confirms prior field observations that southern clam stocks (FL for instance) are more susceptible to QPX infection than northern (MA and NY) stocks. Susceptibility to QPX infection should be considered when acquiring seed and use of local resistant clam strains in QPX endemic areas should be emphasized.

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