Final Report

Evaluation of putatively QPX-resistant strains of northern hard clams using field and genetic studies

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PROJECT OBJECTIVES:

1. Identify high quality QPX-resistant hard clams based on the clam's ability to grow fast and resist disease in Mid Atlantic and New England conditions.

2. Characterize molecular mechanisms associated with superior performing hard clams to assist in broodstock development.

3. Help hatcheries to produce fast growing, QPX resistant clams by providing broodstock developed in this study and by dissemination of knowledge concerning selection of broodstock in other states.

ANTICIPATED BENEFITS:

The work demonstrated a source of QPX-resistant broodstock, with good growth potential, that can be used to produce hard clam seed for aquaculturists who are economically impacted by QPX disease. Broodstock are being maintained at the MBL at ARC hatchery, and in the field for future use. Molecular tools were developed by the University of Washington that can identify direct and indirect molecular mechanisms for disease-resistance that could be exploited for genetic selection practices.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Growth

Growth rates of the primary strains of interest, the BX and MASH stocks in Massachusetts, Rhode Island and New Jersey were virtually the same throughout the experimental period with one exception. The BX strain was significantly larger than the MASH strain at Scudders Lane at final sampling. The ME strain was smaller when the experiment started and appeared stunted throughout. All lines showed little or no growth for the winter, October to April period in both years.

Mortality
In general, both the BX and MASH lines experienced higher mortality during the early sampling periods and then reduced mortality during the last periods with no significant difference with notable exception. The BX strain in Massachusetts survived significantly better than the MASH strain in Barnstable Harbor and Scudders Lane. The ME strain experienced higher mortality than the other strains, and was completed gone in most plots by the end of the project except for some in NJ. The FL strain was only grown at Scudders Lane but did not survive the entire project.

Condition Index
A condition index based on the dry weight of meat and shell length was constructed to provide a comparison between the strains. There were no differences between the BX and MASH strain in NJ. While BX strain had a higher condition index than the MASH strain in Massachusetts, it wasn't significantly different. The values for the ME strain were less than half of the others and, as with the growth and mortality data, suggests that this group had no advantage for growing in more southerly climes.

Characterization of molecular mechanisms associated with superior performing hard clams to assist in broodstock development.
As part of this research effort we have significantly contributed to the genomic resources available for this species. Raw data is available to the public via NCBI. Comparing gene expression between the two strains (BX and MASH) provides important insight into the processes associated with superior performing broodstock. Of the 203 differentially expressed genes associated with an enriched process, a majority were expressed at a higher level in BX These data suggest that successful implementation of these responses is associated with increased survival such as we found evident in Barnstable Harbor. Finally, 145 putative SNP markers were discovered that will assist in the identification and selection of superior performing clams in future.

Disposition of broodstocks developed from this project
After the final sampling of broodstock at the Scudders Lane site in the fall of 2010, the MBL selected 150 of the largest and presumably fastest growing BX broodstock. These were housed in bio-secure facilities, conditioned and spawned in the spring 0f 2011. Some were offered to a commercial hatchery (ARC) for introduction to their broodstock improvement program. The MBL has cultured the next generation of these clams and has re-stocked them in the same location of QPX disease pressure in Barnstable Harbor for further testing and selective breeding.

Impacts: This study has reaffirmed that clams selected from areas under intense disease pressure can be a good source of select disease-resistant broodstocks. Selection of a more notherly strain did not produce more winter tolerance, survival or better winter growth but that strain may have been compromised by a smaller start to begin with.
Recommended follow-up activities: Other clams that have been archived from past NRAC studies (Kreauter et al.) could be examined for the same genetic markers to assess those strain's usefulness in terms of disease-resistance, and future marker assisted selection. Fund a more concerted genetic selection program for hard clam culture.

Introduction

Disease resulting from infection of *M. mercenaria* with QPX continues to cause severe mortality of cultured clams and significant economic loss to aquaculturists. This is especially true in Massachusetts. An outbreak of the disease in cultured hard clams in the fall of 2005 in Wellfleet, MA, resulted in a bold move by the culturists there to prevent further disease. They removed all animals that could be found from the infected plots within the leases. The cost was over $500K for just 3 growers, and many others were also affected. The culturists in Wellfleet appear at this point to have prevented continued significant outbreaks. However, several other locations, in MA and RI especially, continue to experience significant disease resulting in often devastating economic losses since clams usually die just a few months before they attain market size.

Previous studies of clam strains planted in more northerly sites than their origin have shown that these clams are more susceptible to QPX (Ragone-Calvo et al. 2007). While the mechanism hasn't been proven, it is widely believed that southern strains may be immuno-compromised until later in the spring than native stocks and thus at greater risk of infection by QPX. Therefore we sourced some of a most northerly strain (ME) to transplant to the southerly sites to see if the reverse was true, and might these have better survival. Another favored strain (BX) were selected for their "proven" resistance to QPX by virtue of being selected from severely effected plots We selected two "naïve" strains for a comparison, with the MASH strain being a local control, and the FL strain being a definite QPX susceptible control. This project was launched with an aim of testing the disease-resistant and growth characteristics of four strains of hard clams with the hope that at least one of them would prove hardy and fast growing for local culture.

Methods

Clam seed was purchased and planted in the Fall of 2008 from ARC in Massachusetts from two different sources of broodstocks; one had been subjected to and survived severe QPX outbreaks in Barnstable Harbor (denoted here as BX), and the other was from a presumably naïve strain on the southern shores of Cape Cod in Mashpee (denoted here as MASH). After failing to secure a northern strain promised from Canada, we procured seed from the Downeast Institute's Goose Cove, Trenton, Maine hatchery/nursery (denoted as ME). Seed were planted at typical commercial average densities of 50 per square foot. Our control strain (presumably susceptible to QPX) came from Southern Cross hatchery in Florida (denoted as FL). The sampling protocol dictated sampling of 10 per plot /40 per stock for histology, 10 per plot /40 per stock for Condition Index (CI), and 30 per stock for hemolymph (HLPH) (from histo sample) at most specific sampling periods (spring, summer and fall).

Research plots were placed in 2 locations where QPX is known to be a significant disease problem: Scudders Lane, Barnstable Harbor, MA and Dry Bay, NJ. Research plots wer 7 ft x 7 ft. A total of (4 plots x 4 strains of seed) 16 plots were planted at Scudders Lane experimental site and (4 plots x 3 strains of seed – not FL) 12 plots total, in Dry Bay, NJ.

Clams were also planted in two commercial aquaculture sites that have experienced

significant QPX disease in the past. These plots were on the leases of Scott Laurie in Barnstable Harbor, MA and Jeff Gardner in Watch Hill, RI. Three 10 ft. x 10 ft plots per strain (total of 9 plots per aquaculture location) were planted and maintained under typical commercial conditions.

At each sample time, research plots were overlaid with a grid and replicate cores of five 8" diameter in MA and ten 4" diameter in NJ (the smaller size is required due to mud bottom in NJ), were randomly taken for dead/live counts in October 2008, April, August and October of 2009 and June, August and October 2010. Live animals from these were sampled pathologically for QPX disease prevalence and severity in July and October, 2009, and June, August and October 2010. The disease was expected to be fully developed in the populations by 2010.

Condition index evaluations were conducted on 60 animals/strain/experimental site in April and August 2009 and June, August and October 2010 according to the following formula: $CI = DW_{soft} / W_{shell} \times 100$
Where CI is the condition index, DW_{soft} is the dry weight of the soft tissue (grams) and W_{shell} is the width of the clam at the hinge (mm) (Smolowitz et al. 1998).

Genetic Studies – see separate sub-report at the end.

Results

Growth in Massachusetts and Rhode Island

At the Massachusetts research site, MASH and BX stocks started at similar size, and grew with a slight early advantage to MASH but final harvest size advantage for BX (Figure 1). ME stock survival was very low, and what was picked up in sampling remained small. The Maine stock was smaller when the experiment started and never achieved the same sizes as the two Massachusetts stocks. FL stock started strong but growth weakened in the second year with low survivorship. At Massachusetts and RI commercial farm sites, growth of the MASH and BX stocks were virtually the same throughout the experimental period, and final sizes were nearly identical (Figures 2 and 3) The majority of individuals of MAX and MA strains were market size at the last sampling in October 2010.

Figure 1. Scudders Lane Research Site, Barnstable, MA

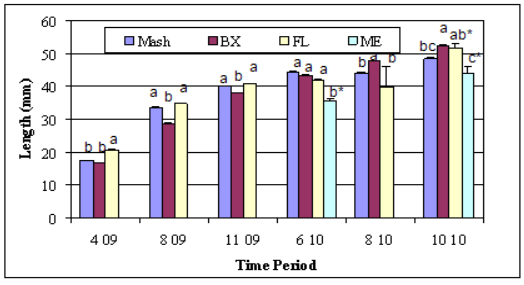
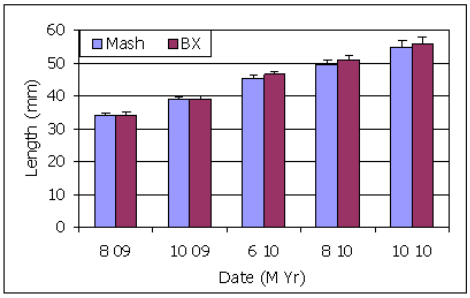
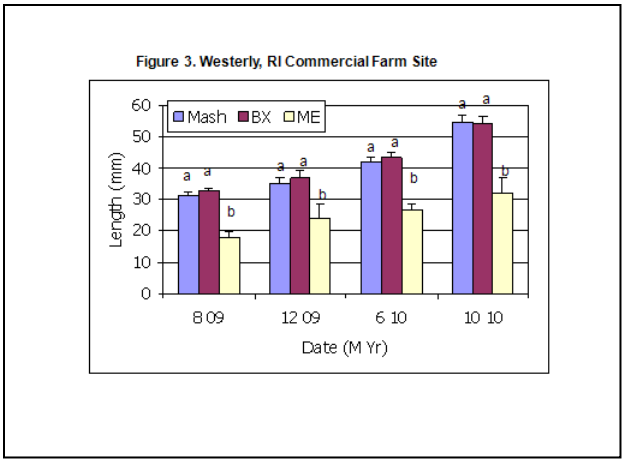
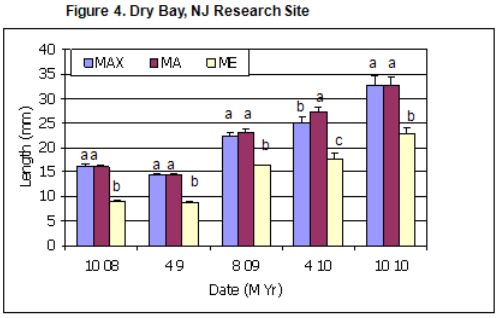


Figure 2. Barnstable Harbor Commercial Farm – no sig. diff.







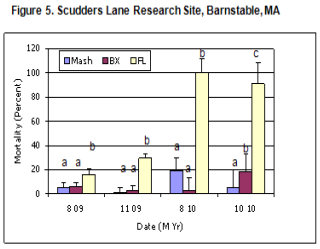
MAX = BX, MA = MASH

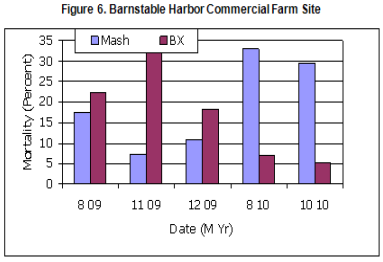
Growth in New Jersey

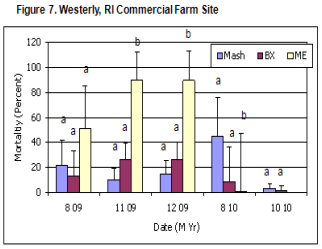
Growth of the MASH and BX stocks in Dry Bay, New Jersey was virtually the same throughout the experimental period, and final sizes were identical (Figure 4) The Maine stock was smaller when the experiment started and never achieved the same sizes as the two Massachusetts stocks. Average daily growth rates throughout the period (0.042, 0.038 and 0.029 mm/day) for the BX, MASH and ME stocks, respectively suggest that the BX stock may have performed slightly better than the MASH line, but the difference is not significant. Interval growth clearly shows high growth of all stocks from April to August in 2009, but the ME stocks experienced significantly poorer growth in the August to October period. The ME stocks also appeared to grow well for the April to August 2010 period, but during the fall growth these same stocks significantly underperformed the BX and MASH lines. All lines showed little or no growth for the winter, October to April period in both years. Less than a dozen ME and FL clams were found and measured at the final harvest at Scudders Lane (see * in Figure 1).

Mortality in Massachusetts and Rhode Island

Mortality is shown at each interval at the three sites (Figures 5, 6 and 7). The cummulative mortality was much higher, nearly 100% for FL, and 100% for ME at sites in Massachusetts post-planting, and eventually 100% in Westery, RI. (Figure 8a).







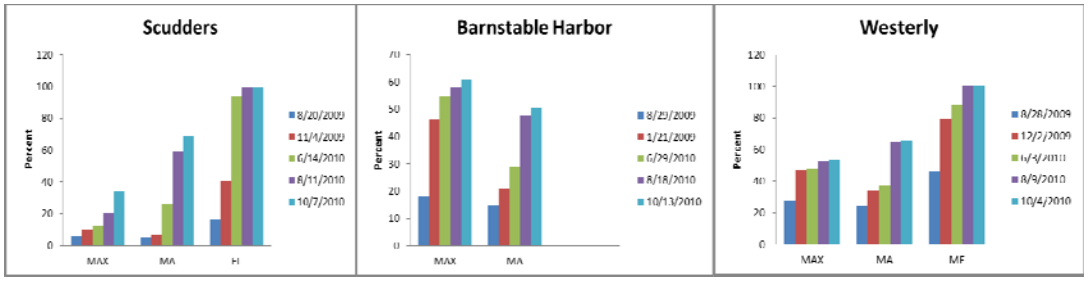
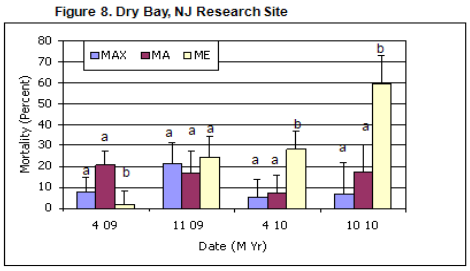


Fig 8a. Overall cumulative mortality for strains at the Massachusetts and Rhode Island sites followed the same basic trend. Both FL and ME (not shown for Scudders and Barnstable Harbor sites where there was 100% mortality), have significantly higher mortality at all sites as compared to both the MAX (=BX) and MA (=MASH) strains.

Mortality in NJ

As above, we have analyzed the mortality data in two ways. First we compared the numbers harvested to the planted numbers for each stock, or the interval mortality. We have not corrected these data for clams that were removed by sampling and thus they represent higher mortality than actually occurred by a few percentage points. The data clearly show that the ME stocks did not perform well in NJ, and that the MA and MAX stock were not different (Figure 8b). One replicate of the MAX stock was nearly completely destroyed by rays between the last sampling period and the harvest. Figure 8b includes this replicate in the bar labeled, but they are excluded in the labeled MAX 2. When this is taken into account the mortality for MA and MAX 2 was identical.



Second, we compared cumulative mortality based on live and dead counts from core samples (Figure 9). These data allow comparison of the three stocks throughout the time period, and do not require correction for individuals removed by sampling because they are subsamples including both the live and dead individuals. These data suffer from potential loss of dead clams in the smallest size categories, and thus these data should underestimate the overall mortality. As with the harvest data, the ME stocks suffered higher mortality. ANOVA analysis on Arcsine transformed percentage data confirm that the ME stocks had statistically higher mortality. The differences between the MA and MAX stocks are more difficult to explain. The MA core mortality data are essentially identical to the harvest estimates of mortality. The MAX data show substantially less mortality based on core collections (30%) than for the harvest method (50%). The major

difference between the data for the MA and MAX core collections is that fewer dead were collected in the MAX cores while numbers of live remain about the same. Currently, we have no explanation for this discrepancy other than sampling error, this is exemplified by the higher 95% confidence limits on the MAX and MAX 2 samples (Figure 4) Examination of the interval mortalty graph (Figure 9) shows a different pattern of mortality for the ME stocks relative to the other two groups. In general, both the MA and MAX lines experienced higher mortality during the first three sampling periods and then reduced mortality during the last three. This is particularly evident for the MAX stock. The ME stock experienced higher mortality that the other stocks in all but the first sampling period, and instead of being reduced, interval mortality increase during the last two sampling periods.

Because little of no QPX was found in any stock in NJ the above mortality cannot be ascribed to that disease. Other studies have shown that the MA lines, when grown in NJ experienced only light QPX infections.

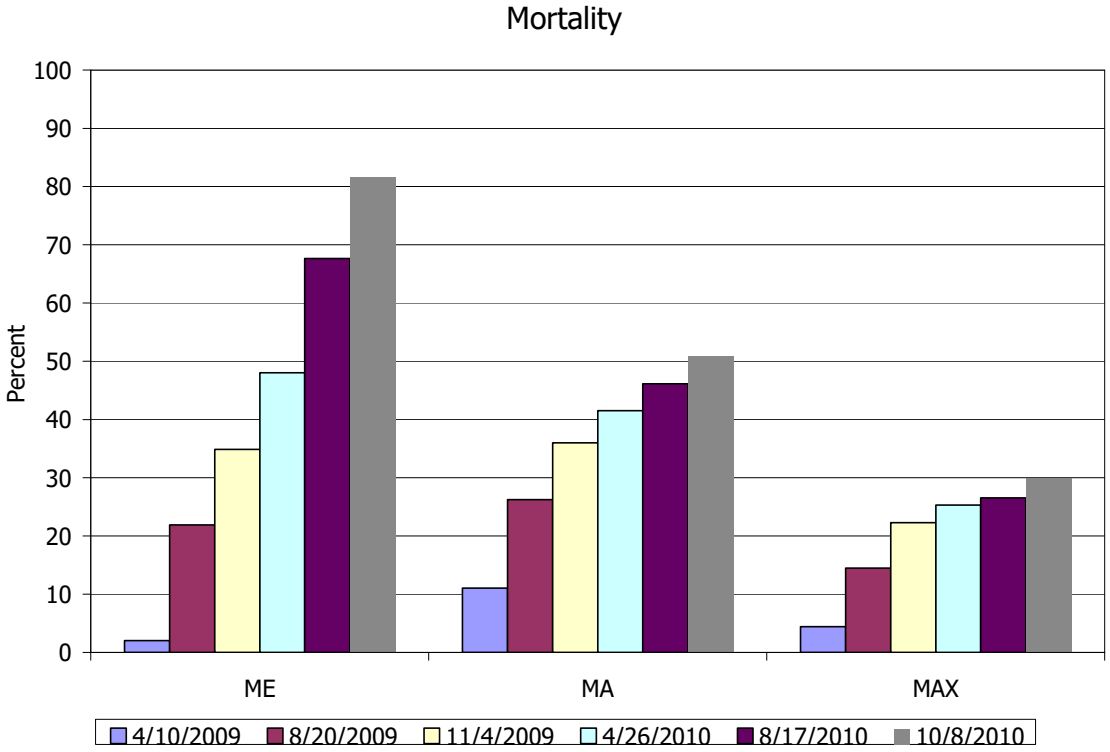


Figure 9. Average cumulative mortality based on live/dead counts from core samples of three stocks of hard clams (*Mercenaria mercenaria*) grown in Dry Bay, NJ. Stock are: ME = stock derived from parental lines collected in Maine, MA (=MASH) = selected Massachusetts line, MAX (=BX) = Selected line of QPX survivors from Massachusetts lines.

Condition Index

A condition index based on the dry weight of meat and shell length was constructed to provide a comparison between the stocks in Massachusetts and RI (Figure 10), and New Jersey (Figure 11). There were no differences between the MASH and BX

stocks until harvest at Scudders Lane research site where BX has a significantly better CI. The values for the ME stocks were less than half of the other stocks and, as with the growth and mortality data, suggests that this group was not doing well.

Figure 10. Scudders Lane Research Site, Barnstable, MA

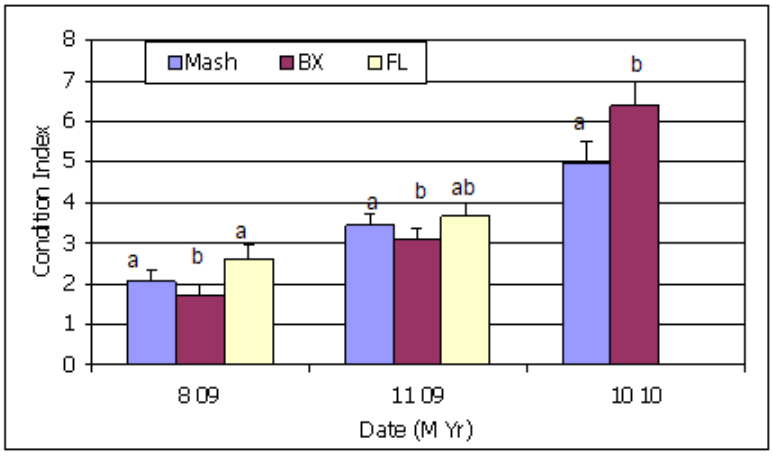


Figure 11. Dry Bay, NJ Research Site

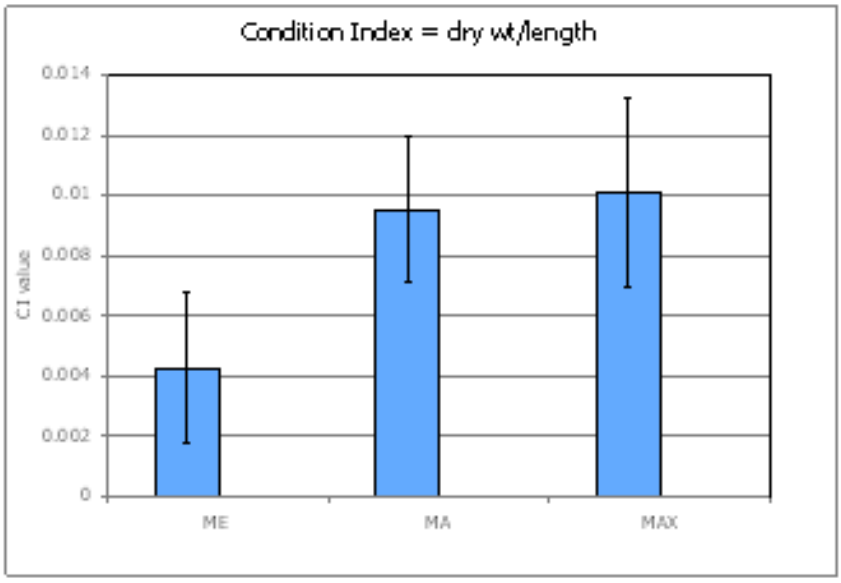


Figure 10. Average and 1 standard deviation error bars of condition index (CI) as dry meat weight relative to shell length for three stocks of hard clams (*Mercenaria mercenaria*) grown in Dry Bay, NJ. Stock are: ME = stock derived from parental lines collected in Maine, MA = MASH = selected Massachusetts line, MAX = BX= Selected line of QPX survivors from Massachusetts lines.

GENETICS RESEARCH SUB-REPORT
Characterizing molecular mechanisms associated with superior performing hard clams to assist in broodstock development.

Methods

Biological Collection

Hard clam (*Mercencaria mercenaria*) seed from two different broodstock sources were planted in Scudder's Lane, Massachusetts in Fall 2008. One broodstock cohort experienced severe QPX outbreaks in Barnstable Harbor, Massachusetts (Smolowitz, Karney personal communication). The second broodstock cohort was obtained from Mashpee, Massachusetts where there were no reported incidences of QPX. Seed clams were planted in 4 separate plots and will be referred to as BARN (also referred to as BX or MAX elsewhere in this report) and MASH, respectively. In August 2010, 40 clams were harvested from each cohort for histological analysis. Gill tissue was removed from a subset of clams (n=30) using sterile procedures and stored in RNAlater. RNA was extracted using TriReagent (Molecular Research Center) following manufacturers recommended protocol and stored at -80 for RNA-seq analysis..

RNA-seq Sample Preparation and Analysis

Total RNA samples from eight individuals from each cohort (BARN and MASH) were pooled in equal quantity. Samples were enriched for mRNA by processing with two rounds of the MicroPoly(A) Purist Kit (Ambion). Library preparation and sequencing was conducted by the University of Washington High Throughput Genomics Unit (HTGU) on the SOLiD 4 System (Applied Biosystems).

All sequence analysis was performed with CLC Genomics Workbench version 4.0 (CLC Bio). Initially, sequences were trimmed based on a quality scores of 0.05 (Phred; Ewing, Green, 1998; Ewing *et al.*, 1998) and the number of ambiguous nucleotides (>2 on ends). Sequences smaller than 20 bp were also removed. Quality trimmed reads from both libraries (BARN and MASH were *de novo* assembled using following parameters: limit=8, mismatch cost=2, and minimum contig size of 200 bp. Consensus sequences were compared to the Swiss-Prot database (http://uniprot.org) in order to determine putative description. Comparisons were made using the BLASTx algorithim with a maximum of 0.01 e-value threshold. Associated GO terms (Gene Ontology database; http://www.geneontology.org) were used to classify sequences based on biological process as well as categorize genes into parent categories (GO slim).

RNA-seq analysis was performed to determine differential gene expression patterns between the BARN and MASH libraries using the *de novo* assembly as a background. Expression values were measured in RPKM (reads per kilobase of exon model per million mapped reads, see [Mortazavi et al., 2008]). Parameters for RNA-seq analysis included; unspecific match limit = 10, maximum number of mismatches = 2, minimum number of reads = 10. Differentially expressed genes were identified as having ≥ 2 fold change in RPKM expression values and a significance of $p \leq 0.05$.

Significantly enriched GO terms were identified using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (http://david.abcc.ncifcrf.gov/). The Swiss-Prot accession numbers for differentially expressed genes were uploaded as a gene list while all Swiss-Prot accession numbers for annotated contigs (E-value $\geq$0.01) were used as a reference. Significantly enriched GO terms were identified as those with a p-value $\leq$0.05. Swiss-Prot accession numbers for significantly enriched GO terms were extracted for further analysis.

Restriction site associated DNA (RAD) marker library Preparation
Restriction site associated DNA (RAD) marker libraries were constructed to identify distinctive markers among the cohorts. Genomic DNA was isolated separately from the gill tissue BARN (n=4) and MASH (n=4) clams using DNAzol (Molecular Research Center) as per manufacturers recommendations. Libraries were prepared as described by Miller et al 2007. Briefly samples (n=8) were digested Sbf-1 (New England Biolabs), barcoded, and RAD adapters (PI and P2) were ligated on DNA fragments. Size selection of DNA fragments was achieved by running PCR on a 1% EZ gel (Invitrogen) with E-gel 1 kb Plus DNA ladder followed by purification using the MiniElute gel purification protocol. Subsequent library construction and sequencing was carried out by hte University of Washington High Throughput Genomics Unit (HTGU) using the Illumina GAIIx system.

RAD library analysis
Sequences were trimmed for for a minimum base pair phred quality score of 80 and then sorted by barcode to determine the representation of each sample in the library. Sequences associated with each cohort (BARN (same as BX) or MASH) were combined for SNP discovery. Identical sequence reads were aligned and enumerate were aligned and counted using NOVOALIGN. Sequences that were determined to lack any polymorphisms within a cohort were then assembled and SNP discovery performed to identify SNPs that distinguished each cohort (CLC Genomics Workbench v4.0). Any polymorphisms identified represent a SNP locus that is fixed for the individuals in a specific cohort examined.

Results

Clam Mortality and Disease
Field sampling indicated that of the course of the trials, hard clam size did not significantly differ (Figure 1). QPX was first detected in MASH clams in June 2010 (Table 1) and the prevalence increased from 12.5% in June to 45% in August (Table 2). Likewise, increased mortality was observed starting in June 2010 (Figure 2). No QPX was observed in BARN clams.

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Table 1. Results from Pathology report on clams harvested in June 2010.

Site	Stock / Plot	QPX distribution				QPX infection intensity					Tissue infected	Prevalence
		N[1]	F[2]	MF[3]	D[4]	N	R[5]	L[6]	M[7]	H[8]		
SL	BARN	40	0	0	0	40	0	0	0	0		0.0%
SL	MASH	35	5	0	0	35	3	2	0	0	G, Go, F, VM[13]	12.5%

[1] N = No infection detected.
[2] F = Focal - QPX cells clustered in one infection site or lesion.
[3] MF = Multifocal - QPX cells clustered in more than one infection site or lesion.
[4] D = Diffuse - QPX cells scattered throughout infected tissues.
[5] R = Rare (<10 cells/prep)
[6] L = Light (11-100 cells/prep)
[7] M = Moderate (101-1000 cells/prep)
[8] H = Heavy (>1000 cells/prep)
[9] G = Gill
[10] M = Mantle
[11] Go = Gonad
[12] VM = Visceral mass
[13] F = Foot

Table 2. Results from Pathology report on clams harvested in August 2010.

Site	Stock / Plot	QPX distribution				QPX infection intensity					Tissue infected	Prevalence
		N[1]	F[2]	MF[3]	D[4]	N	R[5]	L[6]	M[7]	H[8]		
SL	BARN	40	0	0	0	40	0	0	0	0		0.0%
SL	MASH	22	9	4	5	22	12	6	0	0	G[9], M[10], Go[11], VM[12], F[13]	45.0%

[1] N = No infection detected.
[2] F = Focal - QPX cells clustered in one infection site or lesion.
[3] MF = Multifocal - QPX cells clustered in more than one infection site or lesion.
[4] D = Diffuse - QPX cells scattered throughout infected tissues.
[5] R = Rare (<10 cells/prep)
[6] L = Light (11-100 cells/prep)
[7] M = Moderate (101-1000 cells/prep)
[8] H = Heavy (>1000 cells/prep)
[9] G = Gill
[10] M = Mantle
[11] Go = Gonad
[12] VM = Visceral mass
[13] F = Foot

Transcriptome sequencing

Sequencing of the hard clam transcriptome yielded a total of 72,352,632 and 58,578,559 reads from the BARN and MASH libraries, respectively. After quality trimming, 50,873,441 and 43,972,311 reads remained from the BARN and MASH libraries, respectively, with an overall average length of 36 bp. *De novo* assembly resulted in 59% of the reads assembling into 8,482 contigs with an N50 value of 250, and an average size of 259 bp and 136x coverage (Figure 12 and 13). A total of 2,437 contigs were annotated using the Swiss-Prot database. Of those contigs with associated GO descriptions, the two most represented biological processes include RNA metabolism and transport (Figure 14).

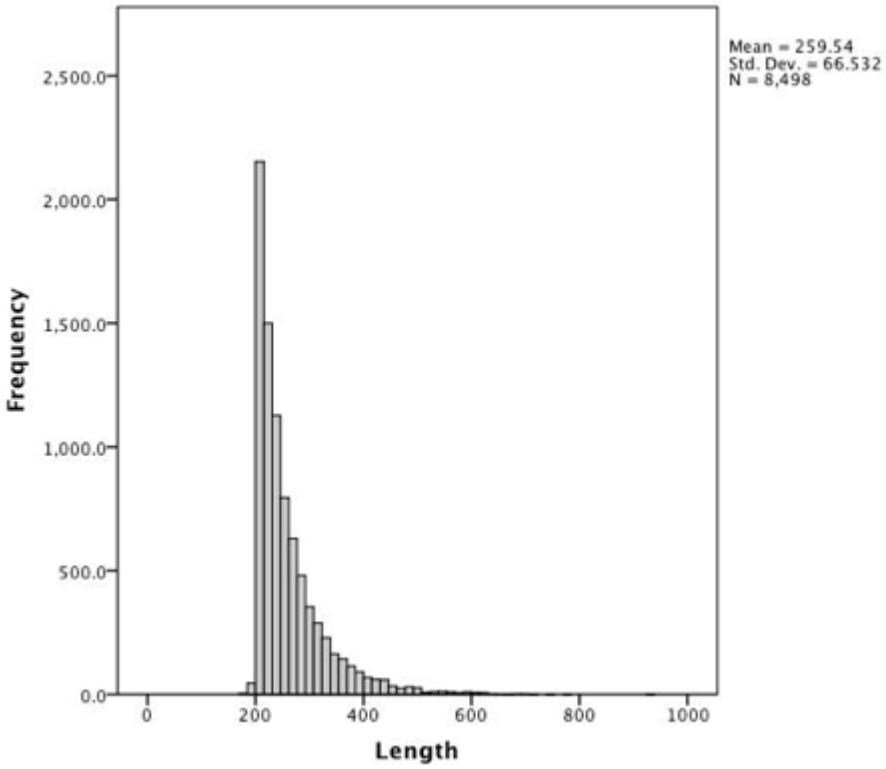

Figure 12. Contig length distribution from *de novo* assembly of reads from both libraries

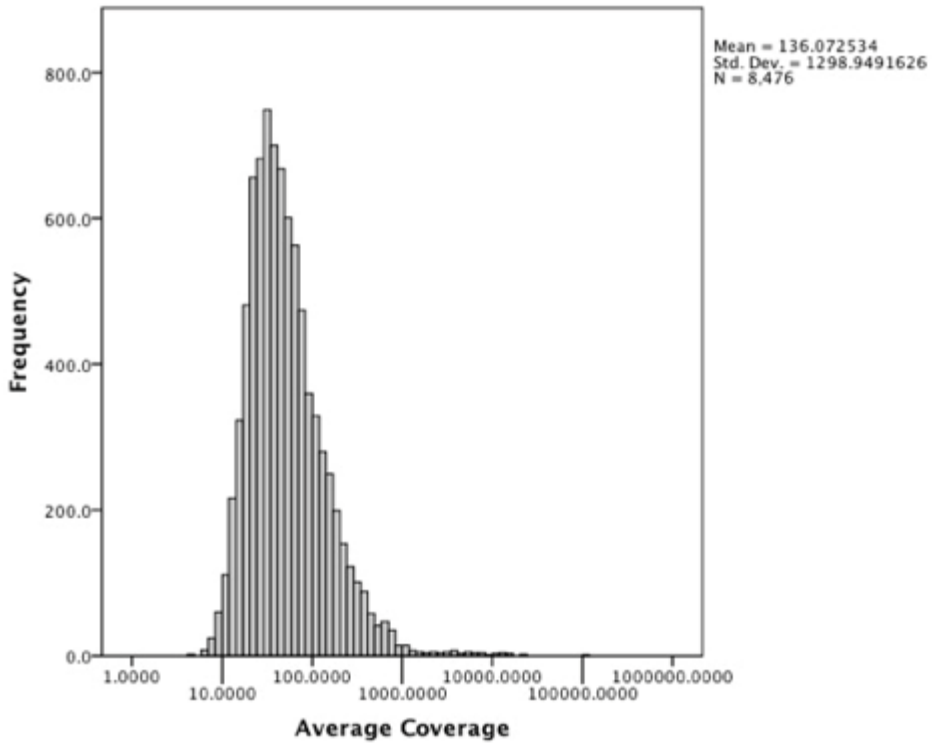

Figure 13. Average coverage distribution from *de novo* assembly of reads from both libraries

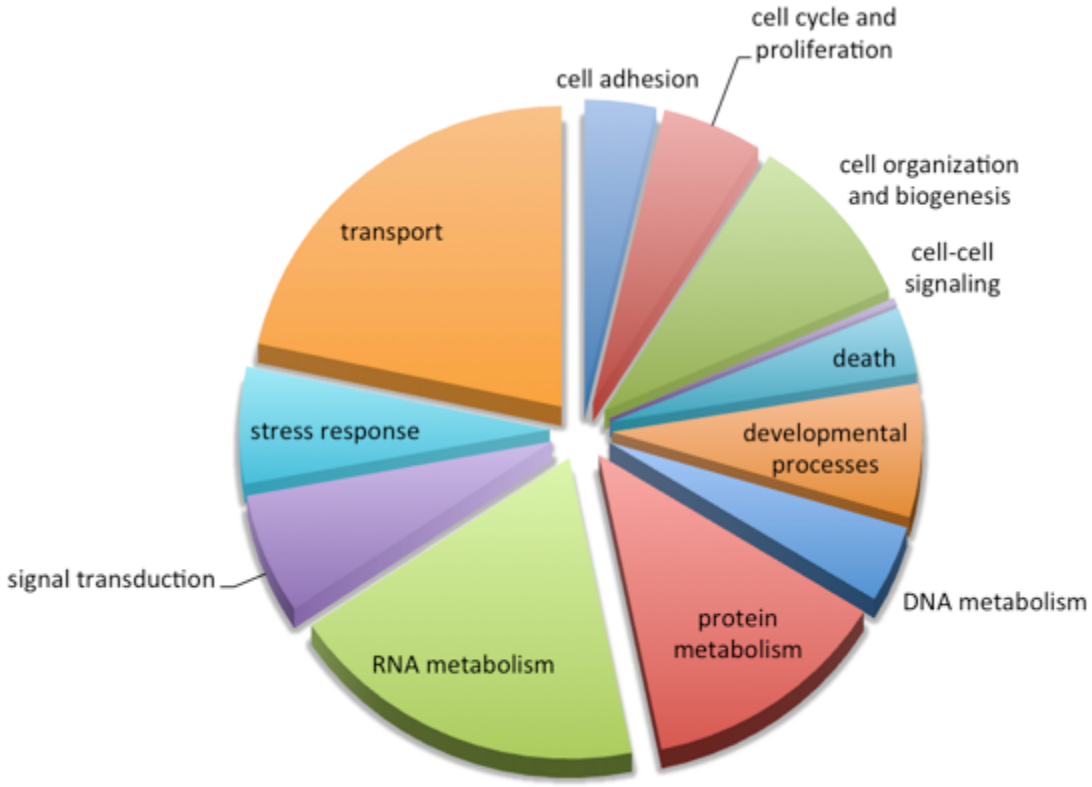

Figure 14. Annotation of contigs from assembly based on biological process.

RNA-seq

A total of 450 of the annotated contigs were differentially expressed across the two libraries with a majority (414) expressed at a higher level in the BARN library. Gene-annotation enrichment analysis revealed that that several biological processes of interest. In total, 203 of the differentially expressed hard clam contigs (corresponding to 193 unique Swiss-Prot ID) were identified as associated with significantly enriched biological processes (See Supplementary Online Table 1). One hundred eighty-seven were expressed higher in the BARN library. Enriched process associated with genes expressed at a higher level in the BARN library including ones involved in *proteolysis* (cysteine proteinase, puromycin-sensitive aminopeptidase, cathepsin) *apoptosis* (baculoviral IAP repeat-containing protein, Apoptosis 1 inhibitor), *toll-like receptor signalling pathway* (TNF receptor-associated factor 3, mitogen-activated protein kinase kinase kinase 7 interacting protein 1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, myeloid differentiation primary response protein MyD88), and *immune response* (superoxide dismutase, 60kDa heat shock protein, peptidoglycan recognition protein). There were relatively few contigs expressed at a higher level in the MASH library though two contigs of particular interest have high protein similarity to interferon-induced protein 44-like and mannose-binding protein, two components associated with histocompatibility complex.

RAD

After quality trimming there were 14.5 million reads from BARN clams (n=4) and 8.4 million reads from MASH clam (n=4), with a read length of 24bp. All but one individuals had between 2.4 and 4.7 millions reads. One MASH sample only had 23,000 reads. To asses overall genetic diversity of the strains, reads were characterized in silico for each strain. A de novo assembly of the BARN reads resulted in 4,491 contigs containing 543 putative SNPs. Assembly of the MASH reads resulted in 9,825 contigs containing 1372 SNPs. In other words, for BARN a SNP was identified at about every 165 bp whereas for the MASH strain, a SNP was identified about every 198 bp. The proportion of allele variation was similar across the strains with, as expected most variations were transitions with and average of 28.2% A/G variations across the two libraries and 24% C/T variations. The proportion of transversion was also similar across strains with A/C and G/T variations occurring approximately 11.5% of time. Interestingly the was a relative high proportion of A/T variation (18.4% in each library) and relative low proportion of C/G variation 7.1% in BARN and 5.6% in MASH.

In order to find diagnostic markers between strains, contigs without any variation within a single strain were compared to each other to identify DNA fragments that were diagnostic of a particular strain. The number of non-variable contigs for each strain was 8606 and 4845, for MASH and BARN respectively. Comparing these two sets of contigs revealed there were 2090 analogous sequences across strains with 1945 matching with 100% identity. The remaining 145 provide diagnostic markers to distinguish the to strains based on the samples used here.

Summary

As part of this research effort we have significantly contributed the genomic resources available for this species. Raw data is available to the public via NCBI (http://www.ncbi.nlm.nih.gov/bioproject?term=PRJNA70975). Over 100 million high quality sequence reads were generated with 8,482 transcriptome contigs characterized. Comparing gene expression between the two strains provides important insight into the processes associated with superior performing broodstock. Of the 203 differentially expressed genes associated with an enriched process, a majority were expressed at a higher level in the BARN. Genes identified are involved in proteolysis, apoptosis, toll-like receptor signaling and general immune function. These data suggest that successful implementation of these responses are associated with increased survival. Analysis focused on gDNA (RAD) characterized fundamental parameters including mean SNP occurrence (1 SNP / 182 bp) and transversion/transition percentages. Finally, 145 putative SNP markers were discovered that will assist in the identification of superior performing clams.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Lindell and Diane Murphy participated in a workshop for growers in Barnstable County, MA in May 2010 to update them on the progress of this and other clam research projects. Presentation will be made at Milford Aquaculture Seminar March 2012.