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Hard clam relocation as a potential strategy for QPX disease mitigation within an enzootic estuary

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Abstract

Monitoring of persistent QPX infections in clams of Raritan Bay (New York) shows certain areas of the estuary have remained without any significant disease prevalence. This study was conducted to investigate the potential to mitigate QPX disease by relocating infected hard clams, *Mercenaria mercenaria* (Linnaeus), from enzootic areas to nearby sites with prevailing environmental conditions suggested to deter infection and favour remission and healing. Clams were collected from a location with consistent disease prevalence in central Raritan Bay and brought to near shore habitats subject to lower salinities and higher summer temperatures. A reduced host density treatment was included in the study to examine the common observation of high clam density in the most persistently infected locales. An additional treatment retained clams above the sediment, since sediments are suspected to represent a QPX reservoir. At the end of the 4-month study all treatments displayed less QPX disease than the control group and the greatest contrast was provided by the disappearance of infections in a tidal creek.

Keywords: Quahog Parasite Unknown, *Mercenaria mercenaria* (Linnaeus), disease, mitigation, transplant, environment

Introduction

Quahog Parasite Unknown (QPX) is a thraustochytrid (Maas, Kleinschuster, Dykstra, Smolowitz & Parent 1999; Ragan, MacCallum, Murphy, Cannone, Gutell & McGladdery 2000; Stokes, Ragone Calvo, Reece & Burreson 2002; Qian, Liu, Allam & Collier 2007) associated with hard clam, *Mercenaria mercenaria* (Linnaeus), mortalities occurring on the Eastern coast of North America from Maritime Canada to Virginia (Whyte, Cawthorn & McGladdery 1994; Ragone-Calvo, Walker & Burreson 1998). QPX infections have been continuously observed in Raritan Bay, New York (NY), since a hard clam mortality event occurred there in 2002 (Dove, Bowser & Cerrato 2004). Nearly, half of NY's hard clam landings are due to a state monitored transplanting operation from Raritan Bay. QPX disease has placed severe restrictions on this programme causing significant economical losses.

Both intrinsic and extrinsic factors have been shown to affect QPX disease development. Several studies have shown a marked difference in susceptibility towards QPX infection according to cultured hard clam type (Ford, Kraeuter, Barber & Mathis 2002; Ragone-Calvo, Ford, Kraeuter, Leavitt, Smolowitz & Burreson 2007; Dahl, Perrigault & Allam 2008; Dahl, Thiel & Allam 2010; Kraeuter, Ford, Bushek, Scarpa, Walton, Murphy, Flimlin & Mathis 2011). Hard clams found to be more resistant to QPX disease have been sourced from areas in the northeastern United States (e.g. MA, NY, NJ) yet considerable QPX infections persist in some of their coastal locales. Ragone-Calvo *et al.* (2007) speculated that other factors may be involved in disease occurrence, such as parasite abundance or environmental conditions, since cultured clams from MA were highly resistant in their study yet epizootics and mortalities persist in grow-out areas of MA. Kraeuter *et al.* (2011) surmise that reports of disease outbreaks in wild populations suggest an environmental component. Raritan Bay contains a wild population of hard clams that continues to display QPX prevalence, although certain areas of Raritan Bay have had little to no QPX disease over the past 10 years.

Previous studies of QPX disease revealed substantial evidence of hard clam healing in naturally infected clams after they were maintained under laboratory conditions for several months (Dahl & Allam 2007). Subsequent trials have shown an influence of environmental conditions on QPX disease development. Infected hard clams held at 21°C and above had significantly less QPX disease and greater healing as compared with those maintained at 13°C (Dahl, Perrigault, Liu, Collier, Barnes & Allam 2011). Salinity was also shown to modulate disease dynamics with previous studies demonstrating greater mortalities in infected clams maintained at 30 ppt vs. 17 ppt (Perrigault, Dahl, Espinosa & Allam 2012), which is in accordance with previous *in vitro* studies that showed optimal QPX growth at 30–34 ppt (Perrigault, Buggé & Allam 2010). *In vitro* studies have also shown an ability of QPX to grow using macroalgae homogenates as a source of nutrients (Buggé & Allam 2007). Thraustochytrids are saprophytic osmoheterotrophs, considered important for degrading detrital organic molecules (Raghukumar 2002). Furthermore, sediments from Raritan Bay (Liu, Allam & Collier 2009) and other enzootic areas (Gast, Moran, Audemard, Lyons, DeFavari, Reece, Leavitt & Smolowitz 2008) have tested positive for QPX and may represent an environmental reservoir. Another factor considered to affect QPX disease dynamics in the field is clam density (Ford *et al.* 2002; Ragone Calvo & Burreson 2002; Walton, Murphy & Smolowitz 2008). High host densities can facilitate transmission of infections and may also pose as a source of stress, as observed by crowding effects on clam growth (Eldridge, Eversole & Whetstone 1979; Hadley & Manzi 1984).

The following study was developed to investigate the potential to utilize favourable environmental conditions suggested to deter QPX disease and promote healing in the field. Clams were collected from the most persistently infected area of Raritan Bay and relocated to sites targeted for lower salinities and higher summer temperatures in an attempt to promote disease remission. In addition, efforts were made to test strategies that can lower infection risk by reducing clam density and by removing clams from the sediment.

Materials and methods

Clam collection and redistribution

Experimental study sites were restricted to the Raritan Bay complex to avoid any potential for introduction of QPX to other clamming estuaries. Hard clams (67 mm average length) were collected in early June 2009 by hand raking ('bull rake' commonly used by commercial harvesters) from an enzootic area in the central portion of Raritan Bay (NY) (Fig. 1) that has been continuously tested positive for QPX since 2002. Clams were checked for 'mudders' (closed shells with no clam) and subsequently distributed into grow-out cages (OBC-1: 0.91 m length × 0.51 m width = 0.46 m^2) for deployment that same day. Clam cages were randomly assigned to three different field sites (Fig. 1) that included near shore habitats that were shallower and had more direct influence from upland fresh water sources; a harbour and a tidal creek. The first deployment site (site 1, 'Bay') was located in the same area where the clams were collected for the study. Three different treatments were implemented at this site with (1) cages deployed on the sediment surface with clams at the same density as the collection area (50/m^2 = 25 clams/cage) to

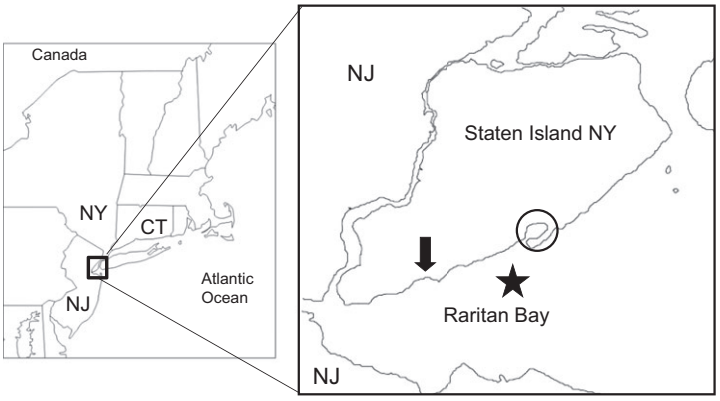

Figure 1 Regional and local maps showing the project area adjacent New York City. Study sites were in Raritan Bay and along the south shore of Staten Island (New York) and are marked as follows; star represents the clam collection area and the Bay deployment site, Great Kills Harbor is circled and Lemon Creek is pointed out by the solid arrow.

serve as a control, (2) cages placed off-bottom at the same density and (3) cages placed on the sediment surface but at half the clam density (25/m^2 = 12 clams/cage). The off-bottom treatment (~1 ft above sediment surface) was accomplished by attaching grow-out cages on top of decommissioned lobster traps. Cages were deployed attached to a long rope set up in trawl fashion, anchored at each end and marked by GPS waypoints. Five replicate trawls were deployed with one cage from each of the three groups for a total of 15 cages at a depth of 7 m. The other two sites were located along the southern shore of Staten Island, NY. Five cages off-bottom (50 clams m^{-2} = 25 clams/cage) attached by ropes to pilings at the end of the docks were deployed in Great Kills Harbor (site 2, 'Harbor') and Lemon Creek (site 3, 'Creek'). Bottom waters in the Harbor get warmer than those measured in the central Bay area during the summer (NYS DEC unpublished). The Harbor has very dense patches of clams (sometimes exceeding 400 clams m^{-2}, NYS DEC unpublished) and yet disease prevalence is extremely low (<0.1%). Cages were deployed in the Harbor at a depth of 3 m. The Creek site is characterized by a mean tidal range of 1.5 m with upstream freshwater sources. Cages deployed in the Creek were frequently exposed to air during low tide. An autonomous temperature data logger (Onset Tidbit) was deployed on a clam cage at each site for the study duration. Water temperature, salinity and dissolved oxygen were measured with a hand held Yellow Springs Instruments (YSI) probe during regular site visits throughout the 4-month deployment (~every 3 weeks).

Clam sampling and processing

A sample of 30 clams was taken from the collection area to ascertain infection during initiation of the study (T-0). After 4 months of deployment, clams from each site were retrieved and processed for the following data collection: total mortality counts, shell length, condition index and infection diagnostics. QPX prevalence and intensity (QPX cell counts) were determined by a quantitative PCR technique (Liu *et al.* 2009) that has shown greater diagnostic sensitivity as compared with standard histological methods. Mantle and syphon tissues from each clam were collected and processed individually for QPCR according to our previously described protocol (Liu *et al.* 2009). The condition index (CI) was calculated following the suggested standard method (eq. 6) in Crosby and Gale (1990): CI = dry soft tissue weight (in grams) × 1000/internal shell cavity capacity (in grams). A total wet weight was measured before diagnostic samples were taken from each clam processed. Once the pathology biopsy samples were removed, all of the remaining tissues were weighed and then placed in an oven (60°C) until the dry weight had stabilized. A dry/wet weight ratio was calculated from all the tissue that remained post biopsy and that ratio was used to determine a total dry weight from the original total wet weight. This allowed for clams to be sampled for QPX diagnosis and have a condition index determined for each individual. Shell length and condition index data did not pass normality tests and consequently were compared using a non-parametric ANOVA on Ranks (Kruskal–Wallis). This was followed with Pairwise Multiple Comparison Procedures (Dunn's Method) as needed. Counts of either disease prevalence (positive or negative) or mortality (dead or alive) in a 2-way row-by-column contingency table were tested for independence of variables by a *G*-test. (Sokal & Rohlf 1995)

Results

Study sites were visited at the end of the 4-month deployment to retrieve clams for sampling. All replicates were intact from the Harbor and the Creek. Forty clams were sampled from each of these two sites (eight clams per replicate). Unfortunately, we were not able to locate all replicate cages at the open water Bay site despite extensive sampling effort, likely as a result of gear displacement in that busy urban estuary. Fortunately, all of the clams in one full trawl replicate (one cage each) were recovered, plus one more cage from the low density treatment. All of the Bay site clams retrieved were sampled and due to difficulties caused by the lack of replicate trawls retrieved from that site, data were pooled within all treatment groups for analysis; N = 24 Control, 24 Off Bottom, 23 Low Density, 39 Harbor, 40 Creek.

Mortality, growth and condition

Mortality measured at the end of the study was not significant; 2–5% for all groups. Clam growth during the study was also not significant. Average

shell length for each group was in the range of 62–67 mm. Condition index was significantly different ($P < 0.001$; Fig. 2) between the different sites. The inshore study sites (Harbor and Creek) had the highest condition index values and the greatest contrast with the control and low density Bay groups. The off-bottom treatment condition index was neatly in between the inshore sites and the other Bay groups; it was not significantly different during any pairwise comparison.

QPX prevalence and intensity

The initial sample (T-0) taken from the clam collection area revealed 23.3% QPX prevalence. After 4 months of deployment at each location, the control group that remained on bottom at the Bay site had the highest QPX prevalence of 16.7% (Fig. 3).

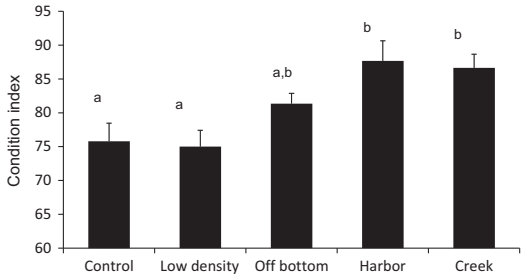

Figure 2 The mean condition index (with SEM) of clams retrieved from each treatment at the end of experiment ($P < 0.001$, ANOVA on ranks). Treatments that were not significantly different during multiple comparison procedures have the same lower case letter (a or b).

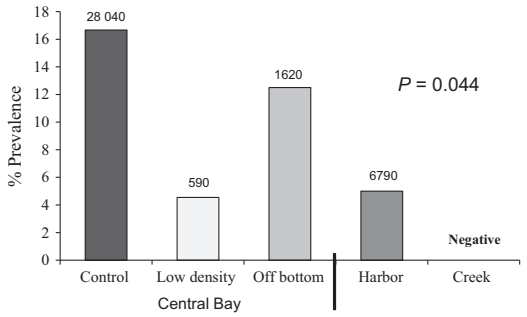

Figure 3 QPX prevalence and intensity in clams retrieved at the end of experiment ($P = 0.044$; *G*-test). The value above each bar is the average QPX cell count (per g wet clam tissue) for the positive clams in that treatment.

The off bottom treatment in the Bay followed with 12.5% QPX prevalence. The low density treatment that remained on bottom in the Bay had the lowest QPX prevalence of that site with 4.5%. Clams from the Harbor deployment had only 5% QPX prevalence. All clams diagnosed for QPX from the Lemon Creek site were negative. QPX prevalence across all sites and treatments were significantly different ($P = 0.044$). Infection intensity, as indicated by the QPX cell count g^{-1} wet clam tissue averaged for positive clams in each treatment (Fig. 3), was greatest for the Bay control group (28 040 QPX cell g^{-1} wet tissue weight). The next highest intensity was for the Harbor group (6790) followed by the off bottom Bay treatment (1620). The only positive clam in the low density treatment from the Bay had a QPX cell count of 590/g clam tissue.

Environmental parameters

The inshore sites appear to shift through a period of increased dissolved oxygen levels before all of the study sites underwent a drop during August (Fig. 4a); the lowest value (2.8 mg L^{-1}) was measured at the Bay site. The highest salinities throughout the experiment were measured at the Bay site with levels maintained above 23 ppt and a maximum of 27.3 ppt measured in October (Fig. 4b). Salinity in the Harbor was lower than the Bay site, with a minimum of 20.5 ppt in July. The Creek had generally lower salinity levels with a minimum of 19 ppt in July. Temperatures diverged and rose more steeply at the inshore locations as June progressed (Fig. 4c). The Harbor had the highest temperature readings during the study, nearly up to 26°C, and one reading in July was almost 5°C greater than that measured in the Bay. The Creek was somewhat intermediate between the Harbor and the Bay, but at times reached similar levels achieved in the Harbor. Temperature logs from the Tidbits that were deployed with the clam cages allow for a more detailed comparison between the two inshore sites (Fig. 5), although not for the Bay as that data logger was not recovered. The Creek temperature log has much greater variability than the Harbor log, which is very likely a direct product of tidal exposure. There were occasions when the Creek site was cooler, over 6°C cooler at a few points, but the majority of the time (82% of the readings) the Creek was warmer than the Harbor, up to 5°C warmer.

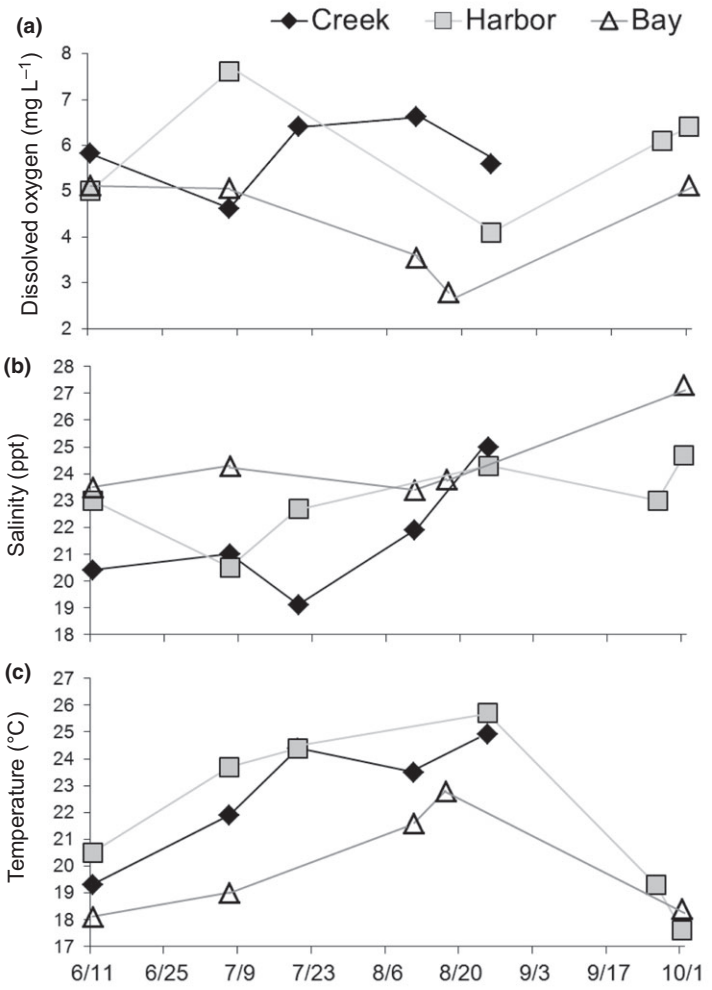

Figure 4 Dissolved oxygen (a), salinity (b) and temperature (c) measured at the study sites.

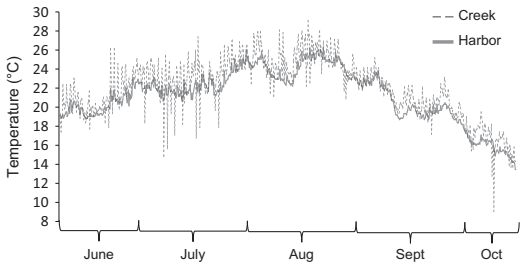

Figure 5 Temperature logs (4 h intervals) from the tidbits deployed at the Creek and Harbor sites during the study period.

Discussion

Clams were moved from a location with persistent QPX infections and relocated within the same estuary to evaluate the potential to promote remission in the wild. After four months of deployment, each experimental group had lower prevalence and lower average QPX cell counts than the control group. This indicates a reduction in infection was achieved with each treatment.

Remaining off bottom appears to have been beneficial for clams in this study. Improved food quality and/or filtration rates could be why all of the clams that were above the bottom had better condition indexes than those that remained on the sediment. Accessible food composition could have been different, with suspended clams having greater access to pelagic microalgal species and the bottom dwelling groups having an increased proportion of benthic algal species and detrital particles. Sasaki, Sanematsu, Kato and Ito (2004) found that infaunal suspension-feeding bivalves on open shallow subtidal soft bottoms are supplied

with few pelagic microalgae. Elevated clams would also have diminished contact with suspended sediment loads as compared with bottom dwelling. Rhoads and Young (1970) used *M. mercenaria* to examine the impact of near bottom turbidity on the growth of suspension feeders. Juvenile hard clams grown near the bottom had significantly less growth than clams grown 45 cm above the bottom. Bricelj and Malouf (1984) showed a decline in *M. mercenaria* clearance rate with increasing suspended sediment concentrations. More significant differences in condition indexes and/or clam growth in our study would have been likely if smaller clams were used or the experiment lasted for a longer period of time.

Reduced contact of elevated clams with sediment and detritus may have effectively reduced the exposure to QPX. Thraustochytrids are ubiquitous in coastal marine environments and considered to play an important role in the degradation of plant matter (Raghukumar 2002). QPX has been shown to grow using macroalgae derived nutrients (Buggé & Allam 2007) and has been detected in sediments from Raritan Bay (Liu *et al.* 2009) and other enzootic locations (Gast *et al.* 2008). Detrital organic matter in estuarine sediments could be sustaining QPX outside of the parasitic pathway in clams and effectively serving as an environmental reservoir for infection. It was interesting to see the same density of clams elevated in the central bay location had lower QPX prevalence as compared with clams that remained on the sediment. Although it was a small difference in prevalence, a more considerable difference was seen in the lower intensity.

The low density group was set on the sediment adjacent the controls and yet had distinctly lower QPX prevalence and intensity. Reduction in infections achieved through manipulation of host density strengthens the notion that hard clam density is an important factor in QPX disease dynamics. Areas in the central portion of Raritan Bay have very high densities of hard clams (e.g. 26–70 clams m^{-2} average) and have suffered some of the most severe infections recorded in a wild clam population (Dove *et al.* 2004).

Suspension above the sediment or decreased host density does not appear to be the only factors involved in QPX disease mitigation in this study. The inshore sites had the same clam density as the controls but had less QPX. They were also suspended in the water column but had less QPX than the off-bottom Bay group. These results suggest site differences to represent a dominant factor affecting disease dynamics. Inshore sites were selected to provide differences in the temperatures and salinities the clams would be exposed to during the deployment as compared with the central open bay.

Great Kills Harbor had lower salinities than the Bay site during early summer and early autumn (Fig. 4). Temperatures in the Harbor were noticeably higher than the Bay site when the study started and remained that way until October. Sustained warmer summer temperatures and periods of reduced salinity could be influential in the lower QPX prevalence observed compared with the similar density of clams on or off bottom in the Bay site. Of particular note is that the Great Kills Harbor area has exceptionally high densities of clams, sometimes exceeding 400 clams m^{-2} (NYS DEC unpublished) but extremely low disease prevalence (only one clam positive for QPX in over 1000 processed since monitoring began in 2002).

Clam samples from all of the replicates deployed at the Creek site were negative for QPX. Cages in the Creek were subject to regular air exposure over daily tidal cycles. Continuous temperature data logging (Fig. 5) revealed this site was warmer than the Harbor for the majority (>80%) of the study time period. Lemon Creek is a fresh water stream that becomes tidally mixed when it reaches the bay. The salinity regime for the Creek is also likely to be more dynamic than what the point measures reflect.

Relocating clams to the inshore sites subjected them to higher dissolved oxygen (DO) as compared with the Bay site, which had the lowest observed levels of the study. Raritan Bay is subject to low DO levels each summer (NYC DEP) and low DO is generally considered a source of stress for benthic organisms (Diaz 2001). Seasonal temperature rise causes water to lose capacity to dissolve oxygen and will simultaneously accelerate the metabolism of the clams, increasing their oxygen demand (Hibbert 1977). Hard clam pumping rates decrease when subject to DO levels below 5 mg L^{-1} (Hamwi 1969). DO has been observed to go below 5 mg L^{-1} in hard clamming areas of Raritan Bay during the summer (NYS DEC unpublished) and it did during this study (Fig. 4A). Clams can respond to hypoxia by closing their valves which negates the ability to feed and impacts physiology further by reliance on anaerobic respiration (Weber,

Hoover, Sturmer & Baker 2008). Compromised respiratory status could serve as an opportunity for QPX to effectively invade a clam and proliferate. Oysters exposed to low oxygen conditions suffered increased *P. marinus* infection related mortalities (Anderson, Brubacher, Ragone Clavo, Unger & Burreson 1998). Production of reactive oxygen species (ROS) is an immune defence reaction that has been demonstrated in *M. mercenaria* (Buggé, Hégaret, Wikfors & Allam 2007) and oysters had a substantial reduction in ROS production under hypoxic conditions (Boyd & Burnett 1999). Lack of oxygen has been reported to impact the immune function of another Venerid clam, *Chamelea gallina* (Matozzo, Monari, Foschi, Papi, Cattani & Marin 2005). Low oxygen stress could also have been reduced for clams suspended in the water column as compared with those that remained on the sediment surface, since previous studies have shown that hypoxia in estuaries can largely result from benthic respiration occurring in the sediment (Lehmann, Barnett, Gélinas, Gilbert, Maranger, Mucci, Sundby & Thibodeau 2009). High seasonal productivity of coastal systems can cause the oxic–anoxic boundary to rise upward, even above the surface of estuarine sediments (Libes 2011). More detailed measurements would be needed to examine a refined vertical relationship of seasonal DO in Raritan Bay.

The influence of environmental conditions on infection dynamics has proven to be crucial in other bivalve-microbial disease systems (Ford & Haskin 1982; Paillard 2004; Villalba, Reece, Ordás, Casas & Figueras 2004). Histological observations of QPX infections from the field provide compelling evidence for the ability of clams to mount a hemocyte-mediated defence reaction (Ragone-Calvo *et al.* 1998; Smolowitz, Leavitt & Perkins 1998; Dove *et al.* 2004). Laboratory challenges that examined constitutive clam immune defence factors demonstrated a strong influence of temperature on cellular and humoral defence parameters which were also affected by salinity (Perrigault, Dahl, Pales Espinosa, Gambino & Allam 2011; Perrigault *et al.* 2012). Infected clams were able to undergo remission of QPX disease at temperatures ≥21°C and suffered less mortalities at 17 ppt vs. 30 ppt (Dahl *et al.* 2011; Perrigault *et al.* 2012).

Clams from southern (e.g. South Carolina, FL) broodstocks have shown greater susceptibility to QPX infection compared with northern (New Jersey, New York & Massachusetts) broodstocks and yet QPX disease has never been detected in clams south of Virginia (Ford *et al.* 2002; Ragone-Calvo *et al.* 2007; Dahl *et al.* 2008, 2010; Kraeuter *et al.* 2011). Higher seasonal temperatures achieved in clamming grounds farther south may be a limiting factor for QPX infections. Kraeuter *et al.* (2011) surmised that 'periods of high temperature may retard QPX development, but there is no field evidence to support such speculation'. Prevalence and intensity were considerably reduced at our inshore relocation sites that had noticeably warmer summer temperatures.

Kraeuter *et al.* (2011) also noted a trend of higher QPX prevalence and intensity in clams from intertidal sites and proposed that intertidal exposure may provide added stress necessary for infection or that when clams remain closed it allows QPX in materials retained in the mantle cavity more time to infect the host. This reasonably implies that increased contact time combined with a lack of ventilation might allow QPX to invade, but our study results are incongruous since the Creek deployment had the best outcome and mimicked a high intertidal site in terms of exposure. Low salinity conditions in the Creek may have been an important factor deterring infection in this intertidal site.

Transplanting bivalve shellfish is common to remediate bacterial contaminants and has been applied to optimize production by providing refuge from predation and infectious disease pressure. Temperate estuarine habitats cover a wide range of environmental conditions. Lower salinity habitats can effectively exclude some major predators (e.g. gastropod and echinoderm spp.) of oysters and clams (Kennedy 1991; Roegner & Mann 1991). Strategic oyster transplanting has been integral to mitigating infectious disease losses in Delaware Bay (Powell, Klinck, Hofmann & Ford 1997). Compared with the central Raritan Bay site, the Harbor and Creek sites provided higher summer temperatures and periods of lower salinity, the same conditions shown to favour clams and deter QPX disease in the laboratory (Dahl *et al.* 2011; Perrigault *et al.* 2012).

Results from this field study encourage strategies to promote clam health within QPX enzootic estuaries by utilizing planting areas that become warmer and receive low salinity pulses during spring and early summer. Achieving temperatures sustained above 21°C and salinities that approach

17 ppt, as observed in laboratory challenges (Dahl *et al.* 2011), may be crucial in deterring infection development early enough. Results from this field study also support reducing hard clam density to lower QPX disease risk and as a possible remediation measure in established QPX infection hot spots. Suspension of clams above the bottom showed benefits although maintaining clams out of the sediment over longer periods of time may become detrimental for an infaunal bivalve, especially when enduring overwinter temperatures. A few of the suspended clams were observed to have considerable recruitment of barnacles, which highlights the potential for competition from suspension-feeding epifauna. The role of sediment in QPX transmission is uncertain and more research is needed to examine this potential reservoir. Understanding the relationship of dissolved oxygen with QPX disease dynamics could benefit from field studies that include *in situ* continuous measures and water column profiles of dissolved oxygen.

Acknowledgments

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