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Ecology of QPX disease in the hard clam *Mercenaria mercenaria*

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Abstract of the Dissertation

Ecology of QPX disease in the hard clam *Mercenaria mercenaria*

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There is increasing recognition of the role of suspension-feeding bivalves in providing important ecosystem services (e.g., water clarity, benthic-pelagic coupling, and habitat structure) within estuarine systems. *Mercenaria mercenaria* (aka hard clams, quahogs) are frequently the dominant suspension-feeder in estuarine systems across the US east coast and have had significant commercial fishery importance and a steadily growing mariculture industry. *M. mercenaria* have generally not had disease problems which contrasts the microbial infections that have plagued oyster fisheries. Quahog Parasite Unknown (QPX) is the first substantial infectious microbial pathogen affecting hard clams across both wild populations and cultured stocks. QPX is identified as a Thraustochytrid, a group of saprophytic protists that are considered to play an important role in detrital organic matter recycling in coastal ecosystems, yet there are some reports of their association with molluscan disease and mortalities. QPX infections can be present in hard clams but without causing overt disease problems. Controlled laboratory experiments and applied field experiments were utilized in an approach to understand the factors that control infection dynamics. Results revealed a significant role of the primary environmental factors of temperature and salinity influencing the balance between the hard clam host and opportunistic pathogen QPX, regulating the resultant disease progression. Counter to most microbial pathogens affecting bivalves, warm temperatures (≥21°C) deter infection progression and promote disease remission. High estuarine salinity (e.g., 30ppt) promotes infection and increases the risk of QPX-related hard clam mortalities. Applied field experiments showed the potential to mitigate disease risk. Areas subject to low salinities and high summer temperatures within an enzootic estuary were utilized to deter infection progression. Reductions of hard clam density also helped reduce disease risk. Clam seed raised from locally sourced wild populations displayed good growth and disease resistance during grow out challenges. The investigations provide fundamental insights for developing management strategies.

Dedication

This work is dedicated to the important role that bivalve shellfish have in the rich maritime cultural history of Long Island, New York.

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Acknowledgments

My wife Kim for her love and support, and our daughter Fiona for the love and joy she brings to our lives. My family gives me strength.

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Introduction

Bivalve shellfish have supported major fisheries along the eastern coastline of North America. The most notable commercially important species have been the eastern (aka American) oyster, *Crassostrea virginica*, and the hard clam (aka northern quahog), *Mercenaria mercenaria*. Oysters typically have greater market value and subsequently greater commercial emphasis historically. Many eastern oyster populations have crashed, having suffered from overfishing and intense infectious disease pressure. Over time there has been a shift of greater commercial focus toward *M. mercenaria*, especially since hard clams have not had the disease problems that plague oysters.

In the early 1970's New York's (NY) waters accounted for roughly half of all the hard clams landed in the nation (NMFS), most of which was harvested from Great South Bay. A substantial contribution to local economy was generated by the landings. A work force was supported as harvested clams were transported from the dock to processors and wholesalers, then shipped to markets and restaurants, and finally purchased by the consumer. In more recent decades hard clam populations have shown declines and overfishing is suspected as a major factor (Bricelj 2009). *M. mercenaria* has been found to be a hardy aquaculture species and their use in shellfish farming has been expanding in many regions especially since the 1990's (FAO). In recent years, NY has had some the greatest hard clam landings among the States in weight and price per pound (NMFS). Since the precipitous decline of the lobster fishery, the hard clam represents the most valuable commercial fishery for New York State.

QPX is the first considerable disease problem affecting the hard clam industry, impacting shellfish farming operations and wild fishery populations. In 1989 heavy mortalities occurred in northern quahogs of a hatchery on Prince Edward Island (PEI), Canada. Lacking a definitive taxonomic identification of the protist associated with the mortality event, it was designated as Quahog Parasite Unknown or QPX for short (Whyte et al. 1994). The same type of organism is the suspected agent of an earlier report of quahog mortality in 1959, concerning a dense population of wild quahogs in Neguac, New Brunswick (Drinnan and Henderson 1963).

A sample of moribund clams from the Chatham area of Massachusetts (MA) in 1992 revealed a parasite very similar to QPX. Since that year, mortalities occurred in the cultured clam leases of Provincetown and Duxbury, MA, culminating to substantial die offs in 1995. Diagnosis from those events revealed a QPX 'like' parasite, the culprit is also suspected in mortalities of hard clams in Barnegat Bay, New Jersey (NJ), back in 1976. (Smolowitz et al. 1998)

QPX has been classified as a member of the Thraustochytridae family within the subphylum Labyrinthulomycota in the phylum Heterokonta (Maas et al. 1999, Ragan et al. 2000, Stokes et al. 2002, Qian et al. 2007). Thraustochytrids are considered important saprophytic osmoheterotrophs for degrading detrital organic molecules (Raghukumar 2002) and are found to be ubiquitous in marine and estuarine environments, even within the bivalve pallial cavity (Maas et al. 1999). They are typically nonpathogenic, although there are a few documented associations

with disease events in wild and cultured mollusks (Polglase 1980, Mclean and Porter 1982, Bower 1987). Typical histologic presentation of active QPX infection (Fig. 1) consists of abscesses or necrotic lesions containing mucoid material regularly exhibited as translucent halos around individual cells of vegetative and spore like stages of QPX (Bower 2010).

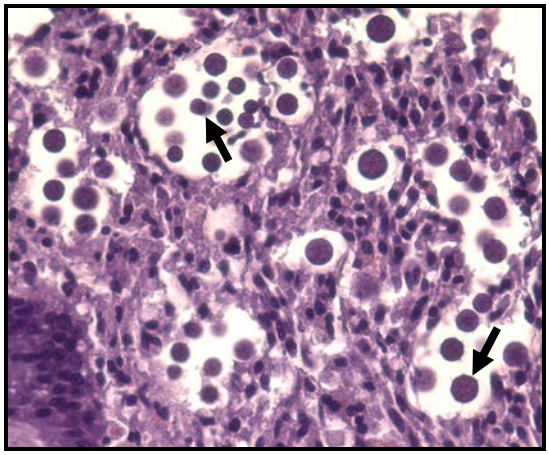

Figure 1. Histological section of clam visceral mass tissue showing QPX thalli (arrowheads) within lesions.

Across geographic regions, diagnostic reports of QPX infections note differences in disease presentation, parasite descriptions, and pathologic severity, which raises the question of possible varied QPX strains existing throughout its known geographic range (Ragone Calvo et al. 1998, Smolowitz et al. 1998, Dove et al. 2004). Basic aspects of the MA outbreak were analogous to the PEI report; such as the age of cultured clams (1.5-2yr), severity of inflammatory response and comparable numbers of endospores within sporangia (Smolowitz et al. 1998). QPX infections from PEI were found to be primarily in muscle (foot and adductor) and connective tissues (Whyte et al. 1994). While the QPX of MA was much more prevalent in the pallial organs (gill and mantle) with mantle swelling, yellow/tan discoloration and the presence of nodules commonly characterized the gross pathology (Smolowitz et al. 1998). Additional comparisons of the QPX examined in Canada (CN) and MA QPX were conducted with use of histological stains, it was suggested that the differences observed from the staining "may indicate slightly different labrinthomorphid" (Smolowitz et al. 1998). The observations of QPX infections from Virginia (VA) seem to fit with the pattern of MA findings, as opposed to the CN findings. A major difference from MA is the fact that no gross lesions were discovered. VA had much lower disease prevalence and not nearly the associated mortality. The investigators suggested that these differences illustrate MA QPX may be more pathogenic (Ragone-Calvo et.al. 1998).

During the summer of 2002 an unusual number of hard clams were turning up dead in the NY portion of Raritan Bay, a source area for a valuable state-managed relay operation: a transplant fishery responsible for nearly half of the annual hard clam harvest in NY State (NYS DEC 2005). A survey confirmed that relatively severe QPX infections were heavily associated with a mortality event across this area of wild hard clams (Dove et al. 2004). Transplant operations were suspended after confirmation of the epizootic. A persistent program of hard clam

infection monitoring in the years following has generated valuable information that guided a process that allowed re-opening portions of the Raritan Bay harvest area (NYS DEC 2005).

The QPX epizootic that occurred in Raritan Bay (NY) was the most significant recorded in a wild population since the first report over 40 years ago. Histological observations from the NY mortality event had a high mantle involvement (62.8%), but there was also a significant visceral involvement (57.1%) including cases of infection in the gonads (Dove et al. 2004). Intensity of NY visceral infections were typically more severe and diffuse than previously documented, equating to a higher parasite biomass even when comparing similar prevalence values for other populations (Dove et al. 2004).

The potentially devastating effects of bivalve epizootics, as seen previously within the eastern oyster industry, lead to precautionary field surveys of hard clam health in areas of VA and Atlantic Canada concerned for their hard clam culture based operations. As a result the known range of QPX is from the Gulf of St. Lawrence down to the Atlantic coast of Virginia. Evidence of QPX in apparently healthy clams from those surveys supports the notion that QPX is widespread in coastal waters where clams are known to grow (Ragone Calvo et al. 1998, MacCallum and McGladdery 2000).

Information concerning QPX disease is first gained from histological evidence through diagnosis of field mortality events (Ragone Calvo et al. 1998, Smolowitz et al. 1998, Ford et al. 2002a, Dove et al. 2004). QPX disease associated mortalities were reported in clams >1 yr old, and QPX was not found during a large survey of seed clams (≤20mm) obtained from multiple hatcheries (Ford et al. 1997), suggesting that cultured clams acquire the parasite in field grow-out sites. The frequent association of QPX disease problems with aquacultured clams led to suspicions of culture practices influencing the establishment of QPX infection and disease progression. Ragone-Calvo and Burreson 2002 suggested that QPX probably infects a host organism that is stressed and therefore less resistant to infection. They pointed to possible sources of stress from high planting densities and poor husbandry. Planting high densities in culture operations is considered to increase stress for the animals as well as aid transmission of infectious agents.

Ford et al. 2002 experimented with different sources of clams grown at different densities in plots with previous QPX activity in NJ. A trend toward higher QPX levels was observed at higher planting densities, although statistical confidence was not achievable due to variation in data. Non-local clam sources (e.g. South Carolina hatcheries) acquired heavy infections and suffered substantial mortalities while the locally sourced clams (i.e. a NJ hatchery) had few infections or mortalities. Ford et al. 2002 raised the issue of whether the problem with the out-of-state seed was genetic or of poor acclimation, or some sort of combination. Poor acclimation is unlikely when considering the disease was not evident until clams in their study spent at least a year in the field. At the same time, a strictly genetic theory was not favored when they discovered that the different clam types had some common parental stocks. They concluded that QPX only caused disease in certain groups of clams, somehow disadvantaged, speculating that the disadvantage may derive “perhaps from an unfavorable genotype-environment interaction” (Ford et al. 2002).

In a subsequent study, Ragone Calvo et al. 2007 deployed different strains of clams at sites known to harbor QPX in NJ and VA. They demonstrated that Florida (FL) and South Carolina (SC) seed clams were significantly more sensitive to QPX infection than seeds from MA or NJ. They concluded that a clam genotype may be predisposed toward infection by QPX. Consensus among researchers is that QPX is probably an opportunistic facultative parasite.

Laboratory investigations of QPX began with isolating the parasite from infected clams (Whyte et al. 1994) and long term propagation as an *in vitro* culture (Kleinschuster et al. 1998). This precedent allowed for investigations of taxonomy (Maas et al. 1999, Ragan et al. 2000, Stokes et al. 2002) and aspects of its biology, including sporulation and mucus production (Brothers et al. 2000, Anderson et al. 2003).

The Marine Animal Disease Lab (MADL), at the School of Marine and Atmospheric Sciences (SoMAS) of Stony Brook University (SBU), successfully isolated QPX from infected clams. The MADL has made it a priority to collect and maintain viable parasite isolate cultures. These isolate cultures have been principal in applications regarding investigations of this parasite's physiology and ecology, genetics, and transmission. Evidence of different QPX strains was obtained as proliferation was compared across QPX cultures grown with manipulated environmental conditions (e.g. temperature); individual isolates were found to have significant differences in optimal growth parameters (Perrigault et al. 2010). Support of the facultative nature of QPX was gained when survival and growth was achieved in cultures composed of macroalgal substrates (Buggé and Allam 2007).

An early initiative of QPX disease research conducted at MADL focused on transmission of infection. An attempt to transmit QPX between infected adults and naïve juveniles by cohabitation was unsuccessful (Dahl and Allam 2007). Adding laboratory cultures of QPX to aquarium tanks containing hard clams did not result in transmission either. Injection of QPX cells into clam tissue led to infection, confirmed QPX disease, and subsequent mortality of hard clams (Dahl and Allam 2007). Inoculation provided a reliable laboratory technique, a reproducible exposure of clams to quantified QPX inoculates, which allowed for subsequent investigations.

The QPX inoculation procedure was utilized for an investigation that simultaneously focused on apparent genetic based variability of host susceptibility and reputed regional variability of parasite virulence. The first objective was to compare susceptibility of different cultured hard clam stocks to QPX infection. A concern with previous QPX field studies is that hard clams from varied geographic sources are inherently different in regards to tolerance of prevailing environmental conditions of transplant locations. Challenging different clam strains in the lab allowed for reduction of environmental variables related to field locations. The second objective was to compare the pathogenicity of QPX organisms collected from the East Coast of the US that were from geographically distinct regions or found to be morphologically distinct if from the same region. The underlying hypothesis tested was that disease occurrence and intensity could be the result of an unfavorable genotype interaction of both the parasite and host. The challenges resulted in QPX disease trends categorized by the seed type, or by the isolate used for inoculation (Dahl et al. 2008). There is a difference in susceptibility of hard clams that can be correlated to the geographic origin of the broodstock. The gradient of resiliency can be described

as a latitudinal trend; highest resiliency in clams from the north, lowest in clams from the south. 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 (suppressed immune response) against the parasite. Acclimation being the source of difference in susceptibility is improbable for those laboratory controlled challenges. Intensity and severity of infection can be heavily dependent on the virulence of a particular QPX strain. It was concluded that strain specific interaction of the parasite and host significantly alters the severity of infection.

Hard clams found to be less susceptible to QPX disease have been sourced from areas in the northeastern U.S. (e.g., NY and MA), yet considerable QPX infections persist in some locales of these areas. Ragone Calvo et al. 2007 speculated that other factors may be involved in disease occurrence, such as parasite abundance or environmental conditions, since MA was highly resistant yet epizootics and mortalities persist in those grow out areas. Exploratory screening of hard clams has detected QPX in apparently healthy populations; locations without a disease problem or mortality events (MacCallum and McGladdery 2000, Ragone-Calvo et al. 1998, Allam unpublished). QPX was first reported subsiding outside of clams in marine aggregates, which suggested a potential vector for this pathogen (Lyons et al 2005). A survey of various sample types utilizing sensitive molecular techniques found QPX to be widely distributed in the environment (e.g., sediment and seaweed), additional evidence supportive of the ‘facultative nature’ of this protist (Gast et al. 2008).

Histological observations of QPX-infected tissues from mortality events and field surveys reveal that some clams are able to mount a hemocyte mediated defense reaction. Observations include reports of giant host phagocytes (multinucleate giant cells), encapsulation, and the association of degraded moribund parasite cells (Dove et al. 2004, Smolowitz et al. 1998, Ragone Calvo et al. 1998). The lack of infection in naïve clams during cohabitation with infected clams illustrates that proximity alone does not facilitate transmission. When naturally infected hard clams collected from the field were maintained for an extended time under uniform laboratory conditions, a discernible amount of healing and disease remission was observed (Dahl and Allam 2007). Clams remitting infection in the laboratory is encouraging, indicating environmental conditions may play an important role in QPX disease development and may actually promote healing and resistance.

QPX disease research has identified differences in host susceptibility and has begun to examine parasite pathogenicity as well as their interactions. The role of environment is a principal component of the classic epidemiological triad or traditional model of infectious disease (Fig. 2). The influence of environmental conditions on the outcome of this hard clam host-protistan pathogen interaction has not been addressed in the focus of studies thus far.

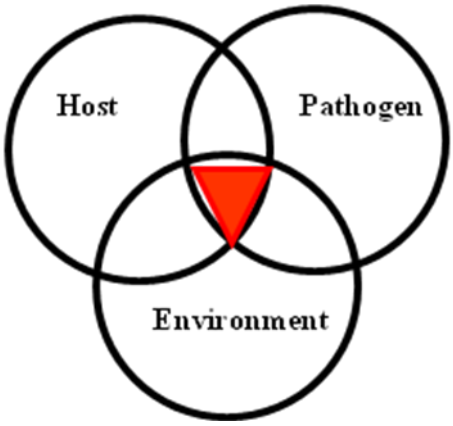

Figure 2.Venn diagram depiction of infectious disease model. Disease occurrence is represented by the central portion where all three circles overlap.

As invertebrates, the influence of prevailing environmental conditions on the functioning of bivalves can be substantial. *M. mercenaria* are relatively shallow dwelling clams that can subsist up into the intertidal zone. Hard clam habitats encompass a wide range of environmental conditions that can be subject to fluctuations seasonally and by daily tidal cycles. The QPX organism appears to be common within such habitats, yet not obligate as a hard clam parasite. There is evidence of host cellular response and resistance to infection yet the circumstances that promote QPX disease remission are ambiguous. The influence of environmental conditions on infection dynamics has proven to be crucial in other bivalve-microbial disease systems (Ford and Haskin 1982, Paillard 2004, Villalba et al. 2004). Huvet et al. 2004 proposed that summer mortality of the oyster *C. gigas* is the result of a complex interaction between the host, pathogens and environmental factors. Investigations are needed to reveal the roles of environmental conditions in this hard clam-QPX relationship and potentially identify drivers of the dynamics of this infectious disease.

Hypothesis

Environmental conditions play an important role in the development of QPX infection in hard clams. Prevailing conditions affect the hard clam host and the QPX pathogen, and ultimately the outcome of their interaction. Certain conditions lead to unfavorable imbalance for the hard clam, an opportunity for QPX which increases disease burden and mortalities. Favorable conditions can foster remission, assisting clams in abating infection.

Principal objectives

1- Investigate influence of environmental variables on hard clam host and QPX parasite interaction, aspiring to discover associated causal factors driving the QPX disease dynamic, increasing severity and leading to mortality, or promoting remission.

2- Examine performance of locally sourced hard clam stocks in two different estuaries.

3- Evaluate transplant applications for potential to mitigate QPX infection intensity and disease risk within an enzootic estuary.

Approach

Laboratory conditions are leveraged to control fundamental environmental variables during pathology trials. Applied field experiments are utilized to investigate potential applications to reduce QPX infection and disease severity. This multifaceted approach will bring about a greater understanding of the ecology of this bivalve disease.

Chapter 1. Laboratory investigations of environmental effects on QPX disease

Results from this chapter were published in:

Dahl S.F., Perrigault M., Liu Q, Collier J.L., Barnes D.A., Allam B. 2011. Effects of temperature on hard clam (Mercenaria mercenaria) immunity and QPX (Quahog Parasite Unknown) disease development: I. Dynamics of QPX disease. Journal of Invertebrate Pathology 106: 314-321. Perrigault M., Dahl S.F., Pales Espinosa E., Gambino L., Allam B. 2011. Effects of temperature on hard clam (Mercenaria mercenaria) immunity and QPX (Quahog Parasite Unknown) disease development: II. Defense parameters. Journal of Invertebrate Pathology 106: 322-332.

Perrigault M.*, Dahl S.F.*, Espinosa E.P., Allam B. 2012. Effects of salinity on hard clam (Mercenaria mercenaria) defense parameters and QPX disease dynamics. Journal of Invertebrate Pathology. 110:73-82. (* the first 2 authors contributed equally).

Abstract

Environmental conditions can play an important role in host pathogen interactions. Seasonality of QPX disease prevalence in the field and changes in QPX growth and survival in vitro suggest a role of temperature in the hard clam-QPX interaction and disease development. Considering QPX disease epizootics are usually observed in field sites with high salinities, salinity is suspected to be an important factor for disease distribution. Hypoxia is increasingly a source stress for coastal benthic organisms especially in systems subject to eutrophication. Separate laboratory trials were conducted using naturally and experimentally infected clams to isolate and examine the effects of temperature, salinity, and dissolved oxygen on QPX disease dynamics. Temperature trial treatments were conducted at 13 °C, 21 °C, or 27°C for 4 months, then all of the groups were brought to a temperature of 21°C for 5 additional months to simulate seasonal changes of temperature in the field and to investigate the effect of temperature variations on QPX disease dynamics. Salinity trial treatments of 17 and 30 ppt were conducted for 4 months. Dissolved oxygen trials were also conducted for four months with a control group approximating 7 mg/l, and low oxygen treatments of 3mg/l and 1mg/l. Temperature trial Results demonstrated significantly higher QPX disease prevalence and intensity, as well as higher mortality, in naturally-infected clams maintained at 13 °C as compared to those held at 21 °C or 27 °C. Similarly, disease development was significantly higher in experimentally infected clams maintained at the colder temperature (70% prevalence after 4 months) as compared to those maintained under warmer conditions (<10%). Additionally, our results demonstrated clam healing and a reduction of QPX prevalence in clams initially maintained at 13°C after transfer to 21°C. Salinity trial results demonstrated higher QPX-associated mortality in naturally infected clams maintained at high salinity compared to those held at 17 psu. Our findings also showed an increase in mortality following experimental challenge with QPX in clams submitted to 30 psu but not in those held at 17 psu. Insufficient disease development across the dissolved oxygen treatments prohibited differentiation of their relationships with QPX disease development. In general, adult hard clams were very tolerant of the low oxygen conditions over the four month trials. Findings from this experimental laboratory approach indicate that high salinity is a risk factor for QPX disease and high temperatures can limit disease progression.

1.1. Introduction

Fundamental environmental conditions have shown to be influential in bivalve host/parasite systems. Environmental conditions such as temperature, salinity, and dissolved oxygen have been documented as having strong influences on the immune function and resistance of marine mollusks (Chu et al. 1996, Reid et al. 2003, Cheng et al. 2004, Paillard et al. 2004). These environmental conditions have also been demonstrated to influence QPX growth and survival in laboratory cultures (Perrigault et al. 2010).

This study was conducted in order to examine the influence of primary environmental conditions on QPX disease development in hard clams. Laboratory based experiments intended to elucidate the effect of temperature, salinity, and dissolved oxygen on the interaction of the hard clam host and QPX parasite. Separate trials isolated the primary environmental variables of temperature, salinity and dissolved oxygen. Identical trials investigated the abiotic factors on two different sets of hard clams; allowing for greater opportunities to observe infection and disease progress. One set harbored natural infections obtained from an enzootic field site. The other set were naïve hard clams subjected to experimental transmission through inoculation to allow for a more quantified and uniform QPX challenge. Clam sources were chosen based on prior experience; cultured clams from FL for challenge by inoculation and naturally infected clams from MA (Dahl et al. 2008, Dahl and Allam 2007). Once experimental conditions were achieved for each trial, hard clam mortality was monitored over the course of four months. Prescribed sampling was conducted to determine QPX disease prevalence and intensity. Results for the same host genotype and infection method are examined according to the varied levels of each environmental treatment and additionally compared across the same environmental trials with different host genotype/infection methods.

1.1.1. Temperature trials

Bivalve mollusks are poikilothermic and therefore their physiological functioning and metabolism are directly influenced by the prevailing temperatures of their surroundings (Grizzle et al. 2001). Compelling effects of temperature, including strong seasonality, on epizootics caused by infectious pathogens are well documented in bivalves (Carnegie et al. 2008, Chu and La Peyre 1993, Ford et al. 1999, Paillard 2004, Villalba et al. 2004). Peaks in prevalence and severity of protistan infections heavily impacting oysters (e.g., Perkinsus marinus and Haplosporidium nelsoni) are typically observed in summer and into autumn when water temperatures are seasonally warmest. This trend seems to fit field reports in the Northeast of hard clams having the highest QPX disease prevalence and mortality during summer months and early fall. A seasonal survey of hard clams in Atlantic Canada found the highest QPX prevalence in August samples (MacCallum and McGladdery, 2000). The highest QPX associated mortalities in MA occurred during the late summer and early fall (Smolowitz et al., 1998). A major hard clam mortality event attributed to QPX in Raritan Bay NY occurred in July (Dove et al., 2004). Monitoring the Raritan Bay clam population since then shows QPX prevalence generally peaking during summer months in New York waters (Liu et al. 2008, Allam unpublished). In vitro growth of QPX was found to be greatest for isolates maintained between 20-23°C, parasite growth was noticeably reduced at 29°C and substantially reduced at temperatures below 17°C (Perrigault et al. 2010).

Hypothesis- Temperatures typical of coastal Atlantic waters in the summer of the Northeastern US (~21°C) will allow QPX to proliferate more profusely, resulting in greater disease burden and hard clam associated mortalities than temperatures that reflect cooler seasons (~13°C) of the same region. The cooler temperature will subdue QPX activity and result in less disease burden and related hard clam mortalities. Sustained at a much warmer regime (~27°C) QPX will become impaired and infections will begin to lose intensity over time.

Objective- Monitor QPX disease progress in hard clams assigned different temperature conditions during a 4 month laboratory trial. Following the initial treatment observations, simulate seasonal temperature changes and adjust all clam groups to uniform moderate conditions and observe disease dynamics for an additional 4-5 months.

1.1.2. Salinity trials

Hard clams are osmoconforming bivalves (Grizzle et al. 2001), ultimately their growth and survival is dependent on the prevailing salinity regime. Hard clams are attributed with a wide salinity tolerance range but below 15 ppt feeding is inhibited, reducing growth and survival (Castagna and Chanley 1973), and when beyond 30ppt, optimal pumping rates begin to decline (Hamwi 1969). A correlation of salinity and infection in oysters by *Perkinsus marinus* has been demonstrated in both laboratory and field studies (Chu et al. 1993, Paynter and Burreson 1991). Investigation of *in vitro* QPX growth was found to be optimal at 34ppt, while growth was clearly deficient at 15ppt (Perrigault et al. 2010). During a survey of clamming areas in Virginia, QPX was found in clams from sites characterized by higher salinities (30-34ppt) but was absent in clams from areas of moderate (15-25ppt) salinities (Ragone Calvo et al. 1998).

Hypothesis- QPX will proliferate and cause more intense infections in clams maintained at high salinity (~30ppt), resulting in disease progression and mortalities. QPX proliferation will become reduced in naturally infected clams and induced infections will be less intense for clams maintained at low salinity (~17ppt). The infection impediment under low salinity will result in greater clam survival.

Objective- Monitor QPX disease progress in hard clams held under different salinity conditions.

1.1.3. Dissolved oxygen trials

There is an apparent increase in the occurrence of hypoxia in coastal estuarine environments across the world which has impacts on their associated ecosystems especially benthic invertebrate communities (Diaz 2001). Under low oxygen conditions a clam may have to close its shell, losing the ability to feed and having to rely on less energetically favorable anaerobic respiratory mechanisms, impacting the clam's overall physiological functioning which is evident in growth reduction (Weber et al. 2008). When subject to oxygen levels below 5 mg/l, hard clam pumping rates subsequently decrease (Hamwi 1969). Dissolved oxygen (DO) levels go below 5mg/l during the summer in Raritan Bay (NYC DEP), which coincides with peak seasonal QPX prevalence (Liu et al. 2008, Allam unpublished).

Oysters exposed to low oxygen conditions suffered increased *P. marinus* infection related mortalities (Anderson et al. 1998). There is evidence of physiological stress from low oxygen conditions that directly affects immune defense functioning of bivalves. Production of reactive

oxygen species is an immune defense reaction that has been demonstrated in *M. mercenaria* (Bugge et al. 2007) and a substantial reduction in reactive oxygen species production was observed in oysters under hypoxic conditions (Boyd and Burnett 1999). Lack of oxygen has been reported to impact the immune function of *Chamelea gallina,* another clam in the Veneridae family (Matozzo et al 2005). Compromised respiratory status may serve as sufficient physiological stress, creating the opportunity needed for QPX to effectively invade the hard clam and proliferate.

Hypothesis- QPX proliferation and infection intensity will be greater for clams subject to low oxygen conditions (3 mg/l), than for clams under normoxia conditions (~7 mg/l). Hypoxic stress will foster disease development and increase QPX related clam mortalities.

Objective- Monitor QPX disease progress in hard clams held under different dissolved oxygen conditions.

1.2. Methodology

1.2.1. *Mercenaria mercenaria*

FL cultured clams were obtained from a commercial source. Naturally infected clams were located with assistance from Cape Cod Cooperative Extension agents that were knowledgeable of hard clam areas with persistent QPX infections in MA. Timing of acquisition of clams from MA was targeted for early autumn, during reported seasonal peak QPX prevalence (Smolowitz et al. 1998). The targeted size range for clams was greater than 30mm and avoid too large (<50mm) as it would inhibit having enough individuals per tank. Clam size consistency was dependent on what was available from the commercial sources; FL 30-35mm, MA 40-50mm.

Clams were fed daily with commercial algae (DT's Live Phytoplankton, Sycamore, IL; Pales Espinosa and Allam 2006) during acclimation and throughout the duration of experiments. Clams were acclimated for 1 week in 150-L tanks with re-circulating water (28–30 ppt) at 21°C ± 1°C. Clams were sampled (30 from each population) for pathology screening (see Histopathology) and to determine initial QPX prevalence and intensity. Following the 1-week acclimation, clams were distributed into the tanks to be used for experimental trials; see next three sections concerning arrangement of each trial. All tanks were aerated and individually equipped with re-circulating water filtration systems. Water quality (e.g., ammonia level) and salinity were monitored weekly and adjusted when necessary. Clams were monitored twice daily for mortality.

1.2.2. Temperature trials

Twenty clams were distributed to each 40 L tank. A group composed of 18 tanks (6 controls FL [FL-c], 6 QPX-challenged FL [FL-q] and 6 MA [MA]) for each one of the three temperature treatments (13°, 21° or 27°C), with a total of 36 FL clam tanks and 18 MA clam tanks. Temperature was adjusted by 1°C per day as appropriate for each treatment and controlled by water baths equipped with heaters (27°C) or chillers (13°C). Water temperature was monitored daily. Salinity was maintained at 28–30ppt. After 4 months of the temperature

treatments (13°, 21° or 27°C) all of the clams were brought to 21°C to simulate seasonal change of temperature in the field and monitored for 5 additional months.

1.2.3. Salinity trials

Twenty clams per 40 L tank as described above (6 FL-c, 6 FL-q and 6 MA) at two different salinities for a total of 24 FL tanks and 12 MA tanks. One group of 18 tanks remained at 30ppt and the other set was adjusted 1ppt per day by the addition of freshwater to achieve 17ppt. Temperature was maintained at 21°C.

1.2.4. Dissolved oxygen trials

Exploratory testing demonstrated that our larger tanks were more suitable for the procedures of maintaining proper DO levels (e.g., monitoring with DO probe, nitrogen bubbling, etc.). Sixty clams were distributed to 150 L tanks, 2 replicates each of FL-c, FL-q and MA. One set of six tanks was maintained at 'normal' oxygen levels (~7mg/L) and the other set of six tanks was held under hypoxic conditions (3mg/L). Tanks had an additional plastic tarp cover to inhibit air exchange. Air bubbling was ceased and low oxygen conditions were achieved over a few days. Dissolved oxygen levels were monitored twice daily with a handheld YSI (Yellow Springs Instruments) probe and either nitrogen or air was bubbled as needed to maintain the 3mg/L oxygen treatments. Temperature and salinity were maintained at 21°C and 30 ppt, respectively.

1.2.5. QPX culture

QPX strain NY0313808BC7 was isolated from mantle nodules of an infected New York clam (Qian et al. 2007) and was subcultured in muscle tissue homogenates (MTH) from *M. mercenaria* according to Perrigault et al. (2009). QPX cultures were initiated in 25cm² flasks containing MTH at 1000 µg/ml protein and were incubated at 23°C for 2 weeks (Perrigault et al. 2009). Neubauer chamber and the fluorescein di-acetate technique (Buggé and Allam 2005) were used to monitor growth and determine the concentration of QPX cells. Additional MTH medium, without QPX, was incubated under the same conditions for injection into control clams.

1.2.6. QPX challenge of naïve clams

After 1 week of acclimation at targeted experimental conditions, naïve FL clams were inoculated by injection into the pericardial cavity of sterile culture medium (MTH) into the control clams or 5x10^4 QPX cells into the challenged clams, according to Dahl and Allam (2007). Clams were kept out of the water for 1.5 hrs post-injection before transfer back to their respective tanks.

1.2.7. Sampling of naturally and experimentally infected clams

Clams were selected at random for histological processing, in equal amounts from each replicate, for a total of thirty clams per treatment after 2 months and again after 4 months. This sampling interval scheme was chosen based on results from our previous laboratory trials showing this time period to be sufficient for observing significant disease progress (Dahl and Allam 2007, Dahl et al. 2008). After experimental conditions were achieved, clams discovered to be moribund (gaping) during daily mortality checks were taken for histology sampling. The temperature trials had a final sample taken after the additional 5 months of uniform conditions when the experiment was ended (9 months total).

1.2.8. Histopathology

All sampled clams were processed for histopathology following the general procedures employed previously (Dahl and Allam 2007, Dahl et al. 2008). A transverse slice of tissue roughly between 3 and 5 mm in thickness through the central region of the meat is made in an attempt to include visceral organs, as well as gill and mantle tissues. Particular effort was made to include tissue from the base of the siphon where infections are often found (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'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 intensities were determined based on the number of QPX cells present on the histological section, and recorded as follows: light (≤10 QPX cells on the section), moderate (11–100 QPX cells), heavy (101–1000 QPX cells), or severe (>1000 QPX cells). Histological presence of old lesions and degrading QPX cells associated with the healing processes of hard clams were also recorded (Dahl and Allam 2007).

1.2.9. Statistical analyses

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 (Kleinbaum and Klein 2005). 'Censored' means the values have been lost from view of the study. This compensates for the removal of clams at set points in time for infection diagnosis. A LogRank test was conducted to determine whether survival curves are significantly different (Kleinbaum and Klein 2005). The Holm–Sidak multiple comparison procedure was used to determine which pairs of curves are different. This method applies a sequential adjustment of critical values that compensates for the number of comparison tests (Glantz 2005).

Disease prevalence data for naturally and experimentally infected clams from each sampling date were separately tested for significant differences according to each experimental treatment condition. Counts of QPX-infected and uninfected individuals from each histology sample were arranged in a 2-way, row-by-column (RxC) 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.; Sokal and Rohlf 1995). The first variable was the experimental treatment (i.e., level of temperature, salinity level, or dissolved oxygen). The second variable was infection, with one class for infected and one class for uninfected clams. Counts of individuals with and without signs of healing and remission were tested in the same manner. All counts were pooled from replicate samples of the same treatment group and time period. BIOMstat additionally carries out Gabriel's simultaneous test procedure which identifies 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). All results were considered significant at an overall level of $\alpha = 0.05$. Mortality counts at the end of the FL temperature trial 5 month extension was also tested through the RxC procedure.

1.3. Results: Temperature trials

1.3.1. Mortality - Inoculated (FL) clams

During the initial 4 month exposure to different temperatures, mortality was very low (<4%) across all temperature treatments for both control and QPX-challenged clams (Data not shown). The 5-month extension of the experiment (all groups at 21°C) resulted in minimal (<6%) to no mortality in control clams initially maintained at 13°C or 21°C whereas their QPX-challenged counter parts had mortalities of 16% and 21% respectively (Fig. 3). High mortalities occurred during the extension period in clams initially held at 27°C, with 36% for challenged and 41% for control clams.

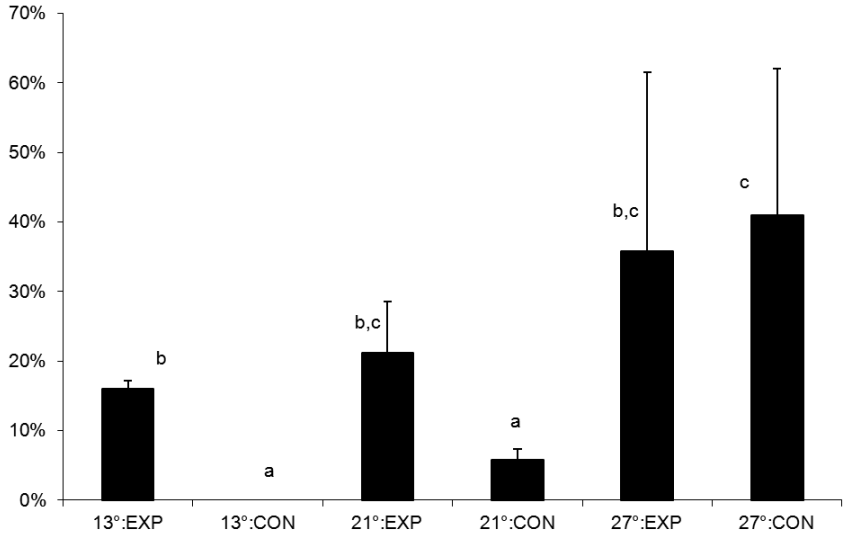

Figure 3. Mortality incurred by inoculated (FL) clams originally submitted to 13°C, 21°C and 27°C for 4 months during the 5 month extension in which all treatments were maintained at 21°C; EXP = challenged with QPX, CON = control. Different letters (a, b, c) designate significant differences between treatments ($p < 0.001$, G-test with Gabriel's procedure).

1.3.2. Mortality - Naturally infected (MA) clams

Clams maintained at 13°C exhibited a steady increase in mortality over the first 4 months (Fig. 4A). After 4 months of temperature exposure, cumulative mortality was significantly higher ($p = 0.01$) in the coldest treatment (19%) as compared to clams held at 21°C (6%) or 27°C (8%). Mortalities continued during the 5-month extension temperature regime of 21°C (Fig**.** 4B), but leveled off after 1 month for clams initially maintained at 13°C (10%) and 21°C (8%). Clams initially maintained at 27°C exhibited a very low level of mortality during the 5-month extension at 21°C (Fig. 4B).

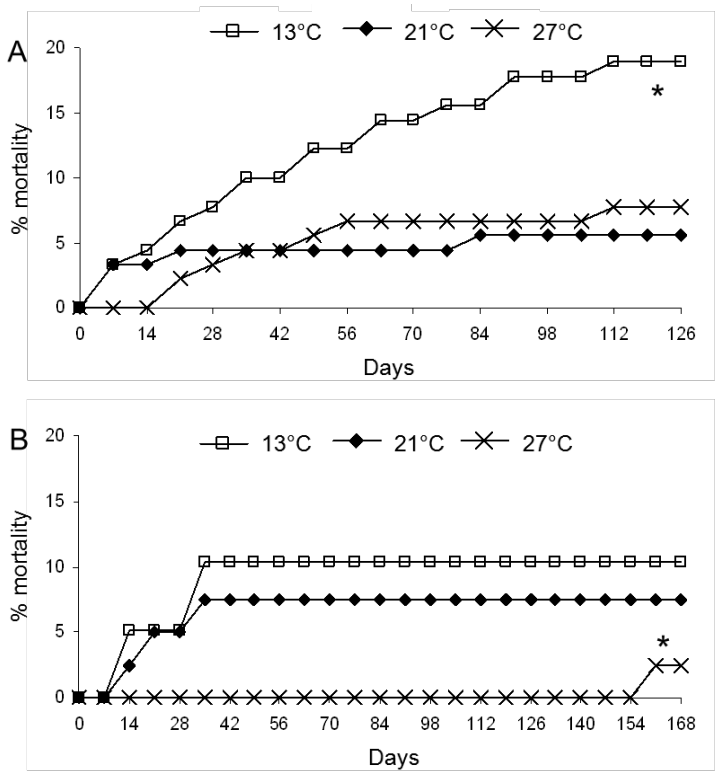

Figure 4. Cumulative mortality in naturally-infected clams submitted to 13°C, 21°Cand 27°C for 4 months (A), after which all treatments were maintained at 21°C (B(day 0 in B represents the beginning of the 5 month extension). * Designates significant difference (LogRank with Holm-Sidak procedure, $p \leq 0.01$).

1.3.3. Infection prevalence - Inoculated (FL) clams

Experimentally infected clams (FL-q) maintained at 13°C displayed significantly higher QPX prevalence ($p < 0.001$), 73% at 2 months and 70% at 4 months, as compared to challenged clams maintained at 21°C or 27°C (≤10%, Fig. 5A). Disease prevalence in FL-q clams remained steady in all temperature treatments during the initial 4-month experiment but a general decrease in prevalence was subsequently observed after all clams were converged to 21°C for the 5-month extension, particularly in clams initially maintained at 13°C (19% at 9 months, Fig. 5A). The decrease in disease prevalence during the extension period coincided with an increase in the proportion of clams displaying healing signs among individuals initially submitted to 13°C and 21°C. The proportion of clams healing in the 27°C peaked at 4 months and there were no additional displays of healing or disease in this group at the end of the 5-month extension. QPX was not detected in any control (FL-c) clams.

1.3.4. Infection prevalence - Naturally infected (MA) clams

QPX prevalence in naturally infected (MA) clams was also significantly modulated by temperature (Fig. 5B). Two months after the beginning of the experiment, disease prevalence was 23%, 10% and 0% in MA clams submitted to 13°C, 21°C and 27°C, respectively (significant difference between 13°C and 27°C; $p < 0.01$). A more pronounced difference of disease prevalence at 13°C (13%) opposed to those at 21°C or 27°C (0%) was noticeable after 4 months

($p < 0.05$, Fig.5B). Disease prevalence decreased over the entire 9-month period and was associated with an overall increase in the percentage of clams displaying healing signs among all treatments (Fig. 5B), particularly among those initially maintained at 27°C (45%, $p < 0.01$).

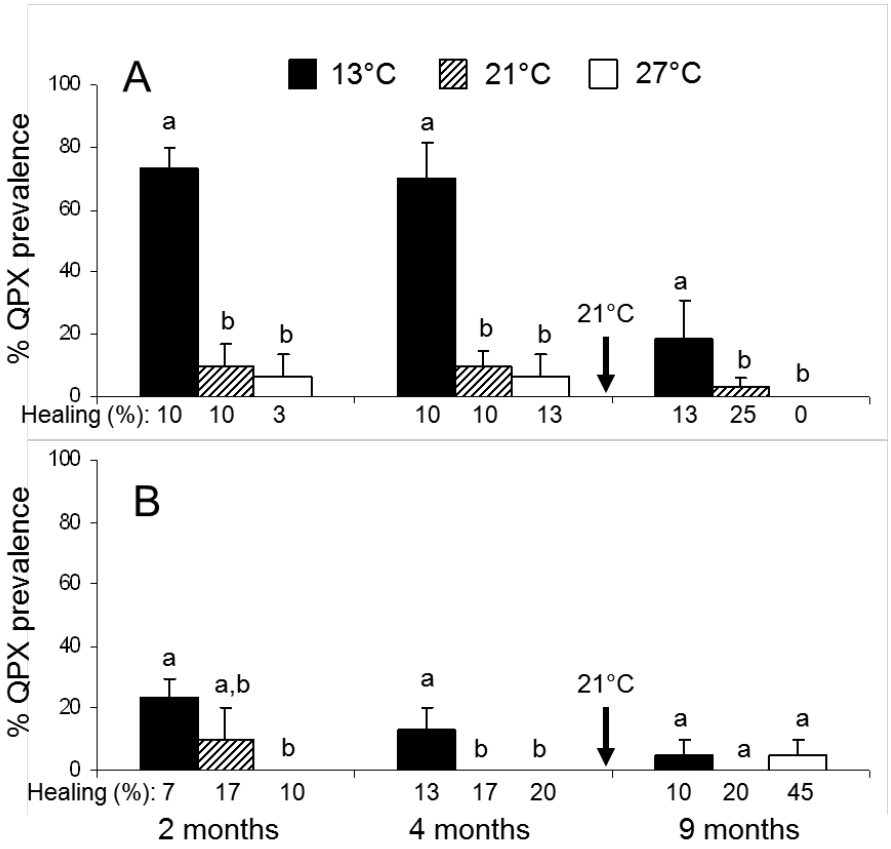

Figure 5. QPX prevalence expressed as mean and standard error (6 tanks/treatment and 5 clams sampled from each tank at each sampling time) determined by histology in (A) experimentally (FL-q) and (B) naturally (MA) infected clams maintained at different temperatures. All clams were transferred to 21°C after the 4-month sampling (arrow). Values along the x-axis indicate percentage of clams presenting healing signs. Different letters (a & b) designate significant differences between treatments ($p < 0.05$, G-test with Gabriel's procedure).

1.3.5. Infection intensity

High QPX prevalence observed in FL-q and MA clams submitted to 13°C was associated with higher disease intensity in these groups as compared to clams maintained at the warmer temperatures (Fig. 6). FL-q clams maintained at 13°C exhibited mostly light infections at 2 months and progressed to include more serious infections over time (Fig. 6A). Moderate and heavy infections constituted most (>75%) of the positive MA clams maintained at 13°C for 2 and 4 months, in contrast to the warmer treatments where low numbers of QPX-positive clams were observed and none of them presented heavy infection (Fig. 6B).

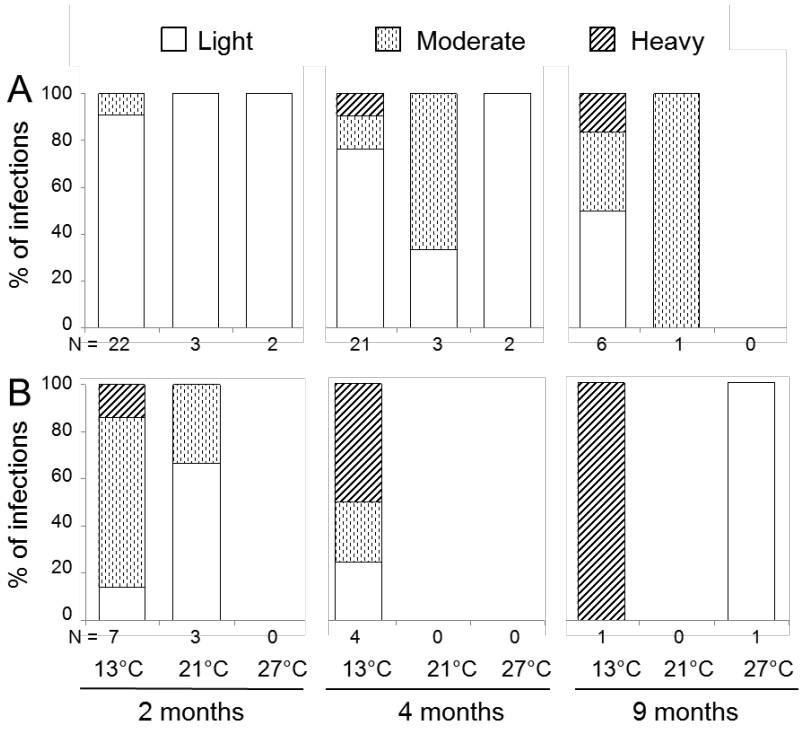

Figure 6. QPX infection intensity determined by histology in (A) experimentally (FL-q) and (B) naturally (MA) infected clams maintained at 13°C, 21°C and 27°C. Number of infected clams is indicated on the x-axis. All clams were transferred to 21°C after the 4-month sampling.

1.3.6. Moribund clams

Five moribund clams were collected for histology among all of the experimentally infected (FL-q) individuals during the initial 4 month experiment (Fig. 7A). Only one was positive from the 13°C treatment with a moderate infection and two infections (1 moderate, 1 heavy) from the 21°C treatment. Disease prevalence was significantly higher among moribund FL-q clams collected during the 5-month extension from the 13°C group (100% prevalence) as compared to the groups initially at 21°C (55%) or 27°C (11%) ($p < 0.001$, Fig. 7A). The difference between the latter 2 groups was also significant ($p = 0.01$). Very low QPX prevalence among moribund FL-q clams from the 27°C group during the 5-month extension contrasted the high mortalities observed in this batch, as well as their controls, which suggests that the mortalities observed at this temperature is not from QPX disease.

In naturally-infected clams, disease prevalence was maximal (100%) at the end of the initial 4-month exposure among moribund MA clams from the 13°C treatment, followed by 80% positive in the 21°C group and 57% in the 27°C treatment ($p < 0.05$, Fig. 7B). The most intense infections were observed in clams maintained at 13°C (4 severe) while mostly moderate infections were detected in those held at 27°C. Trends were not as clear during the 5-month extension as relatively low mortality during that period limited the amount of moribund clams diagnosed (Fig. 7B). Overall, there was a substantial decrease in intensity among positive clams collected during the extension period as mostly light and moderate infections were detected.

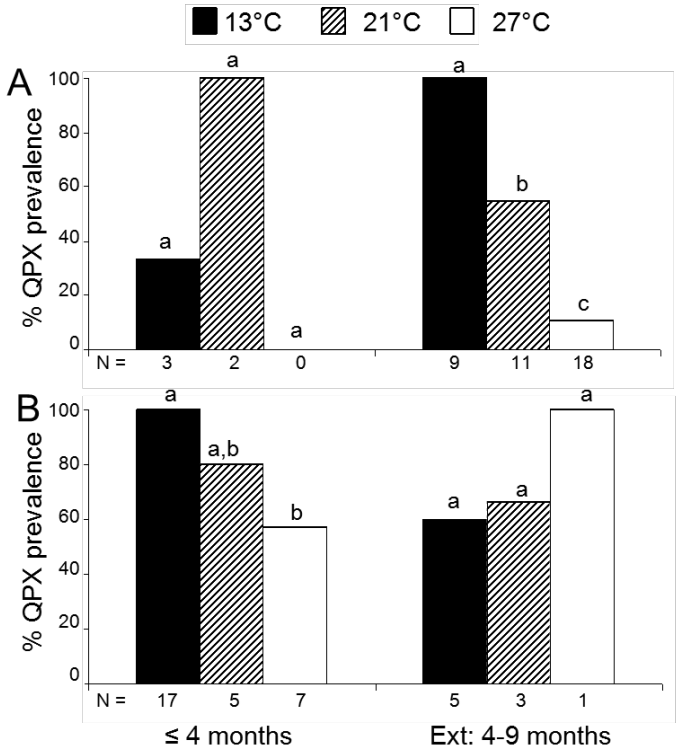

Figure 7. QPX prevalence determined by histology in moribund clams from (A) experimentally challenged (FL-q) and (B) naturally infected (MA) clams, collected during the initial temperature exposure (≤4 months) and during the 5-month extension at 21°C (4–9 months). Number of moribund clams processed is indicated along the x-axis. Different letters (a, b and c) designate significant differences between treatments ($p < 0.05$, G-test with Gabriel's procedure).

1.4. Discussion: Temperature trials

Histological observations of both experimentally and naturally-infected clams exhibited similar trends according to temperature treatment. Clams submitted to 13°C for 4 months exhibited significantly higher QPX infection prevalence than those maintained at 21°C or 27°C. The difference in QPX prevalence between the 21°C and 27°C groups was less dramatic. These results in conjunction with a concurrent study that collected data on the impact of temperature on hard clam immune parameters (Perrigault et al. 2011), comprehensively illustrate a strong modulatory effect of temperature on hard clam-QPX pathogen interactions and resultant disease dynamics.

Temperature is well known to influence clam physiology (Grizzle et al. 2001) as well as the development of infectious diseases in other bivalve species (Haskin et al. 2008; Chu and La Peyre 1993, Ford and Haskin 1982, Paillard et al. 2004), although the modulatory mechanisms are not always clear. Higher QPX disease prevalence and intensity among clams maintained at low temperature (13°C) could result from better performance of the parasite or an immunodepression of clams at this temperature, or a combination of both conditions.

In vitro investigations have demonstrated that QPX grows optimally between 20-23°C (Perrigault et al. 2010) and growth is noticeably reduced below that range, reaching about 60% of maximal growth at 13°C. Despite apparent suboptimal conditions for the parasite at 13°C, disease development was higher in hard clams maintained at this temperature as compared to those maintained at 21°C and 27°C. Investigations pertaining to physiology suggest 20–24°C to represent an optimal range for hard clams while 13°C is noticeably suboptimal. For example, pumping rates in *M. mercenaria* increase at a greater rate when temperatures are 20°C and above, rates are maximal at 24–26°C and start to decline rapidly at temperatures above 27°C (Hamwi 1969). Clearance rate and oxygen consumption in hard clams also increase with increasing temperature although oxygen consumption rates increase faster at temperatures over 20°C and surpass clearance rates at 25°C and above (Hibbert 1977). Similarly, shell growth is greatest between 20 and 24°C (Ansell 1968).

Viewed collectively, it appears that the exposure to 21°C in the current study was the most optimal temperature for clam activity among the three tested temperatures. The 27°C treatment appears to be near a physiological tolerance limit and might become stressful for clams over long periods of time. Stress and exhaustion may have been a leading factor in the mortalities observed during the 5-month extension in Florida clams (both challenged and controls) initially maintained at 27°C. The 13°C treatment represented a suboptimal temperature and was stressful as witnessed by alterations of hemolymph parameters. Perrigault et al. (2011) demonstrated significant immunodepression in control clams maintained at 13°C for 2 and 4 months compared to those held at 21°C and 27°C (same clams used in this study). The same study also showed significant alteration of clam immune response to QPX among naturally and experimentally infected clams maintained at the lower temperature.

Clams submitted to 21°C and 27°C had low QPX prevalence and intensity, and high levels of healing were noted. Failure of QPX to induce infection in clams maintained at 27°C is not that surprising since this temperature is detrimental to QPX in vitro (Perrigault et al. 2010). More interesting is the failure of QPX to induce infections among clams maintained at 21°C. The parallel study showed enhancement of immune response against QPX among clams maintained at 21°C (Perrigault et al. 2011). Therefore, it appears that low QPX prevalence in clams maintained at 21°C is likely the result of an effective immune response to the presence of QPX, while the inability of QPX to establish infection in clams maintained at 27°C derives from the deleterious effect of high temperature on the parasite itself, independent of clam immune response which was minimal among QPX-challenged clams held at this highest temperature (Perrigault et al. 2011).

Differences in disease dynamics were noted between the two clam populations and are likely attributable to the stage of infection at the start of the trials. The naturally-infected clams had well established infections at the start of the experiment and were therefore more likely to achieve advanced disease stages within the initial 4 months, which could explain the observations of higher mortality and greater disease prevalence and intensity among moribund clams. While QPX prevalence in FLq clams tended to be constant, with intensity increasing over time, during the initial 4 month exposure to different temperatures. Prevalence dropped in MA clams in all treatments over time as a result, at least in part, of the death of the most severely infected individuals. Greater numbers of moribund clams were collected within the first 4

months in naturally-infected clams whereas mortality associated with QPX occurred mostly during the extension period in experimentally infected clams.

Evolution of QPX disease in response to temperature variations roughly simulating seasonal changes was observed. Transfer of all clams to 21°C for 5 additional months resulted in a noticeable decrease of QPX prevalence in all treatments, particularly in those initially held at 13°C, confirming the ability of clams to mount an effective response against the parasite under favorable temperature conditions (21°C). This observation is important since it demonstrates the dynamic impact of temperature on QPX disease, favoring disease development at lower temperatures but supporting elimination of the parasite and clam healing at higher temperatures. Temperature clearly affected QPX infection development and the ability of clams to mount an effective immune response (Perrigault et al., 2011).

Previous field reports have found the highest disease prevalence and mortality during summer months or early fall. A seasonal survey of hard clams in Atlantic Canada found the highest QPX prevalence in August samples (MacCallum and McGladdery, 2000). Monitoring of clams in New York waters generally shows QPX prevalence peaks during summer months (Liu et al., 2008, Allam, unpublished). The highest QPX associated mortalities in Massachusetts occurred during the late summer and early fall (Smolowitz et al., 1998) while the first major mortality event in New York took place during July (Dove et al., 2004). The apparent discrepancy in observations from field reports and these lab experiments, of higher disease development at 13°C as compared to warmer summer-like temperatures, could be explained by two main factors. First, QPX disease is a relatively slow and chronic infection and results from the current study and prior investigations show that several months are needed for the parasite to establish infections and progress to overt disease (Dahl and Allam, 2007; Dahl et al., 2008). Similar findings were also made in oysters infected with the mesophilic alveolate *Perkinsus marinus* by Ford and Smolowitz (2007), who demonstrated a lag of more than 3 months between optimal water temperature and maximal disease prevalence. In addition, mortality is an end point for the disease process and the observed mortality peak in summer implies that infection had to be established earlier in the year. Interestingly, there was a noticeable pulse in mortality levels associated with QPX disease after naturally and experimentally infected clams were moved from 13 to 21°C suggesting that the temperature change was detrimental to heavily infected clams. This is not surprising when considering that severely infected clams are less likely to cope with enhanced metabolic demands under higher temperatures, leading to exhaustion of the host and mortality. Increasing metabolic demands during summer have been recognized as an aggravating factor for infectious diseases in marine mollusks, including abalone (Travers et al., 2008) and oysters (Li et al., 2009; Samain et al., 2007; Sauvage et al., 2009). A summer pulse in QPX-associated mortality may reflect both spring and summer processes in the field, with major disease development during mid to late spring (water temperatures around 13°C) and maximal mortality at higher temperatures when metabolic demands on clams increase. Increasing temperature during the summer could be beneficial to lightly infected clams, at an earlier stage of disease, boosting defense response against the infection and leading to remission as observed during the 5-month extension in challenged FL clams initially maintained at 13°C, where mortality alone is insufficient to explain the significant drop in disease prevalence.

A survey conducted in Virginia, the most southern extent (and warmest area) of the known QPX disease range, found the highest QPX prevalence and intensities associated with active division stages of the parasite within clam tissues during May and November, but not during the hotter summer months (Ragone Calvo et al., 1998). Lab results now demonstrate a strong effect of temperature with the greatest disease development at 13°C as compared to 21°C or 27°C, confirming QPX as a ‘‘cold-water’’ infection. These findings have management implications, for example, the timing of large scale clam movements (as in transplant operations or seed deployment) can be selected to reduce the risk of disease outbreaks. Because clams have shown the ability to heal under optimal temperature conditions, a potential disease mitigation strategy for clam stocks sustaining relatively low QPX levels is transfer of clams to shallower portions of the same geographic area (to avoid parasite spread) with generally higher temperatures to foster remission.

1.5. Results: Salinity trials

1.5.1. Mortality

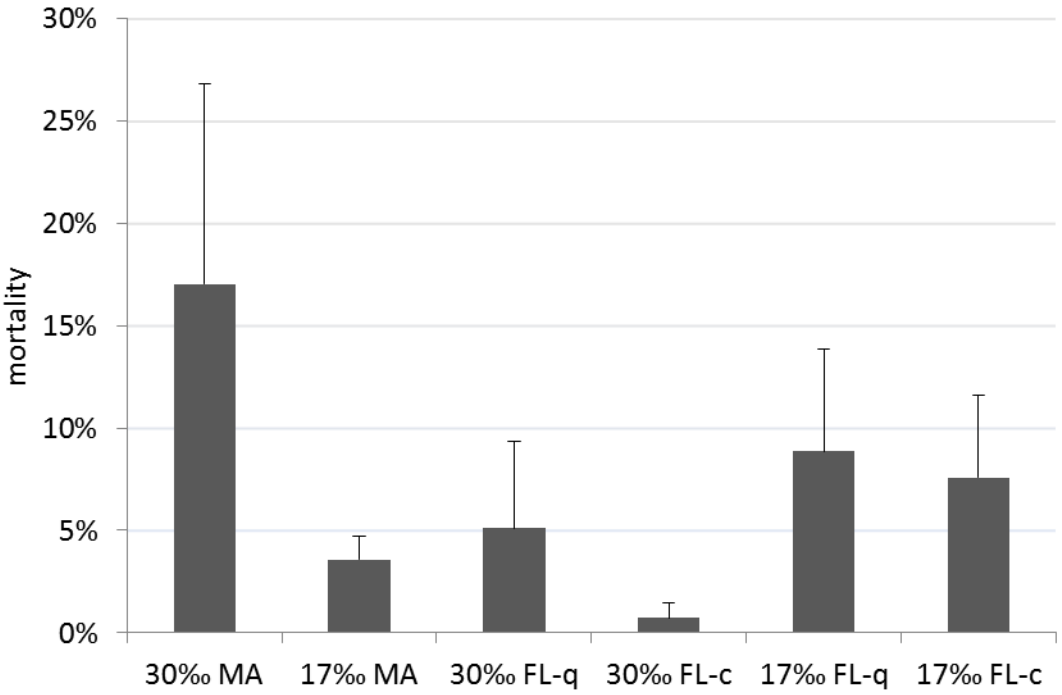

Figure 8. Percent mortalities occurring during the four month trial at high salinity (30ppt) and low salinity (17ppt) treatments for naturally infected MA clams, experimentally challenged FL-q and controls FL-c.

Total mortality levels were relatively low (≤9%) for Florida clams (Fig. 8). Controls (FL-c) had significantly greater ($p<0.01$) mortalities at the low salinity treatment (8%) compared to the high salinity control clams (0.7%). Challenged (FL-q) clams at 17ppt had only slightly greater mortality (9%) than their controls. When challenged at 30 ppt, mortality was significantly higher in FL-q clams (5%) as compared to their controls ($p<0.05$). The largest difference in

mortality between salinity treatments occurred with the naturally infected MA clams, with 17% at 30 ppt and 3.6% at 17 ppt ($p<0.05$).

1.5.2. Infection prevalence and intensity

QPX prevalence was greater in naturally infected MA clams from the 30 ppt treatment at 2 months, 17% compared to 10% from 17 ppt (Fig. 9), although there was no difference in prevalence at 4 months (13% ea). Higher mortality for MA clams at 30 ppt allowed for more moribund clams to be processed, 11 vs 6 for 17 ppt. Prevalence was higher in moribund clams from 30 ppt (91%) than at 17 ppt (67%) and the only severe case observed (>1000 QPX cells) was from 30 ppt. Signs of healing (e.g. old lesions with degraded QPX cells) were noted 7–13% in negative MA clams at 2 and 4 months without a discernible pattern according to salinity treatment.

Diagnosis of experimentally infected FL-q clams did not reveal active QPX infections in samples at 2 months or 4 months. There were signs of inflammatory response and the appearance of some degraded QPX cells.

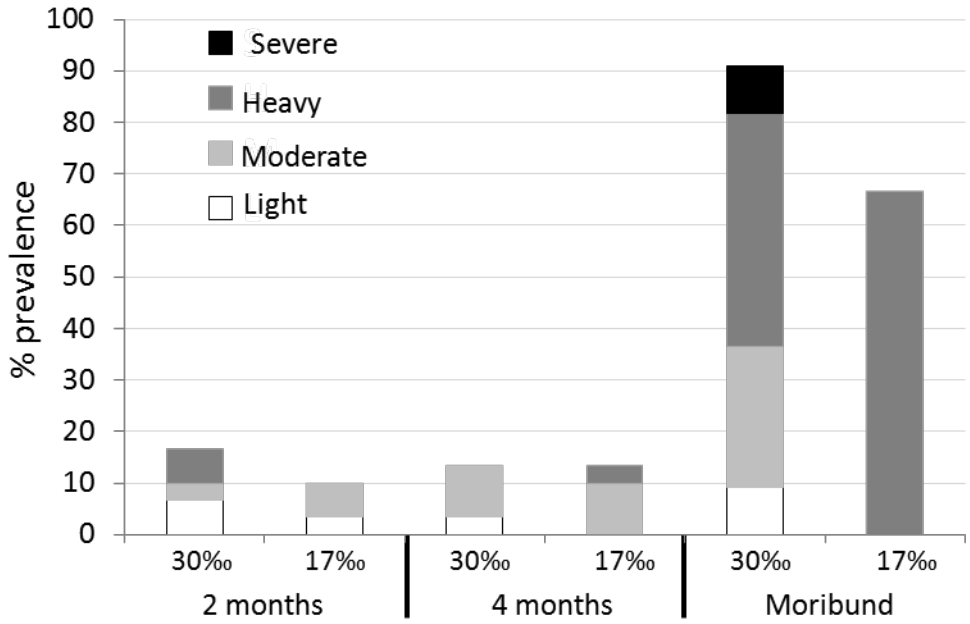

Figure 9. QPX disease prevalence and intensity in naturally-infected clams (MA) sampled after 2 and 4 at 30ppt or 17ppt. N=30 clams per treatment excluding moribund clams where n = 11 (30ppt) or 6 (17ppt).

1.6. Discussion: Salinity trials

The salinity treatments resulted in different QPX disease impacts. Higher mortality was measured in naturally infected MA clams at the higher salinity. The moribund clams sampled from that group had the highest prevalence and greatest infection intensity observed in the study. FL clams challenged with QPX under the higher salinity treatment also had significantly higher

mortalities than their controls. This contrasts the similarity observed between mortality levels of FL challenged and controls at 17ppt.

When considering the high salinity controls had very little mortality, the difference seen in FL clams at 30ppt is likely reflective of QPX challenge. Whereas the FL clams at 17ppt may have exhibited a unified response of low salinity stress; nearly all of the mortality for both challenged and controls happened within the first month. Hard clams are tolerant of a relatively wide salinity range but below 17.5ppt evidence of physiological impacts have been observed as growth is inhibited (Castagna and Chanley 1973). There was a significant increase in the percentage of dead hemocytes observed in this trial under 17 ppt (Data not shown; Perrigault et al. 2012), providing supportive evidence of stressful conditions for those FL clams. The injection process could have caused some injury that aggravated the low salinity stress although the extent of such damage is suspected to be minor when considering the relative lack of mortalities displayed by the high salinity controls.

MA clams were histologically screened prior to the trial and QPX infection prevalence was 37%. Disease prevalences at 2 and 4 months of salinity treatments were lower than the starting prevalence. Prevalence can drop when mortality of the more intensely infected individuals occurs during the trial and evidence from samples taken of moribund clams is supportive of this (Fig. 9). Surviving clams at 2 and 4 months displayed evidence of some individuals under gone healing, another mechanism to reduce infection prevalence. Bringing naturally infected clams into the lab (20-21ºC, 30ppt) has resulted in a reduction of infection over time in a previous experiment (Dahl and Allam 2007). In the current experiment, the higher salinity treatment appeared less advantageous to the clams since a greater amount of infections progressed to mortality.

Experimental transmission of QPX has been achieved previously through injection of parasite isolate culture into the pericardial cavity of challenged clams (Dahl and Allam 2007). The same inoculation technique was used in this experiment with more total QPX cells per inoculate (5×10^4 vs 4×10^4), yet there was a lack of active infection diagnosed after 2 and 4 months. The salinity trials were performed at 17°C and yet the first successful inoculation study was conducted at temperatures around 20-21°C which was found to be more beneficial for clam healing in the recent temperature trials. The first inoculation study did not utilize muscle tissue homogenates (MTH) to culture QPX. MTH has been adopted since it was reported to enhance QPX growth in vitro (Perrigault et al. 2009) and the same culture technique was used for the recent temperature trials that had a considerable amount of infection observed in experimentally challenged clams (see above). Different batches of cultured clams from Florida were used for the temperature and salinity trials. Possible genetic based differences in the clam batches could influence disease susceptibility and affect results of experimental transmission. In parallel, cultures of different pathogens have been observed to lose virulence over time (see discussion in Ford et al. 2002b for review), which leads to questioning whether this has begun to occur with the QPX culture utilized for this challenge.

A field survey observed QPX in sites characterized by high salinities (30-34ppt) but not areas of 15-25ppt (Ragone Calvo et al. 1998). Similarly high salinities *in vitro* promoted QPX growth but became inhibited at 15ppt (Perrigault et al. 2010). In this study, natural QPX

infections were at greater risk (relative risk 4.7 times greater) of leading to mortality at high salinity (30ppt) than at low salinity (17ppt). Lower salinities within brackish environments may restrict and even exclude QPX disease.

1.7. Results: Dissolved oxygen trials

1.7.1. Mortality

Most of the mortalities for naturally infected MA clams (Fig. 10) occurred before the first diagnostic sample at 2 months and were similarly around 12% for low DO (3mg/l) and normoxia. The next two months after the first sample were also similar for both treatments with very little mortality, averaging just over 1%.

Mortalities for FL clams under low or normal oxygen were less than 4% for the whole trial, both challenged and control, nearly all of which occurred within the first two weeks following inoculation procedures.

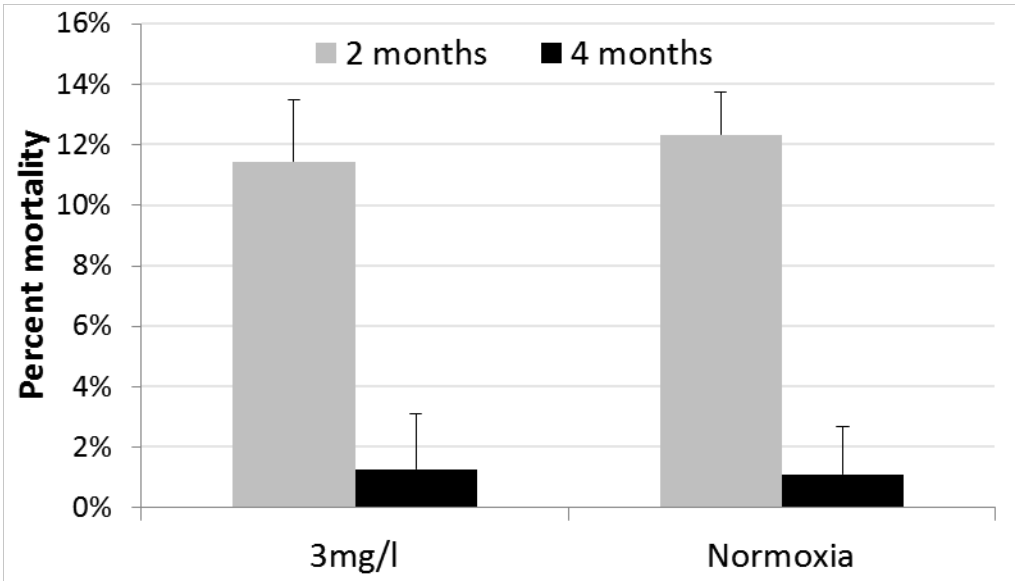

Figure 10. MA clam mortality up to the first sampling at 2 months and then of remaining clams until sampling at 4 months for 3mg/l DO and normoxia.

1.7.2. Infection prevalence and intensity

QPX infection prevalence in naturally infected MA clams after two months of low DO (3mg/l) was mildly greater than the normoxia group; 20% vs. 17% (Fig. 11). At four months this margin switched as the normoxia group had 13% prevalence when there was 10% in the low DO group. Most of the infections at 2 and 4 months were light, with a few more intense individuals in both treatments, one clam had a severe infection at 2 months under the normal oxygen treatment but this was not seen again at 4 months. Moribund clams had relatively similar levels of infection prevalence under low and normal oxygen treatments; 100% and 94% respectively. The majority of moribund clam infections were of a moderate category but there were noticeably more heavy infections among the low DO samples.

Inflammatory response was observed in experimentally challenged FL-q clams during microscopy inspection of histology but no active infections could be confirmed in either the 2 or 4 month samples.

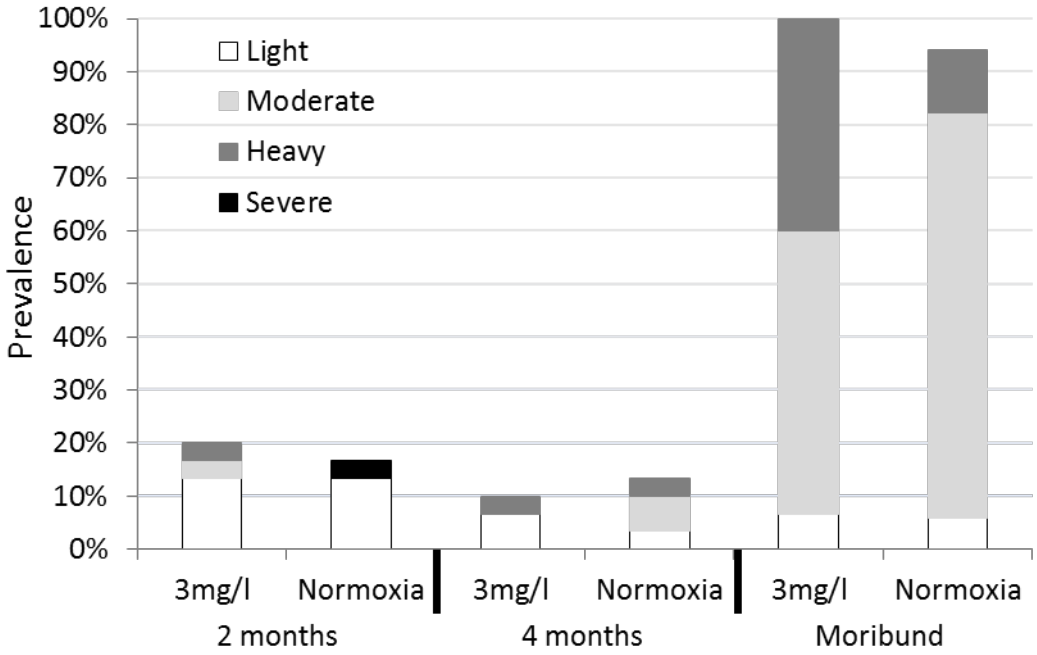

Figure 11. QPX disease prevalence and intensity in naturally-infected clams (MA) sampled after 2 and 4 months of 3mg/l dissolved oxygen (low DO) or normoxia. N=30 clams per treatment excluding moribund clams sampled throughout the trial where N = 15 (3mg/l) or 16 (normoxia).

1.7.3. Second inoculation trial

The lack of significant trends and unsuccessful development of disease for the inoculated (FL) clams inspired a second attempt of a dissolved oxygen trial with inoculated clams only. Aspects of the experiment were the same as the first trial except the low DO treatment was maintained at 1mg/l and all treatments were brought down to 13°C, instead of 21°C, to favor disease development (Chap. 1, Dahl et al. 2011). There were confirmed infections during histology screening of the inoculated FL clams from this second trial (Fig. 12), although there were very few positives and all of the infections were of the lowest intensity (<10 cells). At 2 months the 1mg/l DO treatment had a few more infections than the normal oxygen treatment but this difference disappeared at 4 months. Mortality was very low (2 clams) for the low DO (1mg/l) group and none for the normal oxygen.

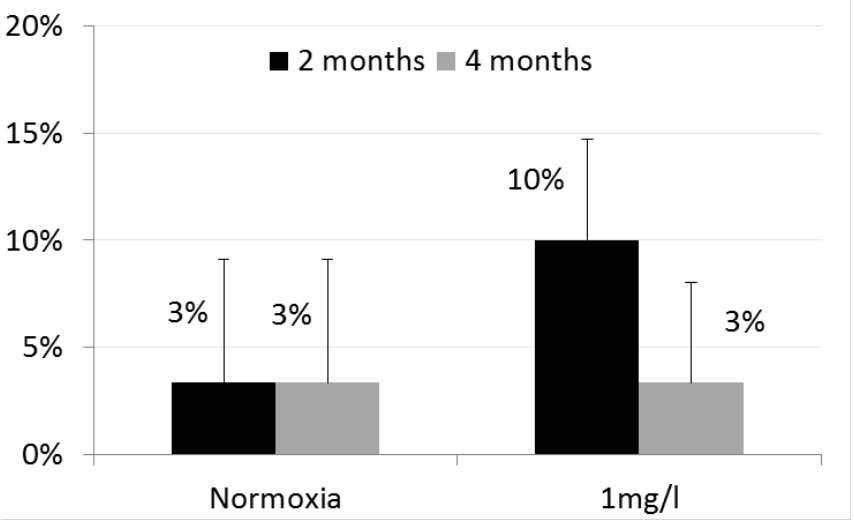

Figure 12. QPX histology prevalence during the second DO trial with inoculated FL clams maintained at 13°C.

1.8. Discussion: Dissolved oxygen trials

Most of the MA clam mortality for both oxygen treatments was relatively early in the trial, which could be a result of well-established infections running their course. Evidence of this is provided by the high prevalence and intensity of the moribund samples. Early mortality of the more infected individuals can contribute to the reduction in MA clam prevalence that was observed over time, similar to the previously described environmental trials. Remission was observed in MA clams and occurred in only one more individual for Normoxia vs 3mg/l in each sampling (7 vs 6 at 2 months, and 5 vs 4 at 4 months). Overall, there was not much of a difference in the results between both DO treatments for MA clams, aside from the observation that more of the low DO moribund infections were of a heavy intensity (although frequency analysis was not significant).

FL clams had minimal mortalities, early in the trials which, could be due to a brief stress related to the inoculation process. Inflammatory response was observed in FL clams during microscopy inspection of histology but no active infections could be confirmed in either the 2 or 4 month samples of the first inoculation trial. This was the same batch of clams and cultured QPX inoculate as used in the salinity trials. Again, the lack of infections using a confirmed transmission technique (Dahl and Allam 2007) raises concerns of whether this cultured parasite is losing virulence or the possibility that we have acquired a uniquely resistant FL clam strain. There were a few confirmed infections in the second inoculation trial at 13°C (vs. 21°C for the 1st trial) in both the 1mg/l treatment and normal oxygen but only of the lowest intensity and no difference between the treatments at 4 months.

Mortality and infection results from both the MA and FL sets of clams did not provide any significant evidence of the dissolved oxygen treatments influencing QPX disease development. An impression gained from these trials is that *M. mercenaria* is quite capable of tolerating low dissolved oxygen conditions even though dissolved oxygen levels below 5.0 mg/l are generally considered stressful to hard clams (Roegner & Mann 1991) as pumping rates and oxygen consumption begin to decline (Hamwi 1969). Anaerobic metabolism is presumed to

become more important with declines in oxygen consumption, although physical activity can be maintained even at dissolved oxygen less than 1.0 mg/l (Savage 1976). Hard clam growth becomes greatly reduced at oxygen levels below 4.2 mg/l but the minimum dissolved oxygen requirement for normal development was found to be 0.5 mg/l (Morrison 1971). Low oxygen tolerance makes sense when considering an infaunal lifestyle in often organically rich sediments of highly productive coastal areas that likely undergo cyclical dissolved oxygen fluctuations, seasonally and possibly even on a daily basis in shallow areas.

Thraustochytrids are commonly associated with detrital matter found in both neritic and deep water sediments (Raghukumar 2002). Such environments are frequently limited in their oxygen content due to organic matter decomposition and/or distance from oxygen sources. Thraustochytrids have likely developed low oxygen tolerance in order to persist and thrive in these places. An *in vitro* study showed that "QPX is sensitive to anoxia and a prolonged anoxic episode significantly altered survival of the parasite" (Perrigault et al. 2010). Limitations in growth of cultured QPX were observed after an anoxic period of 7 days. The ability of QPX to tolerate chronic or acute hypoxia, as opposed to a complete lack of oxygen for a week, is not known.

According to data from a parallel study of the hard clams in the first trial of normpxia and 3mg/l s (Wang et al., unpublished), the dissolved oxygen treatments did impact clam immune parameters, although, observed alterations of the immune response were generally not severe and often transient. The capacity to mount a defense response was observed to be greater for clams under normoxia conditions. Immunosuppression observed under low dissolved oxygen conditions may have allowed more of MA clams infections to progress to a heavy intensity as observed in the low DO moribunds. In general, the dissolved oxygen conditions tested in this study had limited consequences on QPX disease as compared to salinity or especially temperature.

Chapter 2. Performance assessment of local clam stocks in two different estuaries

Results from this chapter were published in:

Dahl, S.F., J. Thiel and B. Allam. 2010. Field performance and QPX disease progress in cultured and wild-type strains of hard clams in New York waters. Journal of Shellfish Research. 29:1.pp.83-90.

Abstract

A field experiment was conducted to compare the performance of different hard clam (*Mercenaria mercenaria*) strains in local clamming waters of New York State. Experimental clams included a *Mercenaria mercenaria notata* seed obtained from a Florida broodstock, and 2 New York seed strains obtained from local hatcheries, including a cultured *M. mercenaria notata* strain and a first-generation ‘‘wild-type’’ strain. Quahog parasite unknown (QPX) was acquired by the Florida clams in less than 2 months of a July deployment of grow-out cages. In contrast, QPX was not observed until the second summer in the cultured New York (*M. mercenaria notata*) strain in which clam survival was high and infection prevalence remained minimal. The New York ‘‘wild-type’’ clams displayed good growth and did not acquire QPX at all, providing evidence for the potential utilization of local wild broodstocks to enhance the resistance of cultured strains. Prior field studies comparing susceptibility of northern and southern hard clam strains observed QPX acquisition after clams had overwintered in the field, raising the question that higher susceptibility observed in southern seed clams could be a result of poor adaptation to winter water temperatures. Our results show that the southern strain acquired QPX after the clams had only been exposed to the warmest period of water temperatures for this field site (22.3°C on average), thus excluding poor acclimation to winter temperatures as the main aggravating factor. Histopathology observations offered further insights to infection dynamics, with early, light infections almost exclusively localized in mantle and gill tissues, clearly supporting the theory that these organs (predominately the mantle) are sites of acquisition for QPX infections.

2.1. Introduction

Some of the earliest experiments concerning QPX disease were field trials that indicated disease susceptibly was related to the hard clam type (cultured strain stock source) used in grow out culture leases (Ford et al. 2002, Ragone Calvo et al. 2007). Ragone Calvo et al. (2007) found that environmental differences can have a marked effect on clam growth, even over very short distances, and particular stocks responded better to certain very local conditions. They proposed that genotype-environment interactions may be involved with variation in QPX susceptibility and to investigate this possibility, they advocated for more “common garden experiments conducted in multiple environments using more than one stock from a region” (Ragone Calvo et al. 2007).

A laboratory controlled study that challenged groups of hard clams from different geographic sources with various QPX isolates confirmed a gradient of resiliency observed in

clam stocks, which can be described as a latitudinal trend; highest resiliency in clams from the north, lowest in clams from the south (Dahl et al. 2008). A proposed explanation for this could arise from clam population selection processes, through historical parasite exposure, as a source of resiliency for a given clam stock which encourages consideration for local brood stock selection. Bushek and Allen (1996) observed Dermo disease resistance in the offspring of oysters that generally corresponded to the duration of historical exposure of the source populations to *P. marinus*. 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).

This study investigates clam host genotype and environment interactions and their influences on QPX disease development. Field sites in different areas of coastal NY waters provide variation in environmental conditions. The performance of two different types of NY seed clams are compared. One batch is from a locally raised hatchery selected broodstock, typical of what is used for municipal shellfish enhancement operations as well as representative of what private operations utilize. The other clam type was spawned from wild collected local broodstock. Prior QPX field trials have not been conducted in NY waters and have not included any clam strains sourced from NY.

Infection pressure of this protistan parasite in NY waters outside of Raritan Bay is unclear. The Peconic estuary has been of particular concern because of its historical reception of clams from the transplant program for bacterial cleansing and because the estuary is actively used for bivalve aquaculture activities. The Suffolk County Shellfish Aquaculture Leasing Program was developed to increase private investment in shellfish aquaculture businesses in the Peconic and Gardiners Bays (Suffolk County Government 2014).

An additional seed type from FL, known to be susceptible to QPX (Dahl et al. 2008) was applied at study sites. This was intended to aid performance assessment of the local NY seed clams and QPX infection pressure of clamming areas; e.g., if no infection was observed, it may not have been discernible that there is a lack of QPX activity in the field site or that both NY strains are highly resistance. There is a lack of understanding regarding QPX disease dynamics in the field and the minimum time needed for disease development in situ remains imprecise. The FL seed clams were also used to augment the potential to gain insight on acquisition of infection and subsequent dynamics.

Hypothesis- The local (NY) clam seed types will incur less QPX infection than the imported (FL) clam seed. The first generation clam seed from wild brood stock will grow slower than the two hatchery selected strains, but as a tradeoff, may be more resistant to QPX infection. The greatest disease pressure of all the field sites will be in Raritan Bay.

Objectives- Compare performance and disease susceptibility of the different local clam seed strains across varied environments within NY waters. Assess infection pressure of important NY growing areas. Observe infection acquisition and early stage infection development.

2.2. Methodology

2.2.1. *M.mercenaria*

Two different hard clam strains were obtained from municipal shellfish hatcheries located on Long Island, New York. One *notata* strain used for municipal clam enhancement activities in NY state (NY notata) for several generations, and a first generation strain from a wild collected NY broodstock (NY white). A third strain ,hatchery raised *notata* variety (FL notata), was obtained from a commercial source in Florida. All seed clams were within 10–12 mo of age and within a size range of 12–17 mm. A minimum of 100 clams per strain was screened for QPX and general pathologies prior to deployment using standard histological techniques.

2.2.2. Field deployments and sampling

Clams were deployed at 4 different study sites (Fig. 13). Three sites were located in different clamming areas of the Peconic estuary. Birch Creek (depth, 1.5 m) has had a positive QPX sample in past monitoring. Northwest Harbor (depth, 4 m) contains publicly accessible bottom waters that are considered important to commercial diggers. Southold Bay (depth, 8 m) has not had any indication of QPX infected clams but the area used to receive Raritan Bay clams for cleansing and is currently an active area for shellfish culture. The final site was located in the Raritan Bay transplant fishery source grounds, within an area that has consistently tested positive for QPX since summer 2002.

Figure 13. Map showing experimental field sites (stars) in Raritan Bay and the Peconic estuary (enlargement).

At each site, 500 clams of a single strain were allotted to individual ADPI (ADPI Enterprises Inc. Philadelphia, PA) grow-out cages (OBC-1) 3/16-inch mesh (5 replicate cages for each strain per site). All 3 strains were deployed in Raritan Bay and Birch Creek. Because of a limited available quantity, only 3 replicates of the NY white seed were deployed in Birch Creek,

and none were deployed in Southold Bay or Northwest Harbor. To ensure the cages rested on the bottom, each side of the long axis was weighted with 1 segment of rebar. Individual cages were attached to a short line (leader) fastened to a longer line in a row (lines were fashioned from commercial lobster ‘‘pot warp’’). The ends of each row were weighted down with cinder blocks or mushroom anchors to keep the deployment gear stationary. Global positioning satellite waypoints were recorded during placement of each row of cages.

Deployment of clams and field gear occurred in the beginning of July. Sampling was conducted 3 times throughout the duration of the deployment and for a fourth time with the final retrieval (October 2005). The first 2 samples were planned for 2 month intervals from deployment, starting in late August and again in October 2004; the third sample occurred the next summer after overwintering (June 2005). Unfortunately, the cages deployed in Birch Creek and Southold Bay were lost during the winter and thus were not sampled in 2005.

During the first 3 sampling trips, each individual cage retrieved was subsampled for mortality counts, and 12 clams were taken for standard histological processing. During the first sample and again the following summer, a sample of 10 clams from each cage was processed for condition indexing (see below). Clam shell lengths were measured during condition indexing or from samples taken for histology. Daily growth rates were calculated as millimeters of shell growth per day for intervals between samplings. Underwater temperature data loggers (Onset Stowaway Tidbit, Onset Computer Corporation; Bourne, MA) were attached to grow out cages and maintained at each site through the duration of deployment. At final retrieval of clam cages, all remaining clams were counted to evaluate overall mortality, all remaining live clams were measured for length, and 30 clams from each cage were processed for histological analyses.

2.2.3. Condition Index

The condition index (CI) was calculated according to the suggested standard method (Eq. 6) in Crosby and Gale 1990: CI=dry soft tissue weight (in grams) X 1,000/internal shell cavity capacity (in grams). This formula is a modification by Hawkins et al. 1987 of the gravimetric techniques of Lawrence and Scott 1982. Clams were placed in an oven (60°C) and weighed over time until the dry weight had stabilized, and then placed in a benchtop muffle furnace (450°C) for 5 hours. This process allows for determination of ash-free dry weight, which is used for dry weight in the CI formula.

2.2.4. Histopathology: Same process described previously (Chapter 1).

2.2.5. Statistical Analysis

QPX prevalence data and total mortality counts were analyzed for significant differences according to clam strain. Counts of QPX-infected and uninfected individuals from each histological diagnosis sample or the final total counts of dead and live clams will be arranged in a 2-way, row-by-column contingency table and tested for independence of variables by means of the G-test with Gabriel’s simultaneous test procedure through BIOMstat (referenced previously). The first variable is classes of clam strain. The second variable is either infection (with one class for infected and one class for uninfected) or viability (with one class for live and one class for dead). Counts were pooled from replicate samples.

CI and growth rate data were analyzed using SigmaStat for Windows (version 3.10; Systat Software, Inc., Chicago, IL). CIs were tested for significant differences of mean values for a particular clam strain across different field sites. Some of the CI data sets failed normality assumptions required for parametric testing of differences, and therefore the Kruskal-Wallis analysis of variance (ANOVA) on ranks test was conducted on pooled CI data; Mann-Whitney rank sum test used if only 2 samples. Daily growth rates were tested for significant differences of mean values for a particular field site according to clam strain as well as for a clam strain across different field sites using 1-way ANOVAs. Significant ANOVAs were followed by multiple comparison procedures: Holm-Sidak for parametric and Dunn's method for nonparametric. All results were considered significant at values of $P < 0.05$. All statistical testing procedures follow methods described by Sokal and Rohlf 1995.

2.3. Results

2.3.1. Shell length and growth rates

FL and NY *notata* clams were of a similar average size at the beginning of the experiment. Both strains maintained similar increases in shell length throughout most of the deployment period in Raritan Bay (Fig. 14A), until the last increment measured between June 2005 and October 2005 which was more than 50% greater for NY (7.7 mm vs 5 mm increment for FL). NY white clams were smaller at the beginning of the experiment than the other 2 strains, but had the greatest growth rate for each interval in Raritan Bay (Fig. 15A) and nearly doubled in length by the final sampling (Fig. 14A). Not surprisingly, all 3 clam strains had relatively poor growth rates during the winter in Raritan Bay (Fig. 15A), and most of the growth occurred during the first and second summers. During the first summer, the growth rate was maximal in NY white clams, followed by FL *notata* and finally the NY *notata*. During the second summer in Raritan Bay, the growth rate of NY white clams remained highest, but the FL *notata* lagged behind the NY *notata*. Growth rates calculated for the entire deployment period were significantly different across the clam strains in Raritan Bay (ANOVA, $P = 0.0011$). Pairwise comparisons showed that NY white clams grew significantly faster than FL *notata* clams, whereas growth rates for NY *notata* was intermediary and not significantly different from the other 2 strains (Fig. 15A).

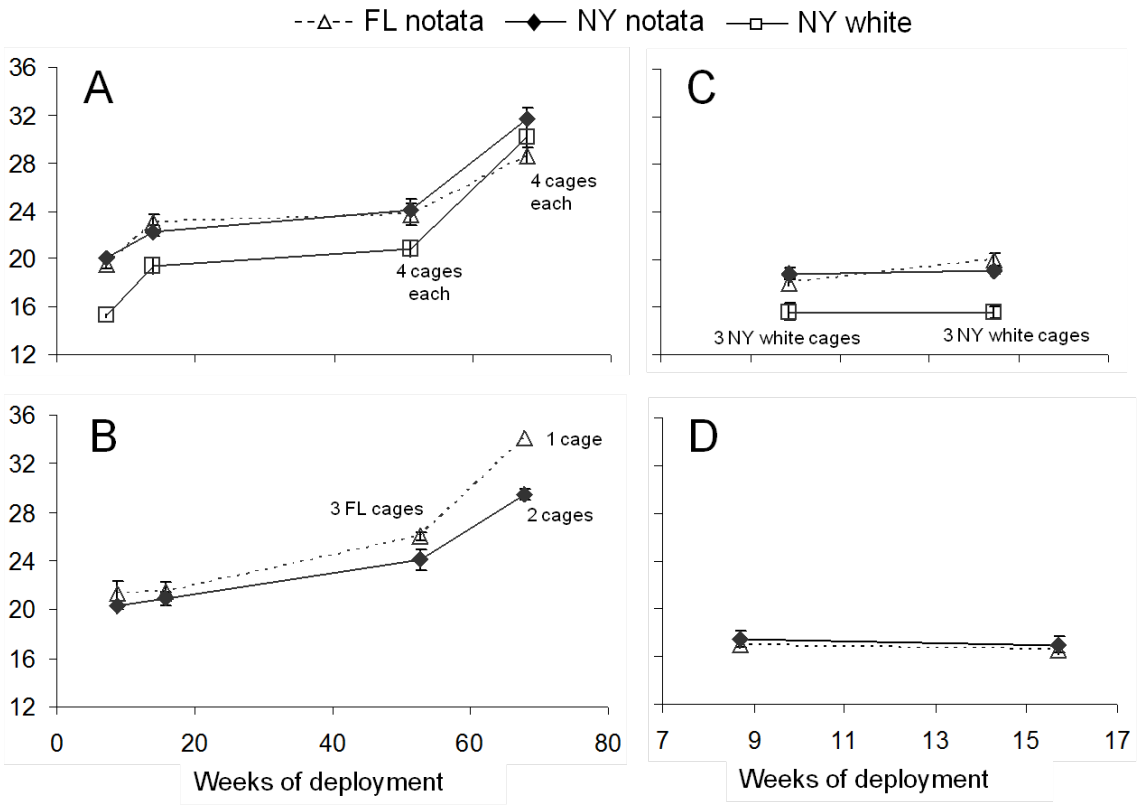

Figure 14. Shell lengths (mean ± standard error) of clams deployed in Raritan Bay (A), Northwest Harbor (B), Birch Creek (C), and Southold Bay (D). Clams were sampled (10–12 clams per cage, 5 cages per strain unless noted otherwise) in August 2004 and October 2004 (all 4 sites), and June 2005 and October 2005 (A, B). Final samples in (A) and (B) represent a minimum of 85 clams per strain.

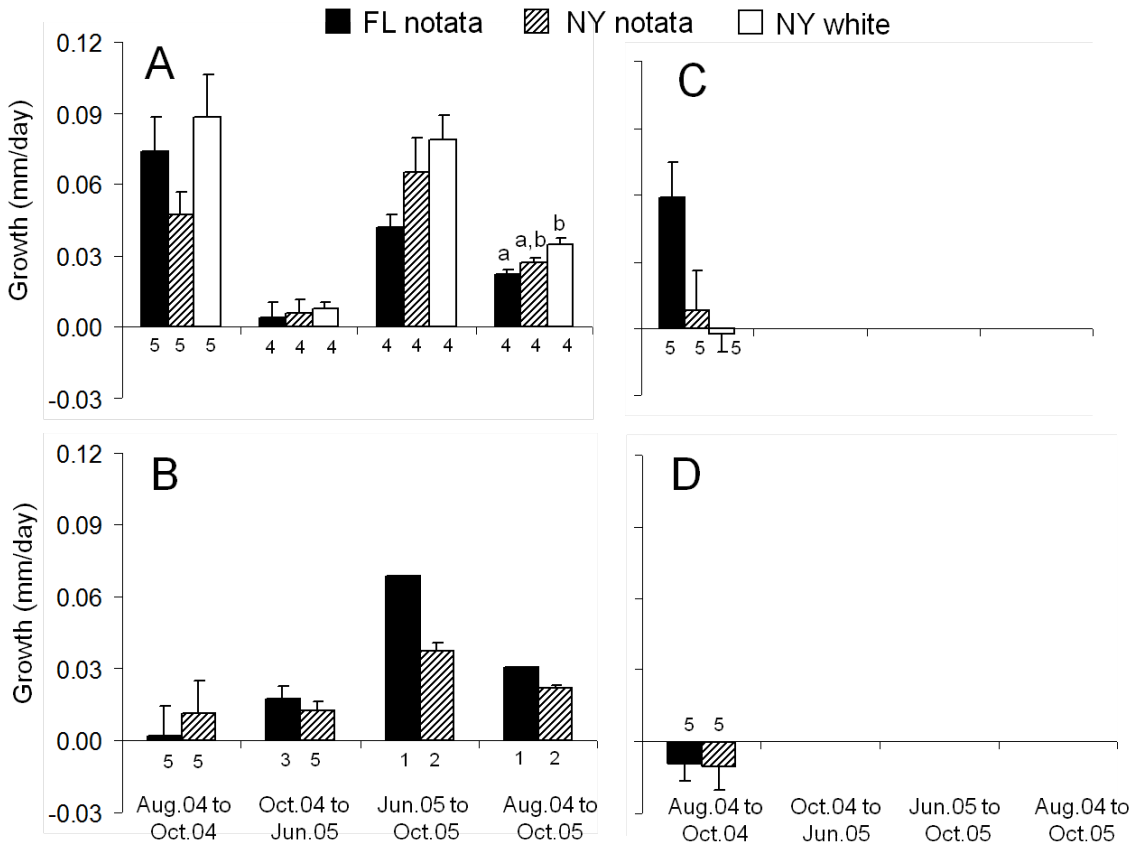

Figure 15. Growth rates (mean ± standard error) of clams deployed in Raritan Bay (A), Northwest Harbor (B), Birch Creek (C), and Southold Bay (D). The number of replicate cages is given along the x-axis, 10–12 clams per cage. Different lowercase letters indicate significantly different growth rates among different strains (Holm-Sidak post hoc test, $P < 0.05$).

In Northwest Harbor, FL *notata* and NY *notata* clams displayed similar increases in shell length during the first summer (Fig. 14B). After the October 2004 sampling, FL notata began to increase at a greater rate (Fig. 15B), resulting in a final average length that is 28% greater than the final average length of NY *notata* clams. Unfortunately statistical testing of growth rates were prohibited due to significant mortalities in Northwest Harbor leaving only 1 cage of FL *notata* and 2 cages of NY *notata* with live clams remaining. Clams deployed in Birch Creek and Southold Bay were lost during the winter (2004-05), thus data presented was collected during the first 2 samplings (August and October, 2004). In Birch Creek, only a small growth increment was noted among the FL *notata* clams (Fig. 14C), and growth rates of the different clam strains were marginally significant (ANOVA, $P = 0.049$; Fig. 15C), although none of the pairwise comparisons were significant. In Southold Bay, neither the FL *notata* nor the NY *notata* clams showed any shell accretion (Fig. 14D). Calculated rates of growth were slightly negative and not significantly different (Fig. 15D).

Growth rates during the first interval (August to October, 2004) were compared for the same clam strain across different field sites. Growth rates for FL *notata* were significantly higher in Raritan Bay and Birch Creek compared with Southold Bay or Northwest Harbor (ANOVA, P

< 0.001). NY *notata* growth rate means among different sites were marginally significant (P = 0.047), with only the values measured in Raritan Bay being significantly higher than those obtained in clams deployed in Southold Bay. Growth rates of NY white clams in Raritan Bay were significantly higher than those in Birch Creek (P < 0.01). Two-way ANOVAs (Sokal & Rohlf 1995) were also conducted to examine for significant interactions between sites and clam strains. Growth rates measured during the first interval were compared across all 4 sites for FL *notata* and NY *notata*, and all 3 clam strains for Raritan Bay and Birch Creek. No significant interactions resulted from either test.

2.3.2. Condition Index

CIs of different clam strains displayed a significant spatial pattern (Table 1). Seven weeks after deployment (August 2004), CIs obtained in Southold Bay were significantly lower than those obtained in Northwest Harbor (NY *notata*), and Raritan Bay and Birch Creek (FL *notata* and NY *notata*). In Raritan Bay, CIs displayed a significant increase (P < 0.001) over time for each clam strain (13% for NY white, 16% for FL *notata*, and 29% for NY *notata* clams). The opposite trend was observed in Northwest Harbor, with a significant decrease (P < 0.001) in CIs (38%) for both the FL *notata* and NY *notate* clams, which were significantly lower than those measured in Raritan Bay (Table 1). The condition of NY white clams was significantly lower in Birch Creek than in Raritan Bay.

Table 1. Condition index of clams (mean ± standard error) collected in August 2004 (all sites) and June 2005 (Raritan Bay and Northwest Harbor). The number of cages is as in Fig. 13; 10 clams/cage.

Field site-Date	Florida	NY *notata*	NY white
Raritan Bay-Aug.04	120.61 ± 2.12 [a]	124.49 ± 2.9 [a]	130.24 ± 3.28
Birch Creek-Aug.04	122.7 ± 2.73 [a]	132.19 ± 0.48 [a]	117.34 ± 0.95
Northwest Harbor-Aug.04	130.36 ± 28.32 [a,b]	135.32 ± 14.25 [a]	Rank Sum Test p < 0.001
Southold Bay-Aug.04	109.59 ± 7.41 [b]	110.19 ± 6.9 [b]	
	ANOVA on Ranks p < 0.001	ANOVA on Ranks p < 0.001	
Raritan Bay-Jun.05	137.63 ± 2.66	160.64 ± 13.74	147.57 ± 6.75
Northwest Harbor-Jun.05	80.66 ± 0.48	89.93 ± 1.0	
	Rank Sum Test p < 0.001	Rank Sum Test p < 0.001	

Different lowercase letters designate a significant difference among sites for each strain.

2.3.3. Mortality

In Raritan Bay, cumulative mortality (Table 2) measured at the end of the experiment was higher for NY white and FL *notata* clams when compared with NY *notata* clams (P < 0.001). Similarly, mortality levels were higher in Northwest Harbor for FL *notata* clams compared with NY *notata* (P < 0.001). FL *notata* endured significantly higher mortality in Northwest Harbor than in Raritan Bay (P < 0.001). Data presented for Birch Creek and Southold Bay was collected during the October 2004 sampling before the loss of the clam deployments over winter. In Birch Creek, mortality levels were highest in NY *notata*, followed by FL *notata* and finally NY white clams (P <0.01). In Southold Bay, FL *notata* clams had significantly higher mortality than NY *notata* (P < 0.001).

Table 2. Cumulative mortality (mean ± standard error) for each clam strain at each site.

Clam strain	Raritan Bay Jul04-Oct05	Northwest Harbor Jul04-Oct05	Birch Creek Jul04-Oct04	Southold Bay Jul04-Oct04
Florida	68.0 ± 4.05 [a]	78.2 ± 5.9	13.45 ± 2.83 [a,b]	20.6 ± 2.31
NY *notata*	59.05 ± 3.3 [b]	60.89 ± 10.54	18.38 ± 4.11 [a]	10.61 ± 1.46
NY white	68.9 ± 3.02 [a]	G-test p < 0.001	7.57 ± 4.14 [b]	G-test p < 0.001
	G-test p < 0.001		G-test p < 0.01	

Raritan Bay and Northwest Harbor samplings cover up to Oct. 2005, Birch Creek and Southold Bay cover up to Oct. 2004. Different lowercase letters designate a significant difference among strains within a site.

2.3.4. QPX Prevalence, Intensity and Distribution in Clam Tissue

QPX was not detected in clams sampled from any of the Peconic estuary sites (Birch Creek, Southold Bay, Northwest Harbor). Diagnosis of the histology samples taken at 7 wks (August 2004) and 14 wks (October 2004) after deployment in Raritan Bay revealed QPX only in the FL *notata* clams (1.7% and 8.3%, respectively; Fig. 16A). QPX prevalence increased substantially in the FL *notata* clam samples taken after 51 wks (June 2005) to 47.9% and remained high (51.2%) in the final sample. Except for the first sample, all of the FL *notata* samples had significantly higher QPX prevalence (G-test, $P < 0.01$) than both New York clam strains. The first infection detected among the NY *notata* clams was after 51 wks, resulting in a prevalence of 2.1%, which remained low in the final sample (2.5%). QPX infection was not observed in any of the NY white clams. Statistical analysis of the prevalence data for the final sample demonstrated significantly higher prevalence for NY *notata* clams when compared with NY white clams (G-test, $P = 0.04$).

The first QPX-positive FL *notata* clam (7 wks post deployment) displayed a light focal infection in the mantle (Fig. 16B).The positive FL *notata* clams after 14 wks displayed mostly moderate infections, with focal infections restricted to the mantle, multifocal infections included siphon tissues, and the single heavy infection additionally displayed QPX in the visceral mass. The majority of QPX infections in FL *notata* after 51 wks were light (48%) or moderate (39%), and the remaining positive clams (13%) were heavy infections. In this sample QPX was observed strictly in pallial tissues in clams that displayed focal infections (48%), often in the gills, but in the mantle and siphon tissues as well. QPX was also observed in the visceral mass in all but one of the multifocal infections (35%) and in all of the diffuse infections (17%). All heavy infections displayed diffuse or multifocal lesions. A range of infection severity, distribution, and tissue combinations was represented in the final FL *notata* sample (67 wk, October 2005). Compared with the prior (51 wks) sampling, the proportion of light infections decreased as heavy infections increased, and reached nearly 25% each. Moderate infections represented the remaining cases (51%). The proportion of focal infections in the final sample decreased nearly in half (from 48% at 51 wks to 25% at 67 wk) as the proportion of diffuse infections increased noticeably (17% to 39%). The first QPX-positive NY *notata* clam (51 wks) had a light focal infection in the siphon. In the final NY *notata* sample, 1 clam had a light focal QPX infection in the gills, 1 clam displayed a moderate focal infection at the junction between the mantle and the siphon, and another had a moderate focal infection in the foot.

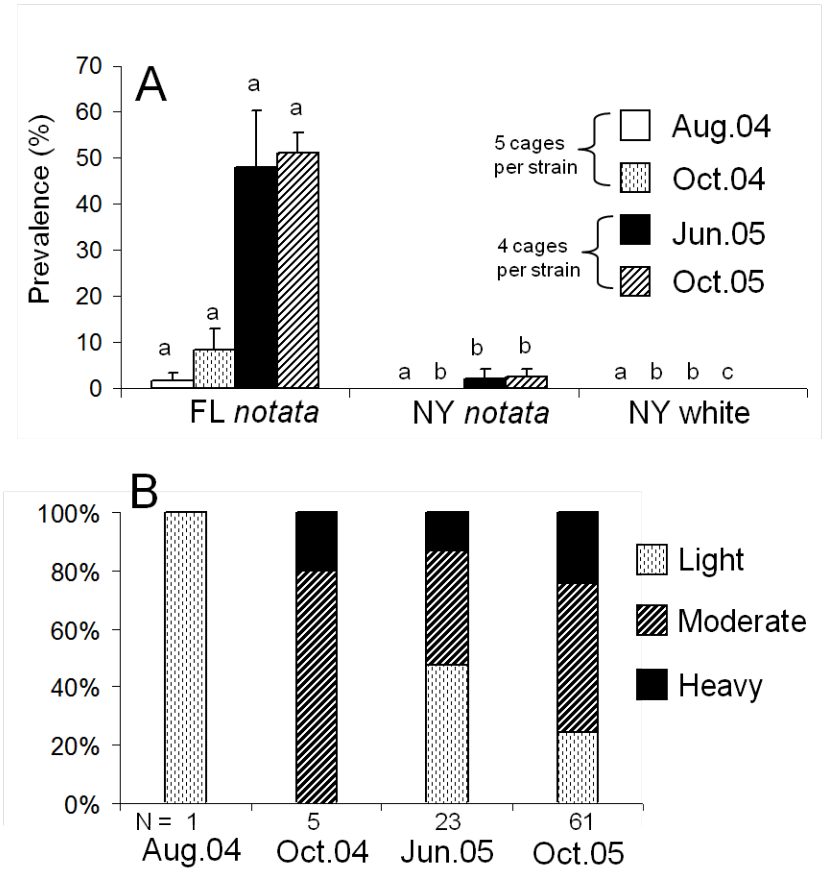

Figure 16. (A) QPX disease prevalence (mean ± standard error) in clams sampled from the Raritan Bay deployment (30 clams per cage final sample, Oct. 2005, otherwise 12 clams per cage). Different lowercase letters designate significantly different prevalence among different strains sampled on the same date (G-test, $P < 0.05$). (B) Proportion of infected FL *notata* clams with different QPX disease intensities (the number of infected clams is given along the x-axis).

2.3.5. Temperature Data

Temperature data were available for all sites between July 2004 and October 2004 (Fig. 17), and throughout the entire experiment in Raritan Bay and Northwest Harbor only. Temperature range was similar between Southold Bay (15.1 to 24.6°C) and Raritan Bay (15.7 to 24.8°C). Northwest Harbor had an approximately 2°C greater range (13.7 to 24.8°C), whereas Birch Creek displayed an approximately 5°C greater range (12.8 to 27.3°C). Compared with an average of the 3 Peconic site temperatures, Raritan Bay remained 0.5°C cooler from mid July to mid September and 1°C warmer from mid-September to mid-October. In general, the Peconic estuary sites appear to fluctuate greater and more frequently than Raritan Bay; this is especially noticeable in the Birch Creek plot.

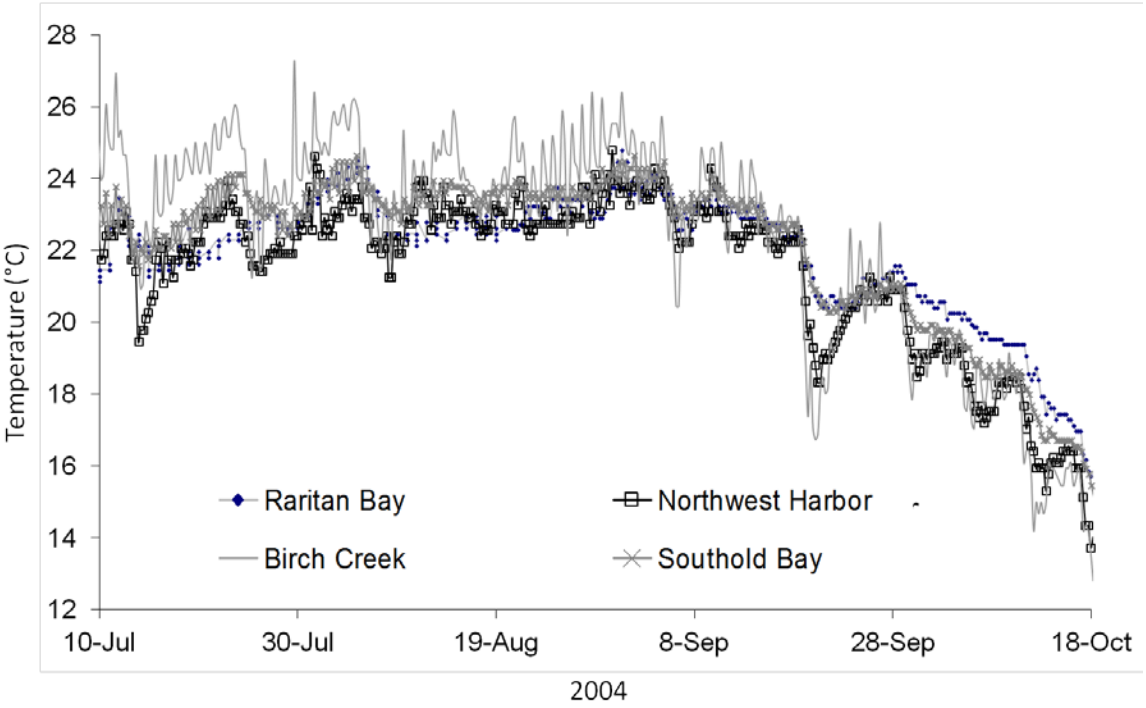

Figure 17. Water temperature recorded by data loggers deployed with clam cages. Plots are restricted to show only the period of time that data are available from all four field sites.

The temperature range through the entire deployment was almost 3°C greater in Northwest Harbor (range, -1.5 to 26.9°C) than in Raritan Bay (range, -0.2 to 25.5°C). A divergent colder trend is observed in Northwest Harbor when compared with Raritan Bay in the fall and winter (average difference of 1.4°C from mid-September 2004 to mid-March 2005). In spring and summer, there was only a slightly warmer trend in Northwest Harbor (average difference of 0.2°C from mid-March 2004 to mid-September 2005).

2.4. Disscussion

This study aimed to compare the performance of locally cultured (NY *notata*) and wild-type (NY white) hard clam strains across New York waters and evaluate QPX disease pressure in clamming areas in the Peconic estuary. The Florida hatchery-raised clam strain was deployed to increase potential sensitivity of QPX infection pressure, based on previously reported susceptibility (Dahl et al. 2008). QPX disease is often reported in cultured clams, which has created concern that culture practices may increase the risk of infection. Clam stocking densities have been a suspected risk factor because they are often much higher than found in natural populations (Ford et al. 2002, Ragone Calvo & Burreson 2002). The stocking density of clam seed allotted to the grow-out cages in this study (1,000/m^2) was higher than previous QPX studies, but still well within the range of planting densities applied within aquaculture practices (550-1,650/m^2) for clams in that size range (12-17 mm) (Castagna 2001). This application was intended to monitor acquisition of infection and disease dynamics, high mortalities were to be expected, and ample individuals were used to help ensure desired samples could be obtained. Use of juvenile seed to assess QPX infection pressure was validated in the results from the

Raritan Bay deployment. QPX was not detected in histology samples from the Peconic estuary. This is a favorable outcome, providing consolation for concerns of a potential QPX epizootic in the Peconic estuary, especially when considering a historical role of receiving waters for the depuration of Raritan Bay hard clams.

Use of a highly susceptible hard clam seed strain allowed for rapid disease development, facilitating the study of *in situ* dynamics. The first histology sample of FL *notata* clams was taken from Raritan Bay after 7 weeks of deployment and a QPX-positive clam was discovered, representing the quickest acquisition of QPX reported *in situ*. Previous studies described QPX disease in hard clams that have been in the field for 9 months or longer (Ford et al. 1997, Ragone Calvo et al. 1998, Smolowitz et al. 1998, Ragone Calvo et al. 2007). Earlier field studies have also reported clam strains from southern hatchery origins displaying greater susceptibility to QPX disease than northern clam strains after clams had overwintered in the field (Ford et al. 2002, Ragone Calvo & Burreson 2002, Ragone Calvo et al. 2007). Researchers suggested this disparity could be a consequence of the southern strains being poorly adapted to winter water temperatures in the northern field sites (Ragone Calvo & Burreson 2002, Ragone Calvo et al. 2007). In our study, the Raritan Bay deployment was initiated in July, the first sample was in August, the second was at the beginning of October, and both samples tested positive for QPX despite the fact that those periods cover the warmest water temperatures of the year in Raritan Bay (22.3°C average). This new information strongly supports an alternative hypothesis of genetically based susceptibility in southern (e.g., Florida) strains compared with northern stocks (e.g., New York) is in agreement with laboratory transmission experiments reported previously (Dahl et al. 2008).

High frequency of QPX infection prevalence in mantle and gill tissues in previous reports (Ragone Calvo et al. 1998, Smolowitz et al. 1998, MacCallum & McGladdery 2000), as well as in their own study, led Ford et al. (2002) to suggest that those tissues ‘‘are the portals of entry for QPX’’. Early-stage infections throughout this study were consistently found in pallial organs (mantle and gills), providing further evidence that these organs represent sites of infection initiation. In the field study of Ragone Calvo et al. (2007), mild infections were mostly localized in mantle tissue and more severe infections tended to be multifocal. This study had remarkably similar results, because the light focal infections were consistently found in a pallial organ, whereas heavier and more diffuse infections observed QPX in the visceral mass as well.

Seasonality of infection has not been clearly designated from prior field observations. QPX-related mortalities were highest in late summer in Massachusetts (Smolowitz et al. 1998). A seasonal survey conducted in Atlantic Canadian provinces found the highest prevalence in August samples (MacCallum & McGladdery 2000). In the current study, QPX acquisition and initiation of infection seems to occur during early summer and disease severity increases through autumn. For instance, the light infection observed in FL *notata* clams in August 2004 appears to have progressed into moderate and heavy infections that October. A similar scenario is observed for the NY *notata* clams starting during the second summer (June 2005) of deployment. Light infections reappeared in the FL *notata* clams during that second summer (June 2005), likely representing another round of QPX acquisition. The final FL *notata* clam samples taken that autumn (October 2005) also revealed an increased percentage of heavy infections.

Growth rate and condition index results demonstrated a clear influence of field site on the performance of each clam strain. A generalized influence appears unilateral across the clam strains and there were no significant interaction effects as interpreted from the results of the 2-way ANOVAs. Clam performance values observed for growth and condition where good in Raritan Bay. This is not surprising when considering that this area has maintained the most productive hard clam population in New York State. In contrast, clam performance in Southold Bay was very poor. The 'negative' growth rates measured in Southold Bay (Fig. 14D) may be a result of sampling error or probably due to the selective mortality of larger clams. In June 2005, both clam strains deployed in Northwest Harbor displayed a significant decrease in their CI compared with the prior year. The causes of this reduction are uncertain, but it may be worth noting that heavy mortalities in both clam strains were seen during June and October of the 2005 samplings.

In general, the FL *notata* clams displayed some good instances of growth and relatively good condition, yet had high mortalities in most sites and an overwhelming difference concerning QPX disease prevalence. These findings are congruous with other field studies (Ford et al. 2002, Kraeuter et al. 2011, Ragone Calvo et al. 2007) regarding significantly higher mortality and QPX prevalence for their southern clam strains (e.g., South Carolina and Florida) versus northern strains tested (e.g., New Jersey and Massachusetts). Survival of NY *notata* clams was high in most field sites with relatively good growth rates including the greatest CI increase in Raritan Bay. NY white clams had the lowest mortality in Birch Creek yet poor growth and condition there, whereas in Raritan Bay they had significant growth and good condition but higher mortality than the NY *notata*. It may be relevant to note that even though all efforts were made to obtain clams of each strain that were the same size, the NY white clams tended to be closer to the lower size selection limit of 12 mm.

Despite the small difference observed in terms of disease prevalence among both New York stocks, the fact is that the NY *notata* strain displayed less resistance to QPX in Raritan Bay than their ''wild'' offspring counterpart. Differences in resistance to infection between the 2 stocks could be a collateral effect relating to differences among selection processes. The NY white clams are first-generation wild-type and not subject to the selection processes of the cultured *M. mercenaria notata*, and culturists may focus on favoring specific traits that subsequently results in an unintended physiological trade-off in other traits. For instance, the NY *notata* seed has been used for aquaculture for several generations and is generally assumed to be characterized by fast growth, although in Raritan Bay, the NY white clams actually had the best growth rates. Something about the environment of the Raritan Bay site could have reduced the growth potential of the other strains but did not inhibit the NY white clams in the same regard. QPX disease likely decreased growth performance of more susceptible clams. The striking decrease in growth performance of FL *notata* measured during the second summer (Fig. 13A &14A), when compared with the first summer, coincided with, and probably resulted from, an increased disease burden. Hard clams diagnosed from the field in previous studies found heavily infected clams to be smaller and showed reduced growth when compared with clams that had little to no infection (Smolowitz et al. 1998, Ford et al. 2002). When considering the 2 New York strains tested, a better genotype environment match could be the case, even though no statistical ''interaction'' was found (2-way ANOVAs). Ragone Calvo et al. (2007) found that ''particular stocks responded better to certain very local conditions''. The fact is that the NY white clams

showed great potential for field applications, particularly with regard to QPX disease resistance, but also in potential for growth proven in Raritan Bay.

Observations of *in situ* dynamics from this field trial reinforce the concept that infections are initiated in the pallial organs (Ford et al. 1997 and 2002, Smolowitz et al. 1998 and 2001). A few weeks were sufficient for susceptible, naive seed clams to acquire infections, which became severe less than 4 months after deployment. Findings also demonstrate that differences in QPX susceptibility based on hard clam genotype, as observed in previous field trials (Ford et al. 2002, Ragone Calvo & Burreson 2002, Ragone Calvo et al. 2007), is not primarily contingent on winter temperature stress and likely results from a lack of selection for resistance. These findings corroborate results of QPX susceptibilities associated with clam genotype from our experimental transmission trials (Dahl et al. 2008) that were conducted in the laboratory under consistent warm water temperatures (20-21°C). Performance of the NY white clams illustrates the potential utilization of wild broodstocks in enhancing resistance of cultured strains.

Chapter 3. Experimental transplanting of clams to areas with favorable environmental conditions to assess disease mitigation within a QPX enzootic estuary

Results from this chapter were published in:

Dahl, S. F. and Allam, B. 2015. Hard clam relocation as a potential strategy for QPX disease mitigation within an enzootic estuary. Aquaculture Research. doi: 10.1111/are.12793

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*, from persistently infected areas to nearby sites with prevailing environmental conditions suggested to deter infection and favor 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 heavily infected locales. An additional treatment retained clams above the sediment, since sediments are suspected to represent a QPX reservoir. At the end of 4-months, 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 that subjected the clams the most extreme summer temperatures and salinities of the study sites.

3.1. Introduction

Previous studies of QPX disease revealed substantial evidence of hard clam healing in naturally infected clams collected from the field, after they were maintained in the laboratory for several months (Dahl and Allam, 2007). Those trials may have provided favorable environmental conditions for the clams which allowed them to successfully fight infections; in particular temperature was maintained at 21°C (see Chapter 1). 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. Oyster habitats subject to fresh water inputs that lower salinity have been shown to reduce Dermo infection and oyster mortality risk (La Peyre et al 2003). In vitro studies showed optimal QPX growth at high salinity (34ppt) and reduced grow at temperatures above 23°C (Perrigault et al., 2010). QPX disease challenges (Chapter 1) have shown greater mortality at high salinity (30ppt) vs low salinity (17ppt) and significantly less disease with greater healing at temperatures of 21°C and above

Another factor considered to affect QPX disease dynamics in the field is clam density (Ford et al., 2002; Ragone-Calvo and Burreson, 2002; Walton et al., 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 et al., 1979; Hadley and Manzi, 1984). Trends of higher QPX prevalence have been observed at higher hard clam planting densities (Ford et al. 2002, Walton et al. 2008). Areas in Raritan Bay have very high densities of hard clams (several

personal observations of >100/m²) and have suffered some of the most severe infections recorded in a wild set population.

The following study was developed in order to investigate the potential to utilize environmental conditions 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 the highest summer temperatures in an attempt to promote disease remission. Additional efforts were made to test strategies that can lower infection risk by reducing clam density and by removing clams from the sediment. Sediments from Raritan Bay (Liu et al., 2009) and other enzootic areas (Gast et al., 2008) have tested positive for QPX and may represent an environmental reservoir.

Hypothesis- Relocation to habitats with 'favorable' conditions can assist clams in abating QPX infection, by impairing parasite function/physiology and/or aiding clam physiological functioning, and subsequently reducing disease burden.

Objective- Evaluate potential to utilize gradients in environmental conditions to aid QPX disease remission and hard clam healing, mitigating infection severity and reducing hard clam losses to mortality.

3.2. Methodology

3.2.1. 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 (67mm 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. 18) 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: 0.91m length x 0.51m width = 0.46m^2) for deployment that same day. Clam cages were randomly assigned to 3 different field sites (Fig. 18) that included near shore habitats that were shallower and had more direct influence from upland fresh water sources; a harbor 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/m²=25 clams/cage) to 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/m²=12 clams/cage). The off-bottom treatment (~1ft 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 meters.

The other 2 sites were located along the southern shore of Staten Island, NY. Five cages off-bottom (50 clams/m²=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/m²; personal observation) and yet disease prevalence is extremely low (<0.1%). Cages were deployed in the Harbor at a depth of 3m. The Creek site is characterized by a mean tidal range of 1.5m 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 YSI (Yellow Springs Instruments) probe during regular site visits throughout the 4-month deployment (~every 3 weeks).

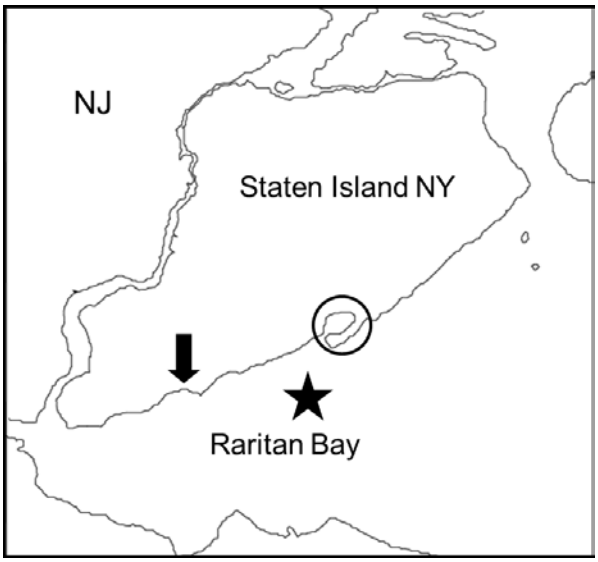

Figure 18. Study sites in Raritan Bay and along the south shore of Staten Island (NY), marked as follows; star represents the clam collection area and the Bay deployment site, Great Kills Harbor is circled and Lemon Creek is pointed out by the solid arrow.

3.2.2. Clam sampling and processing

A sample of thirty 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 to standard histological methods. Mantle and siphon 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) x 1,000/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 and Rohlf 1995)

3.3. Results

Study sites were visited at the end of the four 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 2 sites (8 clams per replicate). Unfortunately, we were not able to locate all replicate cages at the open water Bay site despite an extensive search 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 was pooled within all treatment groups for analysis; N= 24 Control, 24 Off Bottom, 23 Low Density, 39 Harbor, 40 Creek.

3.3.1. 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. 19) 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.

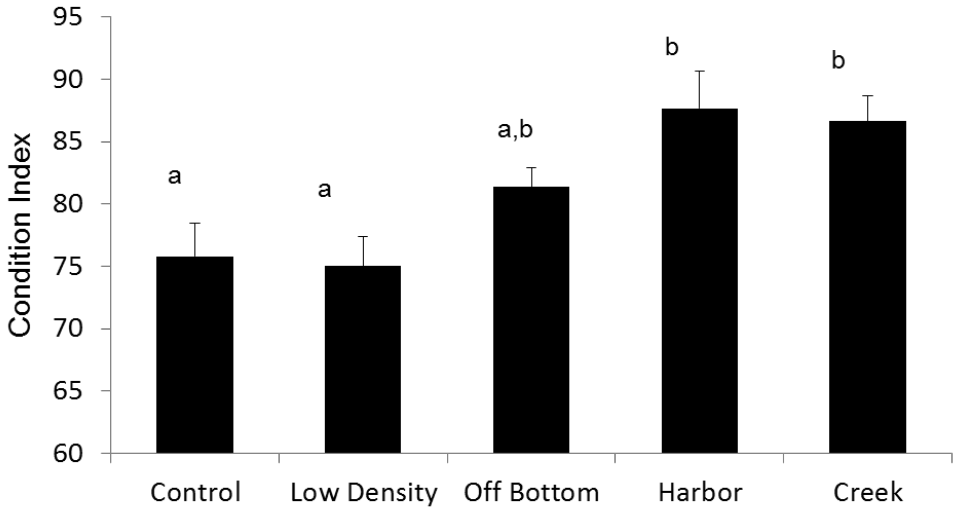

Figure 19. Mean condition index (+SEM) of clams from each treatment at the end of experiment ($P < 0.001$, ANOVA on ranks). Treatments that were not significantly different during multiple comparison procedures have the same lower case letter (a or b).

3.3.2. QPX prevalence and intensity

The initial sample (T-0) taken from the clam collection area revealed 23.3% QPX prevalence. After four 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. 20). 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 wet clam tissue averaged for positive clams in each treatment (Fig. 20), was greatest for the Bay control group (28,040 QPX cell/g wet tissue weight). The next highest intensity was for the Harbor group (6,790) followed by the off bottom Bay treatment (1,620). The only positive clam in the low density treatment from the Bay had a QPX cell count of 590/g clam tissue.

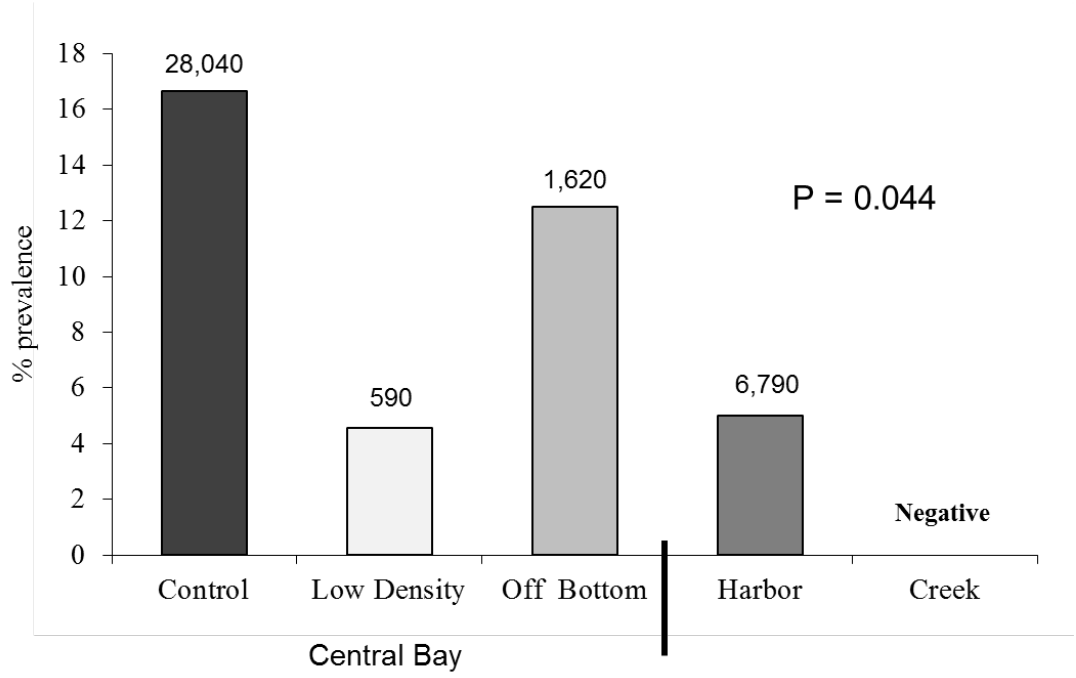

Figure 20. QPX prevalence and intensity in clams retrieved at the end of experiment (P=0.044; G-test). The value above each bar is the average QPX cell count (per g wet clam tissue) for the positive clams in that treatment.

3.3.3. 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. 21A); the lowest value (2.8 mg/l) was measured at the Bay site. The highest salinities throughout the experiment were measured at the Bay site with levels maintained above 23ppt and a maximum of 27.3ppt measured in October (Fig. 21B). Salinity in the Harbor was lower than the Bay site, with a minimum of 20.5ppt in July. The Creek had generally lower salinity levels with a minimum of 19ppt in July. Temperatures diverged and rose more steeply at the inshore locations as June progressed (Fig. 21C). 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. 22), 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.

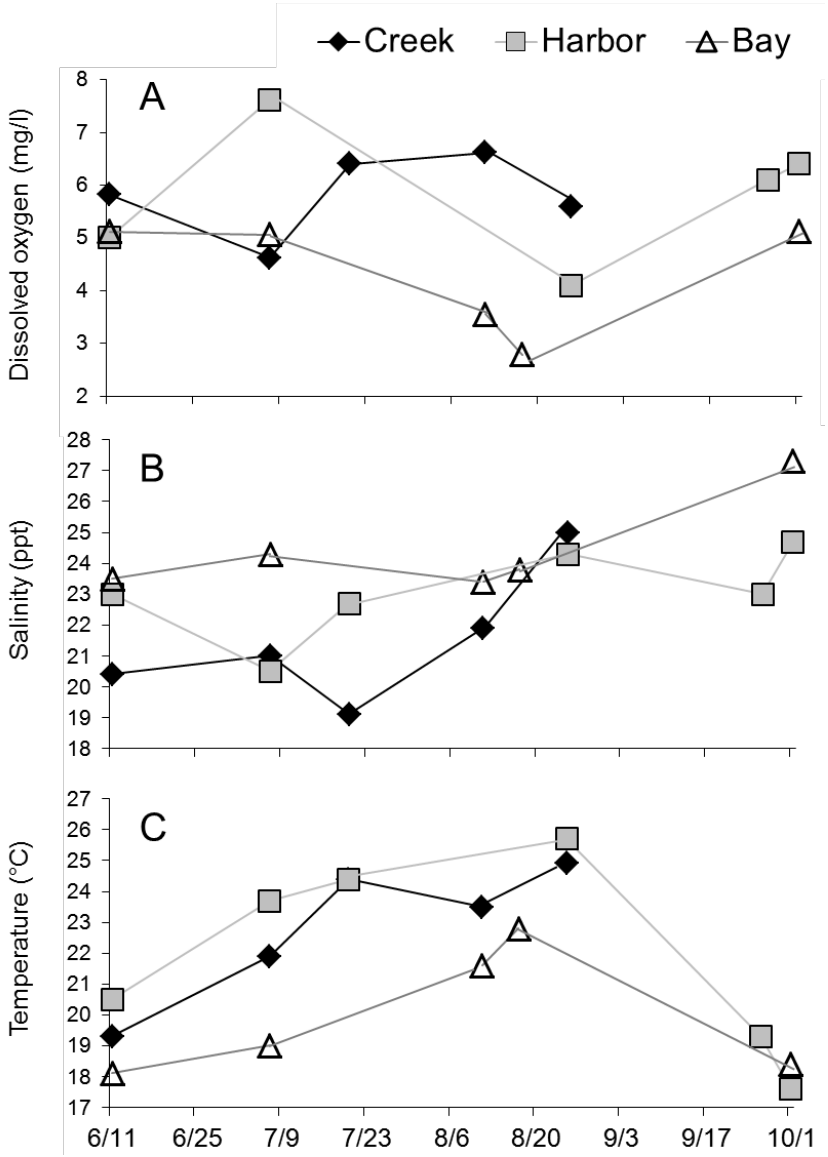

Figure 21. Dissolved oxygen (A), salinity (B), and temperature (C) measured at the study sites.

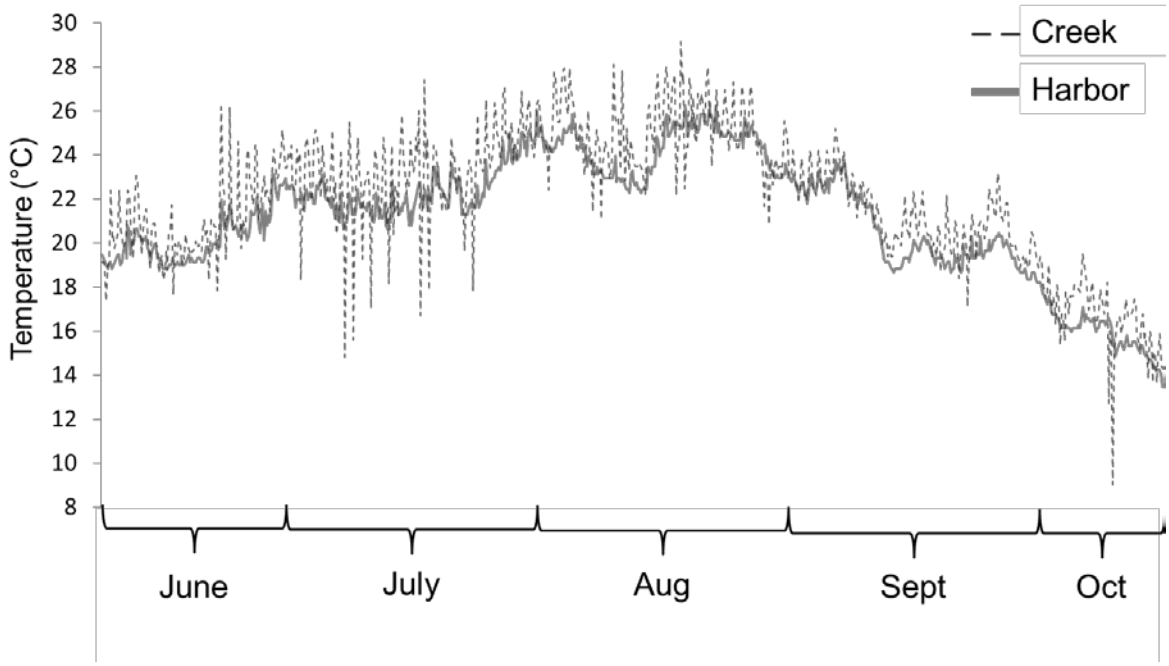

Figure 22. Temperature logs (4 hr intervals) from the tidbits deployed at the Creek and Harbor sites during the study period.

3.4. 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 of 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 et al. (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 to 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 45cm 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 this 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 and 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 to 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 of 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 ave.) 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 to the central open bay.

Great Kills Harbor had lower salinities than the Bay site during early summer and early autumn (Fig. 20B). 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 to 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 1,000 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. 21) 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 to 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 5mg/l (Hamwi 1969). DO has been observed to go below 5mg/l in hard clamming areas of Raritan Bay during the summer (NYS DEC unpublished) and it did during this study (Fig. A). 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 et al. 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 et al. 1998). Production of reactive oxygen species (ROS) is an immune defense reaction that has been demonstrated in *M. mercenaria* (Bugge et al. 2007) and oysters had a substantial reduction in ROS production under hypoxic conditions (Boyd and Burnett 1999). Lack of oxygen has been reported to impact the immune function of another Venerid clam, *Chamelea gallina* (Matozzo et al. 2005). Low oxygen stress could also have been reduced for clams suspended in the water column as compared to 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 et al. 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.

Clams from southern (e.g., South Carolina, Florida) broodstocks have shown greater susceptibility to QPX infection compared to northern (New Jersey, New York & Massachusetts) broodstocks and yet QPX disease has never been detected in clams south of Virginia (Dahl et al., 2008; Ford et al., 2002; Kraeuter et al. 2011, Ragone Calvo et al. 2007). 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.

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. 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 and Mann

1991). Strategic oyster transplanting has been integral to mitigating infectious disease losses in Delaware Bay (Powell et al. 1997).

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. 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.

Summary and conclusions

Thraustochytrids are common in marine and estuarine habitats (Raghukumar 2002), and typically nonpathogenic, although there are a few members of this group that have been associated with disease in wild and cultured mollusks (Polglase 1980, Mclean and Porter 1982, Bower 1987). Infections by the thraustochytrid known as Quahog Parasite Unknown (QPX) have been found in otherwise healthy *M. mercenaria* stocks when looked for across clamming grounds, this was true for Atlantic Canada, for Massachusetts and Virginia (USA), and is also true for coastal NY. QPX has been detected in embayments around Long Island at generally low levels without causing any noticeable disease or mortality problems: on the north shore, out east in the Peconic estuary, in the south shore estuary system and in Jamaica Bay to the west (DEC unpublished pathology reports).

One factor that has been shown to modulate the severity of QPX disease problems is susceptibility of the clam stock. Often clams from more southern US locales fared worse in terms of disease and mortality when grown in enzootic northern locales (Ragone Calvo et al. 2007, Ford et al. 2002, Kraeuter et al. 2011). Poor acclimation to cooler northern climes was suspected to play a role in the disease disparity but warm laboratory controlled trials (Dahl et al. 2008) and summer *in situ* exposures (Dahl et al. 2010) support a clam stock (i.e., genetic) based deficiency in resistance. More resistant clam stocks have been sourced from northern locales (Dahl et al. 2008, 2010, Ragone Calvo et al. 2007, Ford et al. 2002, Kraeuter et al. 2011) and yet that is also where some of the worst QPX problems have been (Dove et al. 2004, Smolowitz et al. 1998). In parallel, there appears to be some regional difference in QPX isolate virulence (Dahl et al. 2008), with more pathogenic strains from the northern locales (NY, MA). Within NY there is only one bay that has had any severe problems and this confinement suggests that other factors may be important for infections to progress beyond background levels.

Clam stocking densities have been a suspected risk factor due to trends of higher QPX prevalence observed in hard clams at higher planting densities (Ford et al. 2002, Ragone Calvo and Burreson 2002, Walton et al. 2008). Clam culture densities are often much higher than found in natural populations. The population in Raritan Bay is remarkable for natural clam densities, 26-70 clams/m^2 average (NYS DEC unpublished) for areas in the central Bay which have suffered some of the most severe infections documented in wild clams (Dove et al. 2004). High host density facilitating transmission of infection makes sense in terms of individual exposure rates. The reduction of infections achieved through manipulation of host density (Chap. 3, Dahl and Allam 2015) strengthens the notion that hard clam density is an important factor in QPX disease dynamics. Persistently infected high density clam aggregations may act as QPX infection incubators. Removal of clams early enough in the season should help abate transmission and decrease collective burdens in disease hot spots.

One area of Raritan Bay contradicts the trend of QPX disease within high clam densities. Great Kills Harbor has extraordinarily high clam densities, >100/m^2 average and one observation

exceeded 400 clams/m² (NYS DEC unpublished), but disease prevalence has been extremely low; only one positive in over 1,000 processed for histology since monitoring began in 2002. These counter-intuitive observations indicate that other factors are controlling infections in that harbor.

Observations from laboratory challenges support high temperatures (21°C and above) as limiting infection development (Chap. 1, Dahl et al. 2011). Clams transplanted to sites that get considerably warmer during the summer suffered less from QPX disease than the cooler control site (Chap. 3, Dahl and Allam 2015). The most active QPX infections in Virginia were found during May and November, but not during the hottest summer months (Ragone Calvo et al. 1998). This pattern of high temperature limiting infections may be the reason QPX infections do not occur farther south than VA. Laboratory challenges have shown that it can take several months for QPX infections to manifest disease (Dahl and Allam 2007, Dahl et al. 2008). A time analysis of temperature with Dermo infections in oysters showed a lag of more than 3 months between optimal water temperature and maximal disease prevalence (Ford and Smolowitz 2007). A delay of infection response helps explain the apparent disparity of annual peak prevalence typically occurring during the summer and yet high temperatures restrict infection progress.

Field case studies have observed clams mounting a hemocyte-mediated defense reaction to QPX infection (Dove et al. 2004, Ragone Calvo et al. 1998, Smolowitz et al. 1998). Transmission trials provided evidence of old lesions and degrading QPX cells indicative of a hard clam healing process under laboratory conditions (Dahl and Allam 2007). QPX infections decreased and healing concomitantly increased after clams were transferred from cooler waters (13°C) to 21°C during laboratory temperature challenges (Chap. 1, Dahl et al. 2011). Laboratory studies of constitutive clam immune defense factors demonstrated a strong influence of temperature on the ability of clams to mount an effective immune response (Perrigault et al. 2011). Temperature is well known to influence clam physiology (Grizzle et al. 2001) and of the temperature trial treatments, 21°C appears the most optimal while 27°C is stressful, as reflected in pumping rates (Hamwi 1969) and oxygen consumption (Hibbert 1977). Mortalities suffered by control clams maintained at 27°C for more than 4 months may reflect the impact of chronic respiratory and metabolic stress. The 13°C treatment represented a suboptimal temperature as witnessed by immunodepression in control clams maintained at 13°C compared to those held at 21°C (Perrigault et al. 2011), which could result from lower metabolic rates. Reduced QPX prevalence and disease remission in clams maintained at 21°C is likely the result of an effective immune response to the presence of QPX, while the inability of QPX to establish infection in clams maintained at 27°C likely derives from the deleterious effect of high temperature on the parasite itself as this temperature was shown to be detrimental to QPX in vitro (Perrigault et al. 2010). Temperature has a dynamic impact on QPX disease, favoring disease development at lower temperatures but supporting elimination of the parasite and clam healing at higher temperatures.

There was an upsurge in disease related mortality after infected clams were moved from 13°C to 21°C, signifying that the temperature change was detrimental to a faction of the diseased clams. Clams with considerable infection burdens are less likely to handle increased metabolic demands under higher temperatures, leading to exhaustion and mortality. Increasing metabolic demands during summer have been recognized as an aggravating factor for infectious diseases in

marine mollusks (Li et al. 2009, Samain et al. 2007, Sauvage et al. 2009, Travers et al. 2008). Infection intensity at the time when temperature is accelerating the clam's physiological rates is likely a key factor in determining the outcome. The transition to increasing summer temperatures is probably beneficial to lightly infected clams that are still capable of mounting a heightened defense response. The temperature promoted response against earlier stage infections may not always be effective across the clam population as intraspecific variation in QPX resistance has been observed (Perrigault et al. 2009). When metabolic demands on clams increase at higher temperatures, mortality probably ensues for the fraction of individuals that infection has progressed too far. That fraction may vary year to year based on relative intensity across the population.

Just down the New Jersey coast, the *Haplosporidium nelsoni* (MSX) infection cycle in Delaware Bay oysters seems to have a similar dynamic with temperature. Peak MSX prevalence occurs as temperatures approach 20°C in the spring and then begins to recede as temperatures get warmer. The MSX parasite and the oyster host are inactive at temperatures <5°C and between 5°C and 20°C the parasite multiples faster, above 20°C oysters can suppress infections (Ford and Haskin 1982).

During laboratory challenges the high salinity (30psu) treatment appeared less advantageous to the clams since the natural QPX infections had 4.7 times greater relative risk of leading to mortality compared to the lower salinity (17ppt) treatment (Chap. 1, Perrigault et al 2012). In Virginia, areas with QPX were characterized by high salinities (30-34ppt) and not in areas of 15-25ppt (Ragone Calvo et al. 1998). QPX *in vitro* growth appears optimal at 34ppt and is evidently impaired under low salinity (15ppt) (Perrigault et al. 2010). Long term analysis analyses of Dermo disease (caused by the alveolate *Perkinsus marinus*) in Delaware Bay oysters also show a clear spatial relationship of increasing disease and mortality with increasing salinity (Bushek et al. 2012).

Investigation of the role and relationship of dissolved oxygen with QPX infection dynamics was not as fruitful as temperature or salinity. The dissolved oxygen treatments in the lab were relatively uneventful in terms of disease and mortality trends. The DO treatments did appear to have an impact on clam immune parameters (Wang et al. unpublished). Immunosuppression under low DO (3mg/l) may have allowed more of MA clams infections to progress to a heavy intensity. Generally, observed modifications to the immune response were temperate and transitory. Overall, *M. mercenaria* appeared very tolerant of low dissolved oxygen conditions as observed when maintained for several months at 3mg/l or even at 1mg/l.

The relocation of clams from central Raritan Bay to sites that provided higher summer temperatures and periods of lower salinity (Dahl and Allam 2015) showed great potential to utilize environmental conditions to mitigate disease risk within a QPX enzootic estuary. The applied investigations brought the research closer toward the understanding needed to guide management protocols. Transplanting strategies have been utilized in Delaware Bay to manage oyster harvests under infection pressures (Powell et al. 1997). Transfer of clam stocks sustaining relatively low QPX levels to areas within the same geographic water body (to avoid parasite spread) that become warmer and receive low salinity pulses during spring and early summer could be an effective mitigation strategy. This can be achieved in shallower portions of an

estuary that are closer to fresh water inputs. Achieving temperatures sustained above 21°C and salinities that approach 17ppt, as observed in laboratory challenges (Chap. 1, Dahl et al. 2011, Perrigault et al. 2011), may be crucial in deterring infection development early enough.

The hard clam transplant program in Raritan Bay involves the transfer of clams in a box truck where they remain overnight before they are placed in the cleansing area waters the next morning. Monitoring of the truck's storage area air temperatures and the internal clam meat temperatures show the clams are subject to temperatures greater than in the water. In mid-August for example, clam bushels can be subject to temperatures above 27°C for nearly 12hrs, with clam meat sometimes reaching temperatures (32ºC for over 2 hours) that were shown to be lethal for QPX in vitro. Infection screening of clams just after harvest from Raritan Bay, before transfer in the truck, then again 6 and 12 weeks after the transfer to the cleansing area waters, show significant reductions of QPX infections (Allam unpublished). This de facto heat treatment may have potential to aid clams in ridding themselves of QPX burdens. Additional studies would be needed to confirm the validity of developing this application. Heat treatment temperatures, durations and follow up temperature periods would need to be examined. It would be interesting to see if techniques could be developed for a salinity treatment. One note of caution, prolonged high temperatures (e.g., 27°C) or low salinity conditions (e.g., 17ppt) can be stressful to clams and detrimental to their health regardless of QPX infections, as observed in control clam mortalities after several months (Chap. 1, Dahl et al. 2011, Perigault et al. 2011).

Ambient water temperature can bolster defense response capacity but hard clam stocks with high susceptibility can acquire infections even during the warmest season in enzootic areas (Chap. 2, Dahl et al. 2010). Hard clam culturists should exercise caution in hard clam stock selection if their growing areas have any QPX disease risk. Clam seed raised from a wild NY population displayed great potential for disease resistance and growth (Chap. 2, Dahl et al. 2010). Hard clam culture and restoration efforts could greatly benefit from additional research and development of QPX resistant hard clam strains. Studies linking defense mechanisms to genetic expression could foster development of resistance markers. Breeding of clams that overcome disease pressure for successive generations should improve strain resistance. The natural resistance of northern clam strains as compared to southern stocks may be the result of selective pressure exerted by multi-generational exposure of indigenous clams to environmentally-prevailing QPX. Selective oyster breeding strategies for disease resistance have resulted in promising stocks to help manage fisheries losses to disease mortalities (Ragone Calvo et al. 2003).

Susceptible seed allowed for observation of early infection stages and subsequent disease progression (Chap. 2, Dahl et al. 2010). Light focal infections were first found in pallial organs and later they became diffuse and incorporated visceral organs. A very similar sequence of observations have been described for infections from another coastal system (Ragone Calvo et al. 2007) and the pattern supports the theory that QPX transmission occurs through direct environmental exposure and yet direct transmission has to be confirmed experimentally. Laboratory environmental conditions may be an important aspect toward achieving transmission without an inoculation technique. New experiments may benefit from cool temperatures with high salinity. Aspects of QPX's biology or ecology may be critical in demonstrating transmission that reflects more natural processes. QPX has been shown to survive and even grow on

macroalgal substrates (Buggé and Allam 2007) and has been detected in seaweed samples from the field (Gast et al. 2008). Thraustochytrids derive nutrition from detrital organic matter (Raghukumar 2002). Detritus in estuarine sediments could be sustaining QPX outside of the parasitic pathway in clams and effectively serving as an environmental reservoir for infection. QPX has been detected in sediment samples from Raritan Bay (Liu et al. 2009) and in other coastal areas (Gast et al. 2008). Clams that were kept suspended above the sediment had lower QPX infection prevalence and considerably lower intensity than clams that remained on bottom in the same location (Chap. 3, Dahl and Allam 2015). Detritus can be incorporated into aggregate particles and marine aggregates have been observed to carry QPX (Lyons et al. 2005), suggesting a potential vector for pathogen exposure. Additional investigations are needed to clarify reservoir and vector roles of sediment, detritus, and aggregates.

Studies have shown thraustochytrid biomass that can be equivalent to bacteria in phytoplankton detritus (Raghukumar 2002). Communities with abundant phytoplankton can support productive clam populations such as found in Raritan Bay which is a eutrophic system. High nutrient levels and primary organic matter productivity could predispose a system to maintain high resident levels of QPX and possibly allow for heightened pathogen virulence. Ample resource availability through organic or inorganic enrichment of aquatic environments can transform benign microbial communities into virulent ones which can lead to increased infection risk (Wedekind et al 2010).

QPX isolates have shown differences in virulence (Dahl et al. 2008) and differences in optimal growth parameters (Perrigault et al. 2010). This evidence supports potential diversity of QPX strains across coastal areas and even within Raritan Bay (Dahl et al. 2008). It is not known if QPX disease may be a result of infection by multiple strains simultaneously. Identifying QPX strains and corresponding virulence will improve assessment of disease risk in enzootic estuaries.

In the past several years there has been an increase in public awareness regarding the importance of bivalve shellfish as suspension feeders that provide ecosystem services; improving water clarity, coupling productivity between the water column and benthos, supporting benthic habitat structure, etc. Much of this awareness has been a result of a growing emphasis on shellfish restoration in coastal areas that historically had substantial bivalve populations, from oyster gardening projects in urban estuarine areas to larger scale hard clam spawner sanctuaries in Great South Bay, NY. Strategies for such endeavors often do not consider infectious disease dynamics. Our evolving relationship with bivalve shellfish as a resource and keystone of estuarine ecosystems is even more challenging with changing environmental conditions. More extreme temperature regimes, altered precipitation and drought, eutrophication and hypoxia, rising atmospheric CO_2 affecting aquatic pH levels; they all have potential to stress the physiological functioning of calcifying marine invertebrates, providing greater opportunity for exploitation by parasites and infectious pathogens. A more comprehensive understanding of bivalve relationships with environmental conditions and pathology is imperative considering the potentially devastating effects of bivalve epizootics already seen within the eastern oyster industry.

Environmental conditions have proven to be an important component in QPX disease dynamics, providing advantage to an opportunistic pathogen or favoring the hard clam host. The

predicted effects of climate change to the Northeast region, such as increasing summer temperatures and more precipitation with extreme events causing low salinity phases (Kunkel et al. 2013), may actually help hard clams and deter QPX epizootics. A steady increase in the summer maximum temperature can already been seen in buoy data from the NY bight (Fig. 23). Dissolved oxygen by itself does not seem to have much of an impact on QPX disease dynamics although evidence is not strong enough to completely rule out low dissolved oxygen as a potentially contributing factor in terms of synergistic stressors. The eutrophication trend of coastal systems will likely cause low dissolved oxygen levels to become a greater cofactor for marine animal pathology as time goes on. Other issues associated with degrading estuarine quality will also contribute interactive effects on bivalve health. Harmful algal blooms are on the rise globally and regionally, they can stress clam physiology by direct toxicity or by depriving nutritional needs.

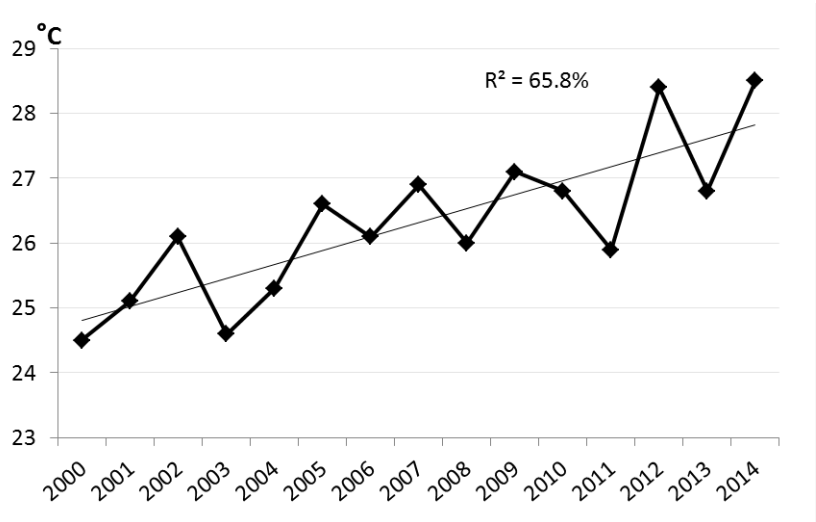

Figure 23. Highest water temperature recorded in the NY bight each year: NOAA buoy station 44025.

The QPX epizootic of 2002 has abated in Raritan Bay (NYS DEC unpublished). The hard clams that remain may have gained some population level resistance as the most susceptible members are more likely to suffer mortality. Additional studies would be needed to confirm the degree to which this may have happened. Although the mortalities were more severe, Haskin and Ford (1979) provided evidence of MSX resistance that developed under natural selection in Delaware Bay. The threat of QPX disease may not be completely gone from Raritan Bay as it seems the right environmental conditions (mild winters, drought) fostered a substantial resurgence of MSX over 14yrs after the first outbreak occurred in Delaware Bay (Ford and Haskin 1982). Another issue to consider is contributions to population recruitment from clams not subject to QPX infection pressure that may have higher susceptibility, from areas of either lower salinity and/or higher temperature portions of the Raritan estuary that could effectively serve as disease refuge or possibly even from adjacent systems. Ford et al 2012 investigated oyster disease refuges and suggest they are likely to impede the development of population resistance if the genetic contribution from the refuge is considerable.

A multifaceted approach of lab and field experiments has identified important QPX infection risk factors and improved the understanding of environmental relationships with this hard clam disease. A general recommendation for the planning of hard clam aquaculture programs or restoration projects in Northeast to Mid-Atlantic coastal areas is to employ precautionary screening. If there are insufficient individual bivalves to sample then pilot scale assays should be conducted to infer potential infection pressure from resident pathogen strains. Inspection of environmental data from the proposed area will benefit risk assessment. In particular it is recommended to observe temperature and salinity trends, ideally over many years of data to aid observation of climatic variation. Environmental assessment is contingent on what data is available and proximate data sources may need to be utilized. Deficiencies in environmental characterization could be supplemented with deployment of autonomous loggers and/or frequent point sampling to better clarify temporal and spatial patterns.

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