Evaluation and Genetic Analysis of Hard Clam, *Mercenaria mercenaria* , Stocks for QPX-resistance.

Project Code: 05-4-6 October 1, 2006 to September 30, 2008
Funding: $71,173

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Reason for Termination: Study end

Project objectives: Objective 1. Compare growth and survival of three selected commercial hard clam strains used in a previous study and planted in MA and NJ.

Objective 2. Compare timing of onset of QPX infection prevalence and intensity in three selected hard clam strains produced in commercial hatcheries and planted at two sites MA and NJ where QPX has been epizootic

Objective 3. Use results from Objective 1 and 2 to extend the range of observations on effects of northward latitudinal shifting of regionally produced seed on susceptibility of hard clams to QPX.

Objective 4. Sample planted clams and survivors, and archive samples, to allow future genetic analysis to identify and map markers showing significant frequency shifts after QPX-caused mortalities;

Objective 5. Provide the hard clam aquaculture industry with information on the use of appropriate seed.

Anticipated Benefits: The hard clam aquaculture industry will become better informed about the importance of QPX x clam strain interactions and plant only the appropriate seed for their locations. The scientific community will develop programs to utilize materials placed in storage for developing marker assisted breeding to reduce the susceptibility to QPX

Principal Accomplishments: Despite delays in funding and difficulty in getting the clams shipped to grow-out sites due to an unknown parasite discovered in one sample (see below for publication) the project completed of the scientific objectives. QPX infections were generally modest, but southern strains (SC) had higher percentages of infected animals in both NJ and MA, and experienced heavier mortality. In the first year, growth rates were similar for all strains, and sizes. During the second year in the field clams in MA grew faster than in NJ. Clams in MA were beginning to reach market size in April and it took the NJ clams until fall to reach approximately the same size. This finding supports the anecdotal information from NJ growers that growth rates in Dry Bay may have decreased in recent years. Whether this is due to the bay reaching carrying capacity or interactions due to the continued planting of animals without allowing a period of fallowing is unknown. Alternatively, the higher survival of all strains in NJ may have increased the crowding and competition for food within the plots. Evidence for this is the lack of difference in growth between NJ and MA locations during the first growth period in the field. Survival of all

strains was greater in NJ than in MA and followed the same pattern with NJ strains exhibiting the best survival followed by MA strains then S. The SC strains in MA had less than 10% survival.

Impacts: This study has confirmed the general pattern found in earlier studies that southern strains become more heavily infected with QPX than NJ or MA strains. It differs slightly in that the SC strain in NJ did not become heavily infected and thus suffered less mortality than the same strain deployed in MA. There was little advantage to using the SC strain in terms of growth as all strains grew at about the same rate when deployed under similar conditions. These data will be presented to the industry at the next NACE meeting. Animals have been archived from the initial samples and at harvest for possible genetic testing. In New Jersey large animals of each of the three lines have been placed in plots to allow further chance for QPX mortality. These animals will be sampled after the current summer growth season.

Recommended follow-up activities: Fund a proposal that will analyze the material placed in storage so an analysis of markers for QPX resistance can be developed and utilized to guide marker assisted breeding.

Introduction

QPX (Quahog Parasite Unknown) has been responsible for significant losses of cultured and wild strains of hard clams, *Mercenaria mercenaria*, from Massachusetts to Virginia. The parasite is acquired after planting, and hatcheries are not the source of the disease (Ford et al., 1997). This is important because the hard clam is relatively unique among bivalves in that all aquacultured seed are hatchery produced, because wild seed are not sufficiently abundant to provide for aquaculture. The disease also occurs in populations of wild clams (Dove et. al. 2004), and thus is not exclusively an aquaculture based disease. The most severe disease outbreaks have been reported in Massachusetts and New Jersey.

Massachusetts experienced a QPX outbreak in Provincetown that essentially ended hard clam culture at that site (Smolowitz et al. 1998). Aquacultured strains in Barnstable Harbor suffered severe mortality from QPX, and an outbreak in cultured hard clams in Wellfleet resulted in the removal and destruction clams from all plots.

In New Jersey, growers experienced significant (80%) losses when southern (South Carolina, and possibly Florida strains) seed were utilized, but similar losses were not found when local (NJ strain) seed were planted nearby (Ford et al, 2002). These losses were virtually eliminated by not using these southern strains, thus saving the industry from the associated economic loss. An outbreak of QPX in wild clams in the Raritan Bay estuary of New Jersey and New York occurred in 2002, and regulatory agencies in both states stopped relay programs (Dove et al., 2004).

These losses have fostered an ongoing series of investigations into the disease and its etiology. Evidence that QPX susceptibility in cultured animals is related to the strain's genetic background was provided by Ford et al. (2002). This has subsequently been confirmed by additional field studies (Ragone-Calvo et al., 2007, Dahl et al. 2010). The Ragone-Calvo et al. (2007) study compared the growth and mortality of five strains of hard clams (Florida, South Carolina, Virginia and New Jersey and Massachusetts) produced in the same hatchery, and planted at commercial densities in replicate plots in Virginia and New Jersey. In this study susceptibility to QPX was related to the strain's genetic origin. By the end of the experiment (approximately 3 years) the southern strains

generally grew faster at both sites than more northern strains, but mortality was higher. Total mortality was 78%, 52%, 36%, 33%, 20% for FL, SC, VA, NJ and MA strains in Virginia, and 53%, 40%, 20%, 6% and 4% for the same strains in New Jersey. The same strains were planted in Massachusetts, but were lost to ice during the first winter. Dahl et. al. (2010) provide evidence that infection of the FL strain occurred within 2 months after deployment in NY. A New York "wild type" and a NY "notata" strain were also deployed. The "wild type" seed did not become infected and the "notata" type seed were infected in the second summer. Both of the NY strains had higher survivorship than the FL strain. These data generally support the strain x latitude interaction found in the prior studies, but the higher mortality at the VA study site relative to NJ did not support a latitudinal gradient in severity of infection. Documentation of the effect of latitudude and strain was still lacking in MA, the area that appears to have shown the highest levels of infection and mortality. The current study was to investigate this effect utilizing the same strains that were used in the Ragone-Calvo et al. (2007) study. Since the evidence for a genetic component to QPX susceptibility was apparent we archived samples of the strains at planting and at project termination to allow for future genetic analysis.

Methods

Seed from populations of the same strains that were used in the Ragone-Calvo et al. (2007) study were reared at Virginian Institute of Marine Science and grown to planting size on the Eastern Shore of Virginia. We deployed three strains (MA, NJ and SC in origin) in the spring of 2008 in Dry Bay, NJ and Barnstable Harbor, MA. Prior to planting 100 clams of each strain were measured and 100 histologically tested for the presence of disease and samples were archived for genetic analysis. Four replicate plots of 5' x 5' (1.52 m x 1.52 m) on each side (25 ft^2 or 2.31 m^2) were established for each strain at each site in a general randomized block design. Clams of each strain were allocated into groups of approximately 990, 723 and 1005 for the NJ, MA and SC strains, respectively, in a haphazard fashion based on weight, and then lots were assigned to each plot in a random fashion. These densities approximate commercially plated densities. The reason for the irregular numbers is highlighted in the results section. Plots were sampled in the spring (April/May) and either the late summer or fall (September to November) depending on when QPX was expected to become prevalent at each site. Sampling within each plot was by random selection of core locations based on a grid that was placed over each plot. Ten samples were collected from each plot using a 10.2 cm diameter corer that was forced into the sediments to a depth of about 15 cm. The clams and muddy substrate were removed and placed in numbered plastic bags. The bags were transported to the lab and retained in a cold room overnight and then clams were removed from the sediment by sieving on a 2mm mesh sieve. At the MA site all cores were sieved in the field and the clams transported to the lab for processing. All live and dead clams, including single shells, were retained for counting and measurement, and individuals were set aside for histological processing and subsequent microscopic examination. On the final sampling date, cores were taken to allow for comparisons with earlier sampling and then the entire plot was dug either by bull rake and hand (NJ) or bull rake (MA). This latter sampling provided a means to estimate the efficiency of the overall sampling effort and to estimate overall mortality.

Data analysis utilized standard statistical tests (ANOVA for growth or X^2 contingency tests of independence for mortality) within each sampling date., The final sampling provided an overall estimate of growth and survival.

Histological Studies: Gross and histological evaluation of 15 clams from each replicate plot were made for each sampling date (60 clams per strain per time). After collection, clams were processed within 96 hours. All animals were measured (length, width and height) and shucked. Shells were examined for chipping and tissues examined for nodules – both traits that have been associated with QPX. A cross section taken from the hinge region through the visceral mass to the mantle edge and a piece of the mantle adjacent to the siphon, were fixed in Davidson's AFA for 48 hours. At initial deployment and in the fall of 2009 a piece of the foot was collected, placed in 70% EtOH and archived at –80ºC for future genetic analysis. Fixed tissues were processed and examined at HSRL according to standard histological procedures (Ford, 2002 and Smolowitz, 1998). The tissues were microscopically examined and scored as percent prevalence (presence or absence of the parasite) and, when present, as (1) focal (single lesion or infection site), (2) multifocal (multiple lesions or infection sites), or (3) diffuse (parasite distributed throughout host tissues). Additionally, the number of viable and apparently non-viable parasites in the entire section was estimated as rare (1-10), light (11-100), moderate (101-1000) or heavy (>1000). The location and number scores were multiplied to provide an index to the severity of infection in each individual. An average of all severity values, including "0"s, was calculated for each strain at each site for each sampling date.

The prevalence data were arc sine transformed and analyzed by ANOVA. The mean severity data were analyzed by Chi Square (Snedecor and Cochran, 1956). Two clams (one each from the SC and NJ strains) from MA in the fall of 2008 were found with only moribund QPX in the tissue. These were considered to be un-infected when the data were analyzed.

Results

The planned fall 2007 planting was delayed because histological analysis of these seed prior to shipment found a previously unknown parasite, which was subsequently described (Ford et al. 2009). Two additional samplings in the spring showed no parasite presence, there were no unusual over wintering mortalities and animals were cleared to be shipped to the test sites. There was no evidence of QPX in any of the seed examined prior to planting. This additional winter of holding the seed resulted in fewer clams than had been anticipated in all groups due to winter mortality. We divided the total numbers of clams in a strain into 8 equal portions and planted the same numbers in each plot in both MA and NJ. All data analysis of survival was based on adjustments that assumed an equal number of each clam strain planted in each plot.

Growth:

For the first three sampling periods the clams were held in Virginia, and the third sampling period represents the size at which clams were shipped and planted (Figure 1). In spite of these size differences, clams of all strains at least doubled in size before the first post deployment sampling in the fall of 2008. At that time, SC clam strains in NJ

were larger than all clams in MA and the NJ clam strain in NJ (Figure 1). All clams in MA were the same size. By the spring sampling of 2009, all clams in MA were still the same size, but were larger that those in NJ, and these in turn were all the same size. By the final sampling in the fall of 2009, all clam strains in MA were larger than those in NJ, and within the MA strains, the MA clam strains were larger than those from the SC strains while the NJ and MA strains were about the same size. At the final sampling all strains in NJ were statistically the same and had reached commercial size (Figure 1).

Survival:

Repeated measures ANOVA of live animals from core samples randomly allocated within each plot found significant differences in numbers of individuals per core between strains, collection dates and an interaction between site and collection date (P= 0.0037, 0.0099 and 0.0199, respectively). The mean number per core for clams from all plots was 3.9, 3.3 and 2.6 for NJ, MA and SC strains, respectively, and the NJ and SC strains were significantly different from each other. The numbers per core from NJ and MA strains were not significantly different, and the MA and SC strains were not different, but the NJ strains had higher survival than the SC strains. Collection date differences were due to the first sampling date having more clams per core (4.0) than the following two (2.9 and 2.86) which were not different from each other. The interaction between site and collection date was primarily due to a large number of clams per core being collected at the MA site during the first collection period relative to the following two collections, which were lower and not different from each other.

Because of the interaction and because of obvious differences in survival when the plots were harvested (Table 1) we analyzed the core data from each site separately. At the MA site there were significant differences due to collection date (p =0.0000) and strain (p= 0.0018). At the first sampling more clams were found than in the following two sampling periods, which were not different. The number per core of live NJ tand SC strains were different, and the MA strain was intermediate and not different than either of the other two. At the New Jersey site there were no differences among collection times or strains based on the core data.

After the last core sampling, the plots were completely harvested and these data were used to estimate survivorship (Table 1). ANOVA analysis showed differences in numbers between the sites (p 0.0000) and strains (0.0003). Overall numbers of clams surviving in each strain, and thus percent surviving, was greater in NJ than in MA. Numbers of MA strains in NJ were not significantly different from the NJ strain in MA, and the numbers of SC strains in NJ were not significantly different from the numbers of NJ and MA strains in MA (Table 1, Figure 2). Comparison of numbers per core normalized to core area and extrapolated to the total plot area showed significantly fewer animals estimated by the cores relative to those collected when the total plot was harvested (Chi square = 84.17 df = 14). In general, the underestimation was similar across all strains and all densities with the exception of the SC strain in MA. This strain had the lowest survival, and the underestimate was greater, probably because so few clams were left in the plot. A similar result was found in Ragone-Calvo et al. (2007).

Table 1. Mean numbers of clams collected at harvest from experimental plots in MA and NJ for 3 strains. All data were normalized to a base of 1000 planted in each plot (N = 4 per strain) and animals removed during sampling. Numbers with the same small letters are not significantly different. Percent survivorship can be obtained by dividing the number of individuals per plot by 10.

Site	Strain	Mean Number per Plot	Standard Deviation	95% Confidence Limit
	MA	418 ab	82.22	176.91
NJ	NJ	534 a	24.46	52.63
	SC	364 bc	16.47	36.02
	MA	261 c	53.61	115.35
MA	NJ	349 bc	80.74	173.71
	SC	66 d	20.63	44.38

Histology:

Prevalence of QPX was significantly higher (ANOVA $p = 0.0022$) in MA than in NJ and at both locations was generally higher in the SC strain ($p = 0.0001$) than in the MA or NJ strains which were not significantly different from each other. In general, only two samples (SC strains in MA from the two fall sampling periods) were significantly different from all others (Figure 3). This reflects the relatively low prevalence of QPX, less than 20%, during the study period.

Infection severity was also relatively low during the study period (Figure 4), and Chi Square analysis of ranked data (Wilcoxon, 1948) indicated no heterogeneity (Chi Square = 14.2 df = 10) in the data. Further analysis of unranked data indicated significant heterogeneity in the data (Chi square = 47.04 df = 10). Separating the data by site also yielded significant heterogeneity at both sites (MA - Chi square = 14.39 df = 4 and NJ - Chi square = 25.52 df = 4).

The infection severity data were then compared against a hypothesis that the distribution of the readings should be equal for all times, sites and strains. The Chi square values indicate very high levels of differences (Chi Square = 301.69 and 46.36 in MA and NJ, respectively (Table 2)). In MA the SC in both fall samplings showed the highest deviation from the expected indicating significantly greater infection severity in these strains in MA.

Table 2. Chi Square values comparing the mean infection severity of QPX to a hypothetical relationship in which all samples were expected to have an equal infection level of 8.39. L = significantly lower than expected, H = significantly higher than expected, * = not significant.

		Fall 08	**Spring 09**	**Fall 09**	**Chi Square**	
Site	**Strain**					
	MA	7.42	7.42	1.80	**16.65**	**L**
MA	**NJ**	4.14	0.00	3.11	**7.25**	*
	SC	146.96	0.15	130.69	**277.79**	**H**
		158.51	**7.57**	**135.61**	**301.69**	
		H	*	**H**		
	MA	7.42	7.42	7.42	**22.26**	**L**
NJ	**NJ**	0.43	7.42	7.42	**15.27**	**L**
	SC	1.80	6.03	1.00	**8.83**	**H**
		9.65	**20.87**	**15.84**	46.36	
		L	**L**	**L**		
		168.16	28.44	151.45	348.05	

It is interesting to note that the mean infection severity for each strain is nearly a factor of 4 higher in MA than in NJ (Table 3). Comparing this 4-fold hypothetical difference to the observed (Table 3) also yielded significant heterogeneity (Chi Square 55.62, but most of this was due to the departure from the relationship in the low level of QPX in the SC strains in the MA spring 2009 sample and the relatively high level of QPX in the NJ strains in NJ in the fall of 2008 (Table 3).

Table 3. Infection severity values of QPX in three strains at two sites for fall, spring and fall samplings respectively. The last column represents a hypothetical 4x more intense severity in MA.

		Fall 08	Spring 09	Fall 09	Total	Total/3	Hypothetical relationship
Site	**Strain**						
	MA	0	0	4	**4**	**1.33**	**1**
MA	**NJ**	2	8	14	**24**	**8**	**8**
	SC	44	10	42	**96**	**32**	**32**
	MA	0	0	0	**0**	**0**	**0.25**
NJ	**NJ**	6	0	0	**6**	**2**	**2**
	SC	4	16	5	**25**	**8.33**	**8**

Discussion:

QPX is a naturally occurring organism and in environmental samples QPX has been found associated with a variety of substrates. Lyons et al. (2005) reported the presence of QPX on detritus. Gast et al. (2008) examined water, sediments, macroalgae, invertebrates and sea grasses, and found QPX in all sample types in MA, and in algae, sediments and invertebrates in VA. In addition, in MA they found a seasonal pattern to the occurrence that differed between sample type, with higher values for seawater and algae in spring and higher values for invertebrates in the fall. The disease organism appears to be restricted to high salinity and data from cultured QPX evaluation of salinity tolerance showed very low growth at salinities at or below 20 psu (Brothers et al., 2000), Perrigault et al.(2010) reported survival of QPX in seawater of 15 psu, but no growth at 15 psu when it was maintained in culture media. QPX has caused significant losses to hard clam aquaculturists in VA, NJ and MA. There is no evidence that clams become infected in the hatchery; rather they are infected once they are planted in growout locations (Ford et al. 1997). Three field studies provide significant evidence that QPX susceptibility is linked to particular strains of hard clams (Ford et al. 2002, Ragone-Calvo et al., 2007, Dahl et al. 2010), and it is likely that these differences are genetically linked, but the reports of disease outbeaks in wild populations suggests an environmental component as well. The Ford (2002) and Ragone-Calvo (2007) studies indicated that as the seed from these strains are shifted latitudinally northward there are significant differences in their susceptibility. There is mixed evidence for a latitudinal shift in QPX related mortality. The Ragone-Calvo et al. (2007) study found greater infection levels in and higher mortality in VA plantings than in NJ. Anecdotal evidence and the current study have shown that QPX infections were greater in MA than in NJ. There is also evidence that QPX is primarily a cold-water disease because it has never been found south of Virginia, and the results of an in vitro study of cultured QPX showed that QPX grows best at 20 to 23 ºC and that mortality of all QPX occurred at a temperature of 32 degrees C (Brothers et al., 2000: Brugge and Allam, 2005, Perrigault et al., 2010). Dahl et al. (2008) used laboratory cultures of QPX to infect seed clams from MA, NY, VA and FL and found that southern seed (FL and VA) were more susceptible to infection thus mirroring the field results. In addition, different QPX isolates had differing levels of

virulence (Dahl et al. (2008). While hard clams are susceptible to QPX, they do have some mechanisms of defense and these mechanism apparently are tissue specific, as extracts from different clam tissues had different inhibitory effects on the growth of cultured QPX (Perrigault et al. 2009), but these defenses may be reduced in the presence of stress inducing factors such as harmful algae (Hegaret et al. 2010).

The data from the current study extend those of the prior studies (Ragone-Calvo et al. 2007, Dahl et al. 2010) indicating that QPX infects different strains at different rates and that strains derived from populations south of VA are much more susceptible than those derived from NJ or MA populations. Although infection levels in all strains were relatively low in the present study compared with those of the Ragone-Calvo (2007) and the Ford et. al. (2002) studies, infection prevalence was still highest overall in the SC strains.

While the link between QPX presence and poor survivorship has been made in a number of studies, there is still no definitive study indicating that QPX is responsible for all of the mortality associated with it, which is typically much higher than prevalence level would suggest. As with the Ragone-Calvo (2007) study, infection rates in NJ were low (never reaching 10%) and infection severity was also low, yet total mortality was between 46 and 64%. This disparity leads to at least three potential hypotheses: 1. QPX develops very rapidly from infection to mortality and our sampling schedule was not frequent enough to detect the link. 2. The mechanism of QPX induced mortality can be expressed at infection intensities lower than those we were able to detect histologically. 3. QPX is a secondary invader that may cause some mortality when it proliferates to high levels, but the underlying stressor/pathogen has yet to be identified.

Given the number of samples in this and the Ragone-Calvo (2005) study that have shown the same pattern of relatively high mortality associated with low prevalence and intensity it seems unlikely that the first hypothesis is true, but here are no studies that have definitively described the onset and build-up of infections in newly exposed clams using closely timed sampling. Most studies have used bi-monthly or spring/fall sampling schemes and there is a need to sample on the order of every two weeks to definitively describe the onset and development of infections and subsequent seasonal patterns of potentially chronic infections. It is possible that in more southern areas such as NJ and VA that periods of high temperature may retard QPX development, but there is no evidence to support such speculation.

Only one study (Ford et al., 2002) has described QPX infection prevalence and severity in dead and dying clams. Prevalence was 86 to 100% and severity was 2 to 3 times greater than in live clams collected at the same time. The Ford et al. (2002) study used SC clams that became more heavily infected with QPX than did those in the present study; nevertheless, the results suggested that lethal QPX infections can be detected histologically, which is counter to the second hypothesis.

The third hypothesis is least consistent with what is known about thraustochytrids, but whether it is the QPX, another stressor, or some combination of the two that causes the mortality deserves investigation. Consistent with the findings of Ford (2001) of the low incidence of pathogen induced mortality in hard clams relative to the Eastern oyster, *Crassostrea virginica*, no other pathogens have been reported. Mortality levels of aquacultured hard clams often reach or exceed 30% within the first year after planting, and most of this is presumed to have been caused by predation, but no definitive studies

have ever been conducted. In general, this mortality lessens as the clams become larger and overall mortality, including losses due to animals being missed during harvest, from planting to harvest in cultured clams ranges from 30 to 60% with "typical" values in the 40 to 50% range. These ranges are consistent with what was experienced for MA and NJ strains in NJ during the present study. Mortaltiy in MA was higher than these ranges for all strains, but especially the SC strain. While mortality levels for the culture period can be approximated, it is assumed that QPX associated mortality would be in addition to this "background" level, but the lack of data on what causes the "background" makes definitive statements imprudent.

There have been many more reports of heavy QPX-associated mortality in MA than in other areas of the northeast, suggesting that infection prevalence and severity is higher in MA than at other sites, and this includes the presence of nodules along the mantle edge. The latter condition is common in MA, but rarely seen in NJ or VA. Our study is the first study that has deployed the same strains at the same time in both MA and NJ. In MA both the percent infected and the intensity of infection was higher, lending support to the contention that QPX induced mortality is more severe in MA than NJ.

The better growth in MA relative to NJ starting in the spring of 2009 and continuing throughout that growing season could be due to a number of factors. Better survivorship in NJ certainly caused the clams to become denser, but the NJ clams in MA were nearly the same density as the SC clams in NJ and growth was better in MA. This comparison is obviously compromised because of the difference in strains. It is possible that the clams experienced different food levels or quality at the two sites. In addition, Dry Bay, the NJ site, is densely planted with commercial clams so it may be that the culture area is reaching its carrying capacity. There is evidence from Dry Bay that at times of low water in the summer dissolved oxygen levels can drop below 2.5 mg l^{-1}, and this would reduce growth at least temporarily.

Setting aside samples for future genetic work will allow this work to proceed without conducting another field study. This is important because the genetic link between QPX susceptibility and particular strains has not been subject to genetic analysis. Using AFLP markers and maps, we have shown in the eastern oyster that disease-resistance genes can be quickly mapped by identifying and mapping markers that show significant frequency shifts after disease-caused mortalities (Guo et al., 2004; Yu and Guo, 2005). Given the time and expense of developing and sampling field trials, it seems prudent that when such studies can provide useful information for breeding programs that setting aside samples at the beginning and end should be part of most protocols.

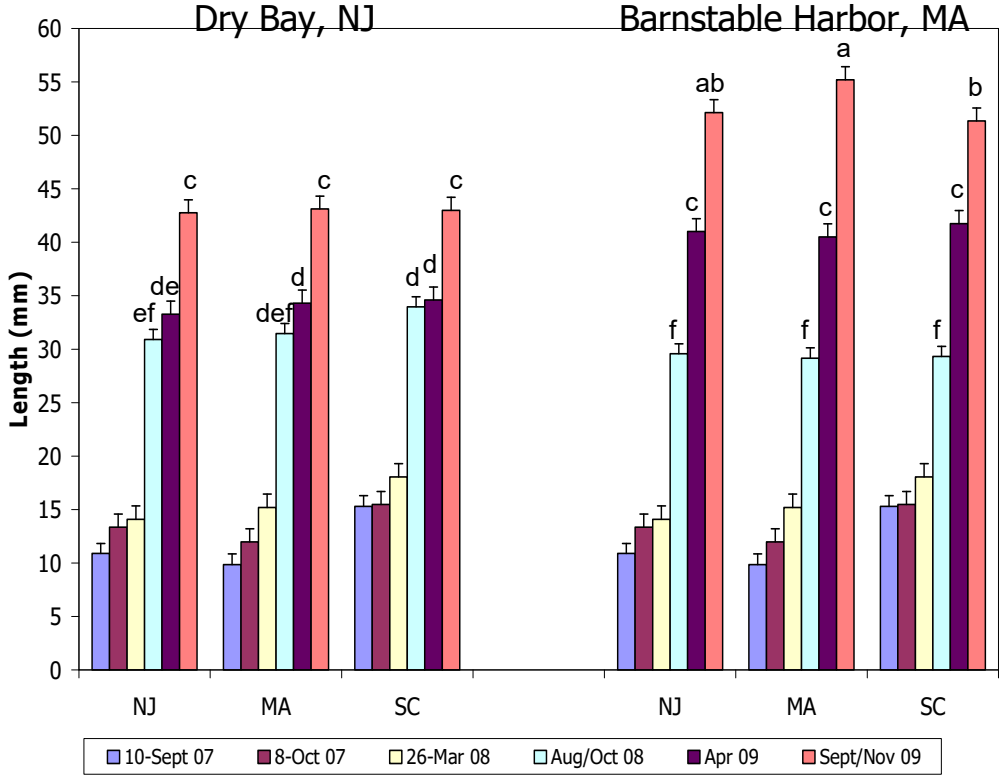

Figure 1. Average size (mm length) of hard clam strains planted in Dry Bay, NJ and Barnstable Harbor, MA. Strains are New Jersey (NJ), Massachusetts (MA) and South Carolina (SC). The first 3 growth periods are identical for each strain and are prior to planting and were not analyzed statistically. Similar letters over the remaining 3 sampling periods denote sizes of animals that were not significantly different. Error bars represent 1 standard error.

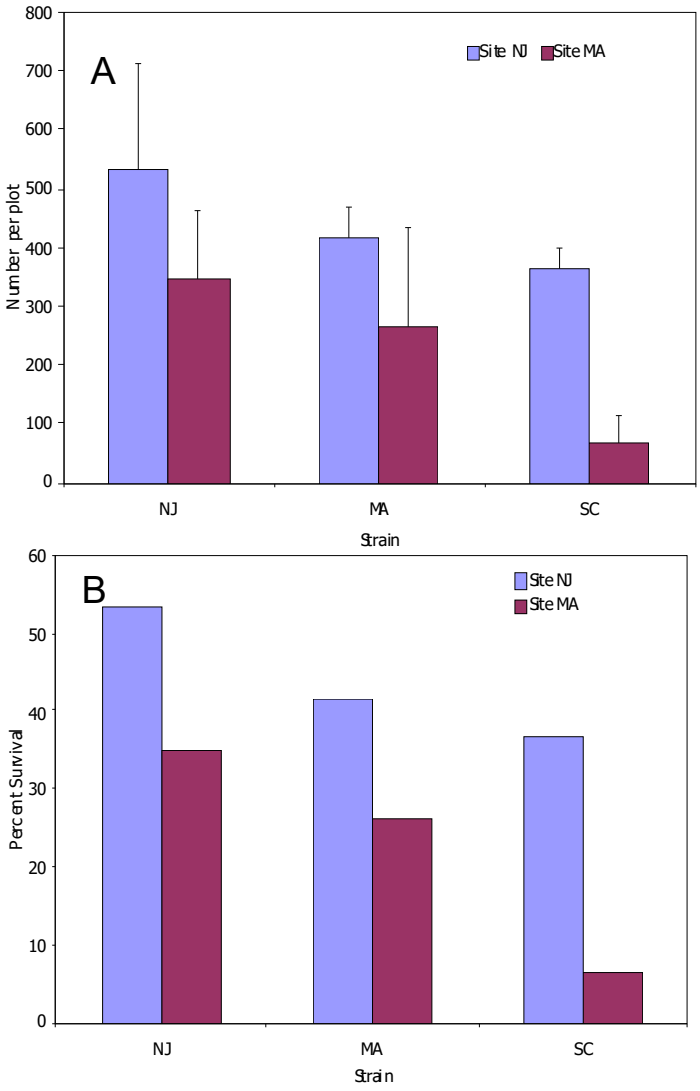

Figure 2. A. Average number of clams harvested from 4 plots for each strain of hard clams planted in Dry Bay, NJ and Barnstable Harbor, MA. Strains are New Jersey (NJ), Massachusetts (MA) and South Carolina (SC). Numbers have been normalized to the same base to account for different planting density and numbers removed by sampling.(see text). Error bars represent 1 standard error. B. The same information as in A except expressed as percent survival.

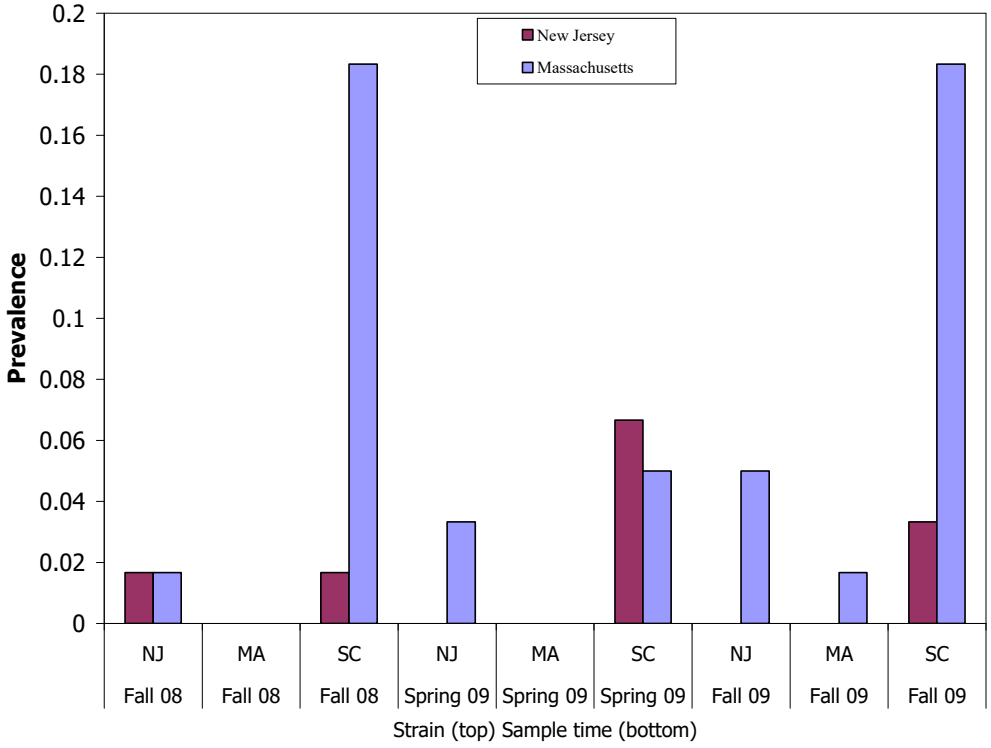

Figure 3. Prevalence (% infected) of QPX in hard clams planted in in Dry Bay, NJ and Barnstable Harbor, MA. Strains are New Jersey (NJ), Massachusetts (MA) and South Carolina (SC). The top line of the Y axis label represents the strain and the bottom line represents the date of the sample.

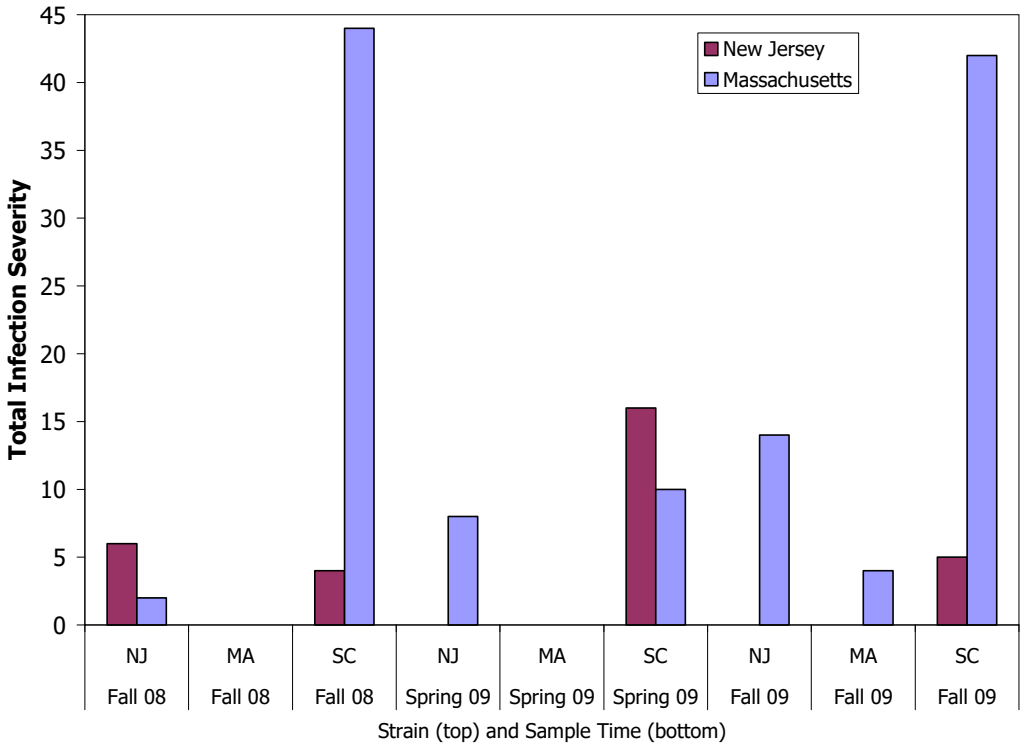

Figure 4. Infection severity (see text) of QPX in hard clams planted in in Dry Bay, NJ and Barnstable Harbor, MA. Strains are New Jersey (NJ), Massachusetts (MA) and South Carolina (SC). The top line of the Y axis label represents the strain and the bottom line represents the date of the sample.

Literature Cited

Brothers, C., E. Marks III and R. Smolowitz. 2000. Conditions affecting the growth and zoosporulation of the protistan parasite QPX in culture. Biol. Bull. 199: 200-201.

Bugge, D.M. and B. Allam. 2005. A fluorometric technique for the in vitro measurement growth and viability in Quahog parasite unknown (QPX). J. Shellfish Res. 24:1013-1018.

Dahl, S.F., M. Perrigault and B. Allam. 2008. Laboratory transmission studies of QPX disease in the hard clam: Interactions between host strains and pathogen isolates. Aquaculture 280: 64-70

Dahl, S.F., J. Thiel and B. Allam. 2010. Field performance and QPX disease in cultured and wil-type strains of *Mercenaria mercenaria* in New York waters. J. Shellfish Res. 29:83-90.

Dove, A.D.M, P.R. Bowser and R.M. Cerrato. 2004. Histological analysis of an outbreak of QPX disease in wild hard clams, Mercenaria mercenaria in New York. J. Aquatic Anim. Health. 16:246-250.

Ford, S.E. 2001. Pests, parasites, diseases, and defense mechanisms of the hard clam, *Mercenaria mercenaria*. Ch 12. In: Kraeuter, J.N. and M. Castagna (eds.). Biology of the hard clam. Developments in aquaculture and fisheries science. Vol 31:591-628.

Ford, S., R. Smolowitz, L. Ragone Calvo, R. Barber, and J. Kraeuter. 1997. Evidence that QPX (Quahog Parasite Unknown) is not present in hatchery-produced hard clam seed. J. Shellfish. Res. 16: 519-521.

Ford, S. E., J. N. Kraeuter, R. D. Barber and G. Mathis. 2002. Aquaculture-associated factors in QPX disease of hard clams: density and seed-source. Aquaculture 208:23-38.

Gast, R.J., D.M. Moran, C. Audemard, M.M. Lyons, J.DeFavari, K.S. Reece, D. Leavitt and R. Smolowitz. 2008. Environmental distribution and persistence of quahog parasite unknown (QPX). Dis. Aquat. Org. 81: 219-229.

Guo, X., Z. Yu, Y. Wang and S.E. Ford. 2004. Strategies for mapping disease-resistance genes in the eastern oyster, *Crassostrea virginica* Gmelin. J. Shellfish Res., 32(1):294.

Hegaret, H., R.M. Smolowitz, I. Sunila, S.E.Shumway, J.Alix, M. Dixon and G.H.Wikfors. 2010. Combined effects of a parasite, QPX, and the harmful-alga, *Procentrum minimum* on northern quahogs, *Mercenaria mercenaria*. Mar. Environ. Res. 69: 337-344.

Li, L. and X**.** Guo. 2004. AFLP-based genetic linkage maps of the Pacific oyster *Crassostrea gigas* Thunberg. *Marine Biotechnology* 6:26–36.

Lyons, M.M., J.E. Ward, R. Smolowitz, K.R. Uhlinger and R.J. Gast. 2005. Lethal marine snow: pathogen of bivalve mollusk concealed in marine aggregates. Limnol. Oceanogr. 50: 1983-1988.

Perrigault, M., D.M. Bugge, C.C. Hao and B. Allam. 2009. Modulatory effects of hard clam (*Mercenaria mercenaria*) tissue extracts on the *in vitro* growth of its pathogen QPX. J. Invert. Path. 100: 1-8.

Perrigault, M., D. M. Bugge, and B. Allam. 2010. Effect of environmental factors on survival and growth of quahog parasite unknown (QPX) in vitro. Journal of Invertebrate

Pathology 104:83-89.
Ragone-Calvo, L.M., S.E. Ford, J. N. Kraeuter, D.F. Leavitt and R. Smolowitz and E.M. Burreson. 2007. Influence of host genetic origin and geographic location on QPX disease in northern quahogs (=hard clams), *Mercenaria mercenaria*. J. Shellfish Res. 26:109-119.
Smolowitz, R., D. Leavitt, and F. Perkins. 1998. Observations of a protistan disease similar to QPX in *Mercenaria mercenaria* (hard clams) from the coast of Massachusetts. J. Invert. Path. 71: 9-25.
Smolowitz, R. Effects of temperature on the infection of hard clams (*Mercenaria mercenaria*) by the protistian organism, "Quahog Parasite Unknown"
Snedacor, G.W. and W.G. Cochran. 1956. Statistical methods. Iowa State University Press. Ames, Iowa. 534 pp.
Yu, Z. and X. Guo. 2003. Genetic linkage map of the eastern oyster *Crassostrea virginica* Gmelin. *Biol. Bull.* 204: 327–338.
Yu, Z. and X. Guo. 2005. Identification and mapping of disease-resistance QTLs in the eastern oyster, *Crassostrea virginica* Gmelin. Presented at the 97th Meeting of the National Shellfish Association, April 10-14, 2005, Philadelphia.

Publications, Manuscripts, or paper presented:

S. Ford, N. Stokes, E. Burreson, E. Scarpa, R. Carnegie, J. Kraeuter, and D. Bushek. 2008. A Novel Hard Clam Parasite: Making Sense of a New Finding. Invited publication, C.M. 2008D 10/ International Council for the Exploration of the Seas Science. Halifax, NS Canada. 5pp.

S.E. Ford, N.A. Stokes, E.M. Burreson, E. Scarpa, R.B. Carnegie, J.N. Kraeuter, and D. Bushek. 2009. *Minchinia mercenariae* n. sp. (Haplosporidia) in the hard clam *Mercenaria mercenaria:* Implications of a rare parasite in a commercially important host. J. Eukaryot. Microbiol., 56:542–551.