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LABORATORY TRANSMISSION STUDIES OF QPX DISEASE IN THE NORTHERN QUAHOG (=HARD CLAM): DEVELOPMENT OF AN INFECTION PROCEDURE

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ABSTRACT Quahog Parasite Unknown (QPX) disease has significantly impacted cultured and wild hard clam, *Mercenaria mercenaria*, populations in the Northeastern United States and is the first formidable disease issue concerning near market sized clams for the industry. Most of what is known about this protistan infection comes from diagnostic reports of mortality events and some preliminary field investigations. Disease dynamics and details of parasite pathobiology are somewhat of an obscurity. This study fostered a laboratory approach towards the experimental induction of infection to confirm direct transmissibility of the disease and to verify trends, observed in the field, of varied host susceptibility based on hard clam stock origin. Evidence of QPX as a directly infective pathogen was achieved, through the utilization of laboratory maintained QPX isolate cultures, as injection of QPX cells into hard clam tissue resulted in infection and subsequent mortalities in matter of a few months. Laboratory conditions did not promote transmission in a trial that aimed to mimic 'natural' methods of infection by the cohabitation of infected adult hard clams, obtained from the field, with naïve seed clams. Histopathology of the adult hard clams, at the end of the cohabitation trial, displayed a significant amount of dead and degrading QPX cells, which suggests that laboratory conditions may have promoted healing and resistance of the host. This study has established an experimental infection method that can be used for future investigations concerning crucial aspects of the QPX/hard clam disease system. Laboratory conditions that led to the healing of field infected animals require more investigations and may promote a better understanding of factors affecting disease development.

KEY WORDS: Quahog Parasite Unknown, *Mercenaria*, inoculation, cohabitation, healing, *in vitro*

INTRODUCTION

Northern quahogs (=hard clams), *Mercenaria mercenaria* (Linnaeus 1758), are commercially important bivalves along the eastern coast of North America, ranging from the Gulf of St. Lawrence down to Florida. Hard clam disease from quahog parasite unknown (QPX) infections has been documented from Maritime Canada to Virginia (Whyte et al. 1994, Ragone-Calvo et al. 1998, Smolowitz et al. 1998, Ford et al. 2002a). Significant mortality associated with QPX has impacted both cultured hard clams (e.g., in Massachusetts) (Smolowitz et al. 1998), and wild hard clams (e.g., in New York) (Dove et al. 2004).

Hard clams are sturdy bivalves, suitable for culture, and the clamming industry has typically enjoyed exemption from industry wide disease issues, QPX being the first. Baseline information concerning parasite and disease status for hard clams is deficient when compared with the amount of studies conducted regarding pathologies of other commercially important bivalves, such as the Eastern oyster for example. As the moniker "QPX" suggests, there is a lack of knowledge concerning this particular parasite and the pathobiology of the disease. Based on morphology and genetic investigations, QPX has been classified as a member of the family Thraustochytridae within the subphylum Labyrinthulomycota in the phylum Heterokonta (Maas et al. 1999, Ragan et al. 2000, Stokes et al. 2002, Qian et al. In Press). QPX disease dynamics are relatively unknown; including the mode of parasite acquisition and infection, and the duration between initial infection and clam death. Consequently, most of what is known about QPX infection comes from histological evidence of field mortality events (Ragone Calvo et al. 1998, Smolowitz et al. 1998, Ford et al. 2002a, Dove et al. 2004), which provides a late "snapshot" of the disease process. Field reports have consistently found that QPX disease associated mortality occurs in clams >1-y old (Ford et al. 1997). These authors did not find QPX in hatchery-raised seed, suggesting that cultured clams acquire the parasite in field grow-out sites. Results from preliminary field investigations indicated that hard clams from different regional stock origins had different susceptibilities to infection (Ford et al. 2002a, Ragone Calvo & Burreson 2002). Similarly, prior investigations documented variability in the presentation and severity of QPX infection from one geographic region to another (Ragone Calvo et al. 1998, Smolowitz et al. 1998, Dove et al. 2004). The field trials have begun to illuminate the role of the hard clam host in QPX disease but uncertainties remain regarding other factors influencing the infection caused by confounded and unknown environmental variables inherent in natural systems that are beyond a researcher's ability to match precisely and control. No indication of the role reservoirs and/or vectors can be inferred, even the pathogenicity of QPX in each field site may not be comparable; available parasite burden may also fluctuate drastically among and within each field site.

Establishment of an experimental QPX infection protocol would facilitate the ability to conduct studies that isolate important factors, investigating the role of potential drivers in this disease system, and providing insight into the pathobiology of this parasite. Initiating infection and recreating the disease in the laboratory is the first stage in the development of an experimental model. Transmission models utilizing experimental infection techniques have been able to reveal significant aspects in other bivalve systems from *Perkinsus marinus* and the Eastern oyster to bacterial pathologies such as Brown Ring Disease of the Manila clam (Chu 1996, Allam et al. 2002, Ford et al. 2002b, Reid et al. 2003). The present study focused on reproducing QPX infection of hard clams in the laboratory

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using two major approaches. First, transmission was attempted from clam to clam through cohabitation of infected adult and naïve seed clams. Cohabitation was conducted to mimic possible ''natural modes'' of transmission in the field. This experiment used different strains of naïve seeds and various sources of infected adults to provide a potential range of host susceptibility and parasite resources. The second approach used *in vitro* cultures of the QPX organism to challenge naïve clams using different exposure methods. One method administered QPX culture to the water column to serve as an environmental exposure. Additional methods used injections, bypassing external defensive barriers, to provide an exposure directly to the pallial cavity and alternately into the pericardial cavity.

MATERIALS AND METHODS

Cohabitation Experiment

Adult hard clams were collected from field sites known to have ongoing QPX outbreaks in New York (Raritan Bay) and Massachusetts (Cape Cod). Three replicates of 70 adults from each field site were placed into separate aerated seawater tables (275 L each; 9 total) with seawater (30 psu, 20°C to 21°C) recirculated continuously through a biological filter. Clams received a ration of commercial algae concentrate (DT's Live Marine Phytoplankton, Sycamore, IL) on a daily basis (10^7 algae cells.adult clam^{-1}.day^{-1}). Naïve seed clams (*notata* variety) of approximately one year of age were acquired from local broodstocks produced by commercial hatcheries in Massachusetts, New York, Virginia, and Florida. Hard clams originating from a Florida raised broodstock have been shown to be particularly susceptible to field exposure of QPX (Ragone Calvo & Burreson 2002), and therefore were assumed to be most likely to acquire infection. In addition to a *notata* variety from each state, a ''white'' variety cultured in New York (first generation derived from wild stock) was used to approximate a wild stock because anecdotal evidence suggests growth rates for selectively bred *notata* varieties are faster than wild clams and may reflect other physiological differences between them. Each seawater table received 80 juvenile seed clams from each of the five varieties for a total of 400 seed clams cohabitating with each batch of adult ''source'' clams. Food rations were then increased to sustain juvenile clams (5×10^5 cells.seed clam^{-1}.day^{-1}). A control seawater table was set up with the same amount of seed clams but without infected adult clams. Seed clam mortality counts were conducted every four to six weeks. Moribund adult hard clams were not removed from the seawater tables to allow their tissue to degrade, which was expected to facilitate the dispersal of the infectious agent and help disease transmission. Ammonia and pH of the recirulated seawater was tested regularly to ensure quality was maintained and partial water changes were made as needed. In addition to an initial histopatholgical screening before the experiment, seed clams were sampled for histopathology after 6.5 mos and at 10 mos of cohabitation. This schedule was established based on field studies that indicate seed acquire active infections within 1 y after deployment (Ragone Calvo & Burreson 2002, Ford et al. 2002a). Each sample aimed for $n = 20$ from all seed clam varieties in each seawater table; totaling 1,000 seed. The second sample was less than 1,000 because of loss of individuals from mortality. Adult ''source'' clams were sampled for histopathology at the end of the 10-month trial.

Inoculation Experiments

Comparison of Different Dosing Methods

QPX-free adult hard clams (''littlenecks,'' ~38 mm) from a culture facility in Florida were held in aquarium tanks (165 L each) with seawater (30 psu, 20°C to 21°C) recirculated through a biological filter and activated charcoal. Clams received a ration of commercial algae concentrate (DT's Live Marine Phytoplankton, Sycamore, IL) on a daily basis (10^7 algae cells.clam^{-1}.day^{-1}). The QPX isolate used in this experiment (clone NY8BC7) was initially isolated from an infected clam collected from Raritan Bay, NY (Allam, unpublished) and propagated in Minimal Essential Medium Eagle (MEM, Sigma M06440) according to methods described by (Kleinschuster et al. 1998). An exponentially growing culture of this isolate was diluted with sterile MEM to obtain a final concentration of 2×10^5 cells mL^{-1}. Microscopical QPX counts were performed as described by Buggé and Allam (2005). Clams were divided into three separate groups (45 clams each) that were exposed to QPX by injection into the pallial cavity (500 µl or 1×10^5 QPX cells clam^{-1}), injection into the pericardial cavity (200 µl or 4×10^4 QPX cells clam^{-1}), or through the water column by directly adding the culture to the seawater contained in clam-holding tank column (1.5×10^5 QPX cells clam^{-1}). The volume of pericardial inocula was smaller than inocula used in other exposure methods, because it was assumed that this route is more efficient in exposing clams to QPX than the other applications based on findings in another clam species challenged with pathogenic organisms (Allam et al. 2002). Injection methods were as described by Allam et al. (2002). Briefly, all of the clams were removed from the tanks and covered with wet towels overnight then transferred to buckets filled with seawater (30 psu, 21°C) to a height of ~26 mm prior to administration of injections. As individual clams began to open, a 21-gauge needle mounted on a syringe was placed into the pallial cavity, then the clam was removed from the bucket and the inoculant was administered. The second treatment batch of clams received a tissue injection at the ligature crease in between the valves, aimed internally for the apex of the visceral mass (pericardial cavity). Once injected, each clam was removed from the seawater for 1.5 h before being placed back into tanks. The remaining clams (not injected) were placed back into the tanks for the final (water column) treatment, which was applied by adding QPX cultures when the clams were feeding with the filtration system turned off to increase the chances of initiating the infection. Pericardial and pallial cavity injections were made only once at the beginning of the experiment. In contrast, QPX cultures were added to the seawater of the water column exposure treatment on a weekly basis (5×10^4 cells. clam^{-1}.week^{-1}) for the entire duration of the experiment (a total of about 74×10^6 QPX cells was added to the experimental tank that initially contained 45 clams). In all cases, control applications consisted of equal numbers of clams exposed to equivalent inoculate volumes of sterile MEM and separately incubated under similar conditions. Clam mortality was monitored daily and moribund specimens were removed from the tank and fixed for histopathology. Clams were sampled for histopathology 2 (6 clams treatment^{-1}), 14 (12 clams

treatment^{-1}), and 31 (10–12 clams treatment^{-1}) weeks after the initiation of the experiment.

Pericardial Cavity Injection in Juvenile Clams

Based on the results of the previous experiment, another experiment was designed to evaluate seed clams (which would allow ''miniaturization'' of assays) and to investigate the reproducibility of infections initiated by the pericardial treatment. Naïve seed clams of approximately one year of age were acquired from a commercial hatchery using local broodstocks, operating in Florida. Seed clams were divided into 4 separate batches of 60 clams each. The first 2 batches were injected in their pericardial cavity with QPX as described above (6×10^3 QPX cells clam^{-1} in 30 µl of MEM), whereas the 2 control batches received MEM. Each batch was placed in separate tanks (33 L each) filled with aerated recirculating seawater that was continuously passed through a biological filtration system (Lee's triple flow, medium corner filter). Mortality was monitored on a daily basis and moribund individuals were removed, recorded on a time log and processed for histopathology. Two prescheduled samplings of clams for histopathology were performed 15 and 27 wks after the beginning of the experiment (each sampling targeted 20 seed clams from each tank).

Histopathology

Seed clams were decalcified (Protocol Decalcifier, Fisher Scientific) prior to fixation in formalin (10%, buffered). Larger clams were shucked and then fixed in formalin. Once individual clams were fixed, a transverse slice of tissue roughly between 3 and 5-mm 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 (5–6 µm in thickness), and mounted on histology slides. Stained (Harris hematoxylin for 2 mins 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). In this classification system, the intensity is ranked 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).

Statistical Analyses

Data from the injection trial of juvenile clams were analyzed for significant differences between replicates, for QPX prevalence, and additionally against the controls for clam mortality. Counts of QPX infected and uninfected individuals from each histological diagnosis sample batch were arranged in a 2-way 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 software program was developed to accompany the third edition of the text *Biometry* by Sokal and Rohlf (1995). One variable concerned the replicate (1 or 2) of injection application with one class for each replicate, and the second variable was infection status with one class for infected and one class for not infected. William's correction for G was determined to obtain a better approximation to the chi-square distribution, Yate's correction for 2×2 tables was also calculated (Sokal and Rohlf 1995). Data consisting of time of death (e.g., 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 used, 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, because 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 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 differences were considered significant at $\alpha = 0.05$.

RESULTS

Cohabitation Experiment

This experiment failed to transmit QPX disease from presumably infected clams collected in the field to naïve seeds as none of the processed specimens displayed QPX infection (1559 seed clams diagnosed; <2,000 caused by mortality and clams discovered empty or not enough tissue during processing). The adult ''source'' clams were processed for histology at the end of the experiment to gain an impression of active infection after ten months. One Massachusetts adult clam was diagnosed with an active infection of a rare intensity. No other active infection was found from a total of 214 adult clams sampled. Signs of what was once active infection were observed during the inspection of the histology samples (e.g., degrading/old QPX cells, tissue disruptions/lesions, and inflammatory response). Almost half (47%) of the Massachusetts adult clams had indications of previously active infection. The New York adult clams had only a few individuals' display features of past infection (9%). These counts of healed clams corroborate prevalences of QPX diagnosed in subsets of clams collected in close proximity (time and location) of the presumably infected adults that served as the ''source'' clams. We received QPX monitoring results, after the trial was initiated that revealed the following prevalences of active QPX lesions: 60% for Massachusetts's adults and 10% for New York.

Inoculation Experiments

Comparison of Different Dosing Methods

Substantial mortality occurred for the pericardial injection treatment: 45% by 16 wk and 76% over the 31-wk trial. Two clams died of the pallial injection group and only one died of the group that had active QPX culture added to the tank water. No mortality occurred in the controls.

QPX infection was observed in a substantial portion of clams submitted to pericardial treatment; 83% at 2 wk, 42% at 14 wk, and 43% at 31 wk after challenge (Fig. 1). Two

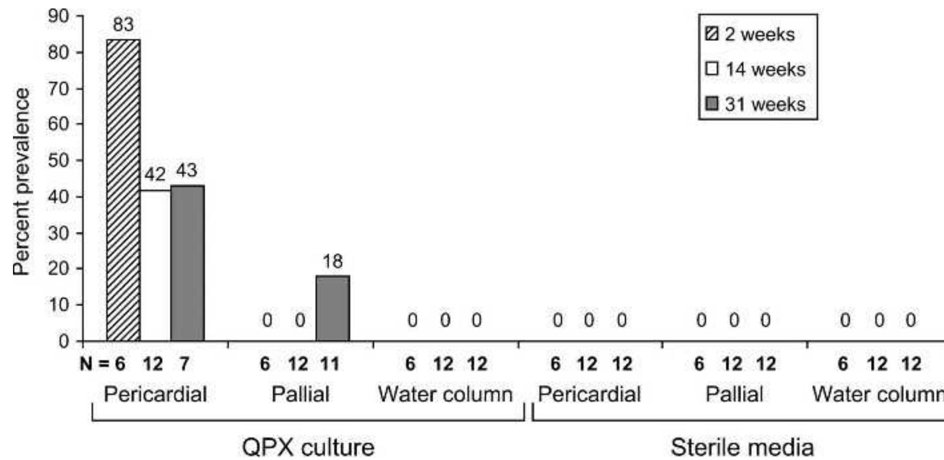

Figure 1. QPX prevalence (%) in adult clams exposed to parasite using different dosing methods.

individuals (out of 11 sampled) were positive for QPX in the sample from the pallial cavity injection group at the end of the trial. No infection was observed for the treatment that received weekly additions of active QPX culture directly to the seawater. Ten of 11 moribund clams processed for histology from the pericardial treatment were positive for QPX infection. Observed infection intensity for the pericardial treatment increased over the duration of the trial (Fig. 2). The two infections from the pallial injection treatment in the sample taken at the end of the trial were moderate. Moribund clams of the pericardial injection treatment were severely infected with abundant QPX cells. Although the injection was made in the pericardial cavity, QPX lesions were observed throughout the soft tissue, particularly in vascular tissues, sinusoidal spaces, and connective tissues of the siphon, mantle, gills, and amidst the visceral mass (Fig. 3). QPX lesions were frequently observed in more than one location of a moribund clam tissue sample. Out of all the lesions noted for the moribund clams, 48% were in the visceral mass and 52% were observed in pallial organs; the mantle was the most affected pallial organ. None of the control clams were infected.

Pericardial Cavity Injection in Juvenile Clams

Clam mortality increased rapidly in juvenile clams injected with QPX (Fig. 4). The second replicate (Challenge-2) exceeded

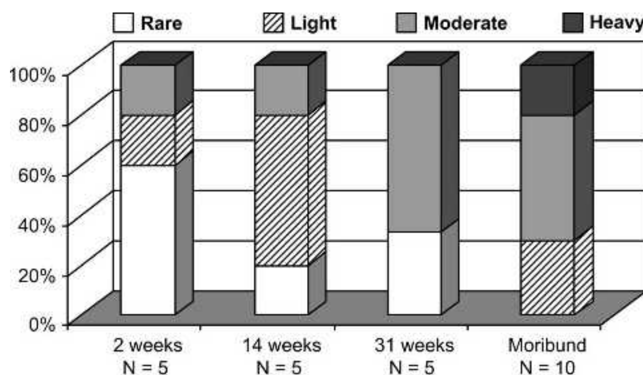

Figure 2. QPX disease intensity (%) in positive adult clams obtained after pericardial injection.

30% cumulative mortality in <1 mo after challenge. The first replicate (Challenge-1) reached that level after an additional month. An apparent disparity in mortality rates is displayed until the last few weeks of the trial when mortality in the first replicate increased and became comparable to the second batch (>60%). Despite this visual incongruity of mortality plots, statistical analyses did not reveal any significant difference between both replicates ($P > 0.05$, LogRank test). Mortality in control clams injected with sterile media showed a quick increase in one of the replicates reaching 10% within 3 wk after challenge. Overall, mortality in both control batches was relatively steady and significantly lower than both challenged batches ($P < 0.001$) reaching 30% to 35% after 7 mos.

Fifteen weeks following challenge, QPX disease lesions were observed in 30% and 60% of clams collected from Challenge-1 and Challenge-2, respectively (Fig. 5). This trend was inverted during the second sampling when prevalences of 50% and 29% were observed. Similar to mortality data, these prevalences appeared very different for the first and second sample among the two challenged batches, and yet they were not statistically different enough to be considered independent ($P > 0.05$). Among moribund clams, 29% of those collected from Challenge-1 were positive, whereas 75% of histologically processed moribund clams in Challenge-2 were positive. The severity of QPX lesions in infected clams included rare, light, and some moderate infections in the first sampling but mostly light and rare intensities in the second sampling (Fig. 6). Moribund clams displayed the most severe lesions. QPX lesions were observed similarly as in the adult injection experiment, typically in multiple locations of the clam tissue sample with 45% of all the lesions noted affecting visceral organs and 55% affecting pallial organs, the mantle in particular was most often affected. None of the control clams, including moribund specimens, were infected with the QPX organism.

DISCUSSION

The results from the inoculation experiments showed that QPX is a directly infective pathogen as injection of cultured cells leads to infection and mortality. The injection into tissue was the only effective application; the most prevalence, substantial increase in infection intensity, and 50% mortality within two to

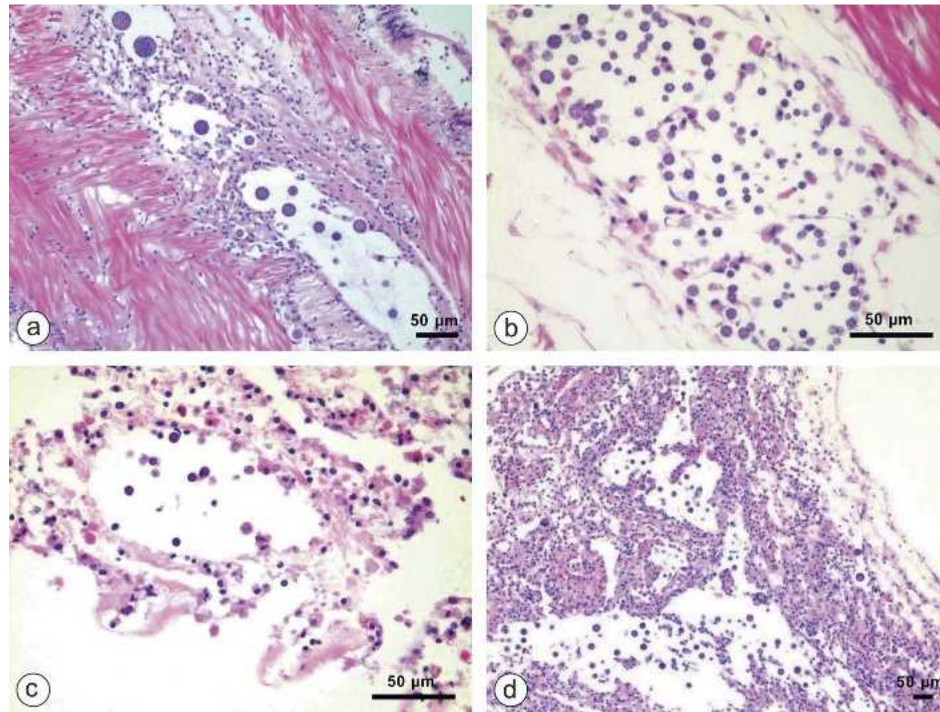

Figure 3. Micrographs showing QPX lesions in siphon (a), mantle (b), gill (c), and visceral mass (d) of clams injected with QPX in the pericardial cavity.

six months. Pericardial injection was also efficient in reproducing QPX lesions and associated mortalities in juvenile clams. In juvenile and adult inoculation experiments, QPX lesions were observed in 30% to 60% of injected animals less than 4 mo after challenge. To our knowledge, this is the first report documenting experimental induction of QPX in hard clams using *in vitro* cultures of this pathogenic protist.

Prevalence of parasites in the first (2 wk) sampling of adults injected in the pericardial cavity seems extremely high (83%) and is probably an artifact of the observation of QPX cells that had been recently injected. However, the consistent prevalence of the second and third adult samples (42% and 43%) are much later in time and therefore undoubtedly depict active infection, especially considering the presence of typical QPX lesions throughout soft tissues. It is noteworthy that lesions obtained after injection of QPX cells into the pericardial cavity were comparable to those observed following injection into the pallial cavity or in prior field investigations; QPX cells were primarily localized in vascular tissue, sinusoidal spaces and connective tissues of infected organs (Whyte et al. 1994, Smolowitz et al. 1998). Thus, although the injection procedure does not mimic natural exposure of clams to the parasite, histologic characteristics of the disease appear to be maintained. The increase in mortality after pericardial injection is consistent with the escalating intensity of the infection and the fact that the most severe lesions were observed in moribund clams. Variability noticed in the development of the infection and associated mortalities in the second injection experiment, between both

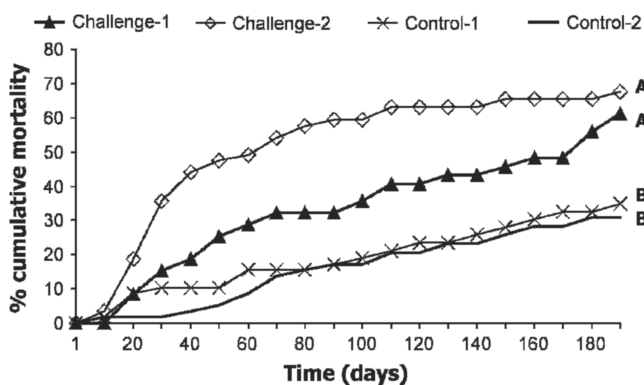

Figure 4. Mortality of seed clams after pericardial injection of QPX. Control clams were injected with MEM. Survival analysis (LogRank test followed by the Holm-Sidak test) showed that the curves labeled with the same capitol letter were similar. The A curves were significantly different from the B curves ($P < 0.001$).

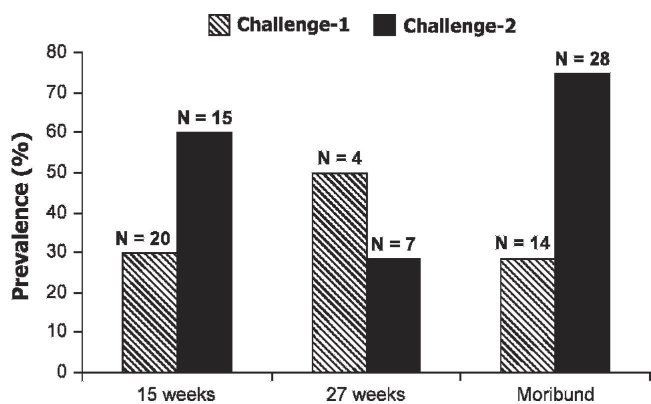

Figure 5. QPX prevalence (%) in seed clams after pericardial injection of the parasite. Differences between both replicates were not significant for clams collected at 15 and 27 wk after challenge (G test of independence, $P > 0.05$).

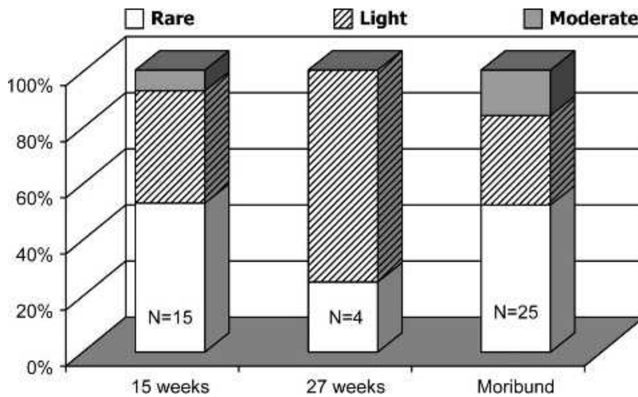

Figure 6. QPX disease intensity (%) in positive juvenile clams obtained after pericardial injection. Results from both replicates were pooled.

replicates, could results from natural variation among clams or might be simply the result of manipulator's effect, because each replicate was injected by a different person. We can speculate that slight differences, over the course of multiple small clams, in depth or angle of needle insertion into the pericardial cavity may modify QPX pathway within tissues (such as rapid dispersion of QPX throughout the circulatory system or, alternatively, its confinement in connective tissues). Such procedural differences may result in observed differences with regard to disease development. It is remarkable, however, that early mortality observed in the second replicate (Fig. 4) was associated with a relatively rapid development of the infection in that replicate (Fig. 5), whereas the infection and resulting mortality appeared to be delayed in the first replicate. Overall, cumulative mortality was roughly the same at the end of the experiment. Similarly, disease prevalence among the first and the second sampling corresponded well; one sample from each was ~30% infected, whereas the other was 50% to 60% infected. This indicates that despite slight differences in the temporal evolution of the disease and resulting mortality in both replicates, the mortality and prevalence values reached after 7 mo were homologous, and not significantly different; relegating manipulator's effect to a minor artifact.

Some infection did result by the end of the trial in the pallial treatment. This finding has potential for further study. Ford et al. (2002a) noted that the mantle and gills are the most frequently infected organs in the hard clam and they may represent the portal of entry for QPX. Additional investigations could focus on the acquisition of the parasite through pallial pathways; it may be a crucial interface in terms of infection establishment or effective resistance to pathogens.

Adding QPX cells to water and the cohabitation of infected and naïve clams were not effective methods of infection in this study. A comparison of experimental inoculation methods of the Eastern oyster with *Perkinsus marinus* (Dermo) achieved transmission for the injection treatments but not for the feeding application (Bushek et al. 1997). Chintala et al. (2002) speculated that endoparasites need to breach host barriers to establish an infection and "such barriers may frustrate attempts to experimentally transmit parasites by 'natural' methods". Mortality from pallial cavity injection of the etiological agent of brown ring disease in the Manila clam by Allam et al. (2002) was insignificant compared with 100% mortality when injected into tissue. Allam et al. (2002) suggested external barriers might be important in the ability of the Manila clam to resist diseases. It is possible that standard QPX culture techniques limit the ability of the pathogens to infect the clam through the water column, by inhibiting virulence factors necessary for breaking epithelial barriers for example. For instance, results from Ford et al. (2002b) demonstrated that freshly isolated *P. marinus* cells from infected hosts were much more virulent than those propagated in culture, "indicating a potential deficiency in the culture medium used." However, results of the cohabitation trial do not support potential culture-related problems, because this experiment used a natural source of the QPX organism (infected clams from the field) and yet infection was not transmitted despite the use of mildly (New York) and heavily (Massachusetts) infected adults. The Massachusetts adult clams were dying early on (up to 50%) in the trial as a result of severe infection and dead clams were left in the tank with the expectation that parasite cells shed from degrading tissues will infect naïve seed. Not only was the pathogen not transferred, but it also appears that the remainder of the "source" clams did not progress in disease severity; instead they abated infection and went into remission under our experimental conditions. Thus, it appears that our laboratory conditions did not promote transmission of QPX infection through the water column for either trial. According to *in vitro* studies of QPX environmental tolerance, temperature and salinity maintained during the trial were well within a favorable range for parasite growth and proliferation (Buggé & Allam unpublished). A more complex ecological relationship may be required to facilitate the transfer of QPX disease; involving factors not reproduced in the experiment. Possible vectors that may help disease transmission, including substrates that could possibly support Thraustochytrids (e.g., sediments or macrophytes) were not incorporated into the seawater tables to limit the variables of the experiment. More intriguing is the fact that QPX did not appear to survive inside the clams already infected, as indicated by the large amount of old and degrading cells observed in the post trial diagnosis. The laboratory environment may have been advantageous for clams: promoting healing and resistance. Environmental factors such as temperature, salinity, and dissolved oxygen have been documented as strong influences on the immune function and resistance of marine molluscs (Chu 1996, Reid et al. 2003, Cheng et al. 2004, Paillard et al. 2004). An effort was made to keep the seawater temperature and salinity stable, and to keep the tanks well aerated to avoid water quality problems that would foster alternate sources of mortality. In addition, the lack of infection in the seed clams illustrates that it takes more than just a close habitation for transmission to occur. All these observations support the advocated theory of QPX as an opportunistic parasite (Ford et al. 2002a, Ragone Calvo and Burreson 2002).

The indication of clams remitting infection in the laboratory is encouraging, for there may be ways to manage or remediate clams infected with QPX. Ultimately, environmental conditions could prove to be significant considerations in QPX disease mitigation. Additional research initiatives are needed to identify the crucial environmental drivers, revealing their roles in QPX disease development. Results from such investigations could provide the foundation for creating new hard clam management strategies concerning QPX infected populations.

In conclusion, QPX transmission to hard clams was achieved in the laboratory and led to hard clam mortalities within a few

months. An experimental infection method, utilizing laboratory QPX cultures, has been established. This represents a major advance for the investigation of this important disease, providing an experimental model that will allow a better characterization of biotic (genetic variability) and abiotic (temperature, salinity, etc.) factors affecting the development of QPX infections.

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