A Focused Literature Search on Potential Effects of Hard Clam Aquaculture on Wild Populations

Delaware Center for the Inland Bays
Shellfish Aquaculture Initiative

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525 × 378 - Hard Clam, *Mercenaria mercenaria*

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Executive Summary

In response to a lack of information pertaining to disease and potential genetic impacts from hard clam aquaculture to wild hard clam populations, Cardno ENTRIX was retained by CIB to perform a focused literature search in an attempt to determine the state of science regarding these concerns.

Relative to the concern of hard clam aquaculture being associated with the cause or spread of disease outbreaks in the wild hard clam population, the literature search revealed the following:

- The primary disease for hard clams along the U. S. East Coast is known as Quahog Parasite Unknown (QPX), an algae-base disease.
- QPX is opportunistic (infects stressed hard clams) and can be found occurring in sediment, plants, and invertebrates.
- The salinity and temperatures within the Inland Bays are sufficient to support QPX, however, the salinity and temperatures are not ideal for QPX disease outbreaks.
- An investigation published in 2007, where hard clam from the Inland Bays were collected and test for QPX, indicated that no hard clams were found to be infected with QPX.
- QPX has not been found to be imported by hatchery-raised hard clam farm stock.
- Although QPX outbreaks have been predominantly associated with farmed hard clam stock, QPX outbreaks have been identified in wild populations.
- Wild hard clam populations tend to have a higher resistance to QPX (are “fitter”) than farmed hard clam stock.
- The review did not find direct evidence that farmed hard clam stock causes disease outbreaks in wild hard clam populations.

Hard clam QPX disease is not a current or historic problem within the Delaware Inland Bays. Based on the above noted, risk of disease outbreaks in wild hard clam populations associated with aquaculture activities appears to be low.

Regarding the potential for farmed hard clam stock causing genetic impacts to wild hard clam populations, the literature review revealed the following:

- Literature regarding genetic impacts to wild clam stock through introduction of farmed stock is limited.
- Hybridization between species may occur (e.g., southern hard clam and northern hard clam).
- Although “foreign” hard clam stock has been demonstrated capable of affecting the genetics of wild hard clam populations, the affects have not been demonstrated to impact survival or fitness of wild populations.

Many states control where the hard clam aquaculture seed sources originate in order to reduce disease-caused mortality and genetic impacts. Requiring seed stock to originate for the Inland Bays could eliminate much of the risk associated with genetic impacts to the wild hared clam populations.

1 Introduction

1.1 Background

During June of 2011, the Center for the Inland Bays (CIB) held a workshop to evaluate the potential for shellfish aquaculture within Delaware's Inland Bays, a practice that is currently illegal. Based on the findings of the workshop and further discussions, a Shellfish Aquaculture Tiger Team (The Team) was formed on March 30, 2012, during a CIB board of directors meeting. The Team, that has met monthly, includes the CIB, the Delaware Sea Grant Advisory Service, Delaware Department of Natural Resources and Environmental Control (DNREC), Delaware Department of Agriculture, consultants, commercial shellfishing interests, and other stakeholder groups representing private and recreational interests. The CIB has defined the Team's mission as follows:

> *"The Tiger Team is working on three fronts:*
>
> *The Policy, Permitting and Funding Subcommittee is reviewing current rules and regulations in the Delaware Code and will propose draft revisions and legislation to permit commercial aquaculture on the Inland Bays for consideration by the State Legislature.*
>
> *The GIS Spatial Planning Subcommittee is mapping existing uses and activities on the Bays to determine the areas that shellfish aquaculture can occur in balance with other Bay users.*
>
> *The Education and Outreach Committee is working to inform the public about the economic opportunities that commercial shellfish aquaculture can bring to our community and the ecological benefits it could bring to the Inland Bays; and build support for legislation permitting shellfish aquaculture on the Inland Bays."*

During this process, certain involved parties expressed an interest in incorporating hard clam aquaculture into the code and regulations, rather than limiting the effort to oyster aquaculture, as originally conceived. The DNREC Division of Fish and Wildlife (F&W) responded to this interest by stating they would support hard clam aquaculture in Little Assawoman Bay, but that they would not support hard clam aquaculture in Rehoboth Bay, Indian River Bay, and Indian River because it would conflict with an established hard clam fishery. F&W later added, to their rationale for not supporting hard clam aquaculture, concerns about the introduction and spread of disease from aquacultured clams to wild clams and the effect this could have on the wild clam population and fishery. Concerns about the genetic impacts to wild hard clam populations were also raised by F&W. However, during initial due diligence evaluation of these concerns, it became clear that supporting literature on these topics was not readily available. In response to this lack of information, Cardno ENTRIX was retained by CIB to perform a focused literature search in an attempt to determine the state of science regarding these concerns.

1.2 Scope of Literature Search

The literature search efforts targeted two areas: hard clam disease and genetic effects. Specifically, the following two questions were developed for the scope of this literature search effort.

1. ***What is the state of science regarding hard clam aquaculture and the spread of hard clam diseases in both wild and farmed populations, during low-level and epizootic occurrences, and within estuarine systems?***
2. ***What is the state of science regarding the potential for impacting wild genetic hard clam stock through the introduction of farmed hard clam populations during aquaculture***

operations? Furthermore, if there are documented genetic effects, did they result in negative effects (e.g., reduce fitness) or positive effects (e.g., increased disease resistance)?

This scope of work is limited to identifying, reviewing and summarizing the state of the science and to provide CIB with information requested for The Team to make informed recommendations and develop a position regarding shellfish aquaculture in the Inland Bays. The information provided herein is anticipated to supplement the information that the Team will consider while making its recommendations.

1.3 Search Strategies and Screening

The complexity of a search strategy varies depending upon the subject matter. For popular or highly studied topics, a focused or specific search strategy is employed. Conversely, for more atypical or less studied topics, a more general strategy is often used; in essence, allowing the researcher to cast a broader net. The search strategies and screening process used for this effort was kept somewhat simplistic, due to the anticipated limited volume of pertinent literature. A separate search strategy was used for each of the questions.

For Question 1, the search strategy use was:

"HARD CLAM" AND AQUACULTURE AND DISEASE

For Question 2, the search strategy used was:

"HARD CLAM" AND AQUACULTURE AND GENETIC

In both cases, "HARD CLAM" was a primary phase, and AQUACULTURE and DISEASE/GENETIC were secondary. The searches were done in combined searches of the 59 Dialog databases (see Appendix A for full listing of databases) that had a significant number of records on environmental subjects. These databases are continuously updated and contain several hundred million indexed records.

The seven databases that had the most records and/or that related most closely with this topic (as of February 2013) were picked to obtain relevant individual records. The following list summarizes those databases that appeared to be most relevant for the topic.

Search: *"HARD CLAM" AND AQUACULTURE AND DISEASE*

```
Items  File
-----  ----
  38    5: Biosis Previews(R)_1926-2013/Feb W1
   9   24: CSA Life Sciences Abstracts_1966-2013/Jan
  42   28: Oceanic Abstracts_1966-2013/Jan
  10   34: SciSearch(R) Cited Ref Sci_1990-2013/Feb W1
  52   44: Aquatic Science & Fisheries Abstracts_1966-2013/Jan
  29   50: CAB Abstracts_1972-2013/Feb W1
   2   78: Aqualine_1966-2013/Jan
```

Search: *"HARD CLAM" AND AQUACULTURE AND GENETIC*

```
Items   File
-----   ----
  37     5: Biosis Previews(R)_1926-2013/Feb W1
   6    24: CSA Life Sciences Abstracts_1966-2013/Jan
  29    28: Oceanic Abstracts_1966-2013/Jan
  10    34: SciSearch(R) Cited Ref Sci_1990-2013/Feb W1
  41    44: Aquatic Science & Fisheries Abstracts_1966-2013/Jan
  25    50: CAB Abstracts_1972-2013/Feb W1
   1    78: Aqualine_1966-2013/Jan
```

Obvious duplicates were removed from the combined results. The following provides a summary of the searches.

```
? S (HARD()CLAM) AND AQUACULTURE AND DISEASE?
    S1    182  (HARD()CLAM) AND AQUACULTURE AND DISEASE?
    S2    106  Removed Duplicates  (unique items)
? S (HARD()CLAM) AND AQUACULTURE AND GENETIC?
    S3    149  (HARD()CLAM) AND AQUACULTURE AND GENETIC?
    S4     78  Removed Duplicates  (unique items)
```

The titles, subject headings and dates were printed out for review by a senior staff member of Cardno ENTRIX familiar with the subject matter. Relevant records identified during the review were screened and prioritized into three categories ranging between most important to least important. The prioritization dictated the order in which the records were reviewed, in case there was insufficient time to review all identified records. Full citations and abstracts were located for the records in these three categories. For most records, the full papers were obtained. Subsequently, all selected records in each category were reviewed. Appendix B includes a listing of literature (records) reviewed and Appendix C includes a copy of each record reviewed.

2 Summary of Findings

2.1 Question Number 1

What is the state of science regarding hard clam aquaculture and the spread of hard clam diseases in both wild and farmed populations, during low-level and epizootic occurrences, and within estuarine systems?

2.1.1 General Information

Quahog or hard clams (specifically, *Mercenaria mercenaria*) are found in native populations along the North American Atlantic shoreline, from Canada south to Florida and into the Gulf of Mexico (Roegner and Mann, 1991). Several variants of hard calms have developed in different geographic regions with slightly different genetic composition (Calvo et. al 2007). Despite its sizeable distribution, hard clam aquaculture has developed to support the associated commercial fishery demand. Virtually all farmed hard calms are produced from hatchery reared offspring (Kraeuter et al., 2011).

Regardless of whether a population is wild (feral) or farmed, the primary disease facing clams is Quahog Parasite Unknown (QPX) (Calvo et al., 2007, Smolowitz et al., 1998, Stokes et al., 2002). QPX has historically been found to cause mortalities in northern waters, from the Mid-Atlantic to Canada, with reports dating back to the 1950's (Smolowitz et al., 1998). Although QPX has been recorded primarily in cultured clams, it has also been observed in wild populations (Stokes et al., 2002). Testing performed on cultured clam stocks indicated the absence of QPX in hatcheries, which suggests a natural occurrence of QPX in the environment (Kraeuter, 2011). Different strains of clams have been shown to have varying resistance to the QPX infection (Dahl et al., 2010, Dahl et al., 2008).

To adequately address the subject question, it is necessary to have an understanding of the prevalent disease etiology/pathology, the base survival requirements (habitats, food web, etc.) of hard clams, and available research evaluating the existing state of diseases for hard clams and aquaculture.

2.1.2 QPX Etiology

The literature search provided information on the etiology of QPX. Provided below is a summary of this information.

> QPX is a widely present protist-based disease that impacts clam populations from Northeastern Canada down to Virginia. Of the literature reviewed, no infections have been reported in southern coastal waters, south of Virginia (Perrigault, et al., 2011).

> QPX is an opportunistic disease (Coen, et al., 2004). As such, QPX typically requires some stressor on the hard clam population in order for outbreaks to occur.

> QPX is not represented by a single species or strain but is believed to result from infections caused by one of multiple strains with varying virulence (Calvo et. al 1998, Dahl et. al 2008).

> It is also becoming more widely recognized that QPX infection occurs after planting and is not present in the hatcheries (Kraeuter et al., 2011).

> Presence of QPX has been identified in many coastal and estuarine waters, but does appear to be limited by salinity (Calvo et. al 1998, Carnegie, 2008). Research has suggested that QPX pathogenicity is greatest at salinities between 30 and 34 ppt, but that QPX can exist (but not thrive) in salinities as low as 20 ppt (Kraeuter et al., 2011).

> Temperature may control the spread of QPX agents, which is most evidenced by the lack of QPX in southern waters (Carnegie, 2008). Lab studies have suggested that the individual growth of QPX protists is optimal between 20 and 23 Degrees Celsius (Kraeuter et al., 2011); however the ability of clams to fight off disease and heal themselves once infected was shown to improve at 21 degrees Celsius (Dahl et al., 2011 and Perrigault, et al., 2011). QPX exhibits a greater pathogenicity between 13 and 21 degrees Celsius, but exhibits no pathogenicity above 27 degrees Celsius (Perrigualt, et al., 2011). It is still unclear as to the extent temperature affects QPX's ability to infect hard clams and the ability of the hard clam to resist or heal from QPX infections. Research has also identified the potential usefulness of relocating infected clams to warm water embayments to help reduce stress and increase disease resistance (Dahl et al., 2011).

> The acidity level of the environment may also contribute to the ability of QPX to survive and reproduce. One study suggested that higher pH values may be beneficial to QPX, whereas lower pH values may slow the growth of QPX (Brothers et al., 2000).

> Most historic outbreaks of QPX occurred in intertidal systems. Dove, et al. (2004), studied one of the first major QPX outbreaks identified in subtidal and wild hard clam populations. The outbreak occurred in the Raritan Bay, and for the first time, the disease presented with visceral and gonadal infections. This occurrence documented that wild hard clam stock is susceptible to QPX in all hard clam habitats. Lyon (2007) also documented QPX infections in hard clams found in both subtidal and intertidal habitats.

> Hard clams in the intermediate size range of 20 to 55 mm (just below market size) are most susceptible to QPX (Lyons, 2007).

> Calvo, et al., 1998, suggested that the QPX pathogenicity may differ regionally. Example, QPX organisms in Massachusetts may be more pathogenic than QPX organisms in Virginia. This suggestion may be misleading because QPX has been present in New England for a longer period of time than in the Mid-Atlantic, therefore, the pathogenicity may be more advanced in the north.

> Acclimatization has not been identified as a stressor that promotes QPX infection. Various studies have found QPX infection to increase most drastically in non-native (out of state) hard clams more than a year after initial planting when the clams should have had time to acclimatize (Dahl et al., 2010, Ford et al., 2002). Another study also found infection of Floridian clams in New York waters, soon after planting despite water temperatures remaining warm the entire time (Dahl et al., 2010).

2.1.3 Hard Clam Environmental Preferences

Many of the studies reviewed suggested a link between the ability of the clam to resist QPX and the presence or absence of additional stressors (Carnegie, 2008, Kraeuter et al., 2011, Dahl et al., 2011).

Several studies have suggested that reducing hard clam density in aquaculture operations, in addition to helping limit the spread of disease, may reduce the stress on the clams (Carnegie, 2008). However, there has been little to suggest the need for a major departure from existing aquaculture standards and densities used in clam culturing (Dahl et. al 2010, Dahl et al., 2011, Ford et al., 2002). Provided below are readily measurable hard clam environmental preferences based on the literature.

> Temperature is known to determine the effective filtration rate of clams, with clam pump rates being their highest between 24 and 26 degrees Celsius (Dahl et al., 2011, Roegner and Mann, 1991). Adult Clams can survive in water at temperatures between -6 and 45.2 degrees Celsius; however the optimal range is between 21 and 31 degrees Celsius. It was also noted that clams may better fight infection at moderate temperatures (21 degrees Celsius) (Dahl et al., 2011).

> The ideal salinity ranges for hard clams is between 26 and 27 ppt. Salinity below 20 ppt may result in reduced abundance (Roegner and Mann, 1991). Hard clams have been known to tolerate large fluctuations in salinity, changes of up to 15 to 20 ppt (Bergquist et al., 2008).

> Hard clams are tolerant to low dissolved oxygen (DO) at all life stages (Roegner and Mann, 1991).

> The ideal pH range for the clams is between 7.50 and 8.50 (Roegner and Mann, 1991).

> Additional water quality parameters, such as sediment loading and pollution are detrimental to clams at high concentrations.

2.1.4 Hard Clam Susceptibility to QPX

Hard clam losses due to QPX are still being documented; both in wild populations and in aquaculture of hard clams. Most documented losses are associated with aquaculture operations, however, there have been documented cases of losses to wild populations as well. For example, mortalities in wild hard clam populations have been noted near Raritan Bay, within the New York portion, with mortality in clams averaging 45-55 mm shell height (Coen et al., 2004).

Several studies have shown trends in prevalence and disease severity correlated with geographic location along the east coast of source populations (Calvo and Burreson, 2002; Calvo et al., 2007; Dahl et al., 2008, 2010, and 2011). Specifically, hard clams derived from the southeast coast were shown to be much more susceptible to QPX than their northeastern (Massachusetts and New Jersey) counterparts (Calvo et al., 2007 & Dahl et al., 2008).

Calvo et al., (2007) studied the correlation between *M. mercenaria* genetic origin and geographic location with susceptibility to QPX disease. Stocks from Florida, South Carolina, Virginia, and New Jersey were cultivated in two cultures, one in Virginia and the other in New Jersey. After two years of monitoring, clams originating from Florida and South Carolina were shown to have significantly higher QPX levels, and the lowest survival rate, relative to stocks from New Jersey and Massachusetts which had the lower QPX levels and highest survival rate. The Virginia stock did not statistically differ from either the northern or southern stocks (nestled in the middle). The Calvo research also noted that the greater prevalence of QPX in cultured hard clam populations may suggest that higher planting densities, such as those in aquaculture environments, may factor into disease susceptibility.

The historic occurrence of QPX in Massachusetts may have permitted the selection of resistant clams in these northern areas (Dahl et al., 2008). When cultured in conditions suitable for QPX propagation (see Section 2.1.2), clams originating in the north have a greater resistance to and lesser mortality from QPX (Dahl et al., 2008). In a similar study to Calvo et al. (2007), Dahl et al., (2008) observed differences in QPX severities between genotypes, using hard clam populations from Massachusetts, Virginia, New York, and Florida. This study also found that clams originating further south were less resistant to QPX than those in the north, as the seed clams from Massachusetts were the most resilient of all strains in the trial. All samples in this study were kept in controlled conditions within a lab, reducing the probability that stress from the placement of southern clams into northern waters is the cause of southern clams' reduced ability to fight off QPX infection (Dahl et al., 2008).

2.2 Question Number 2

What is the state of science regarding the potential for impacting wild genetic hard clam stock through introduction of farmed hard clam populations during aquaculture operations? Furthermore, if there are documented genetic effects, did they result in negative effects (e.g., reduce fitness) or positive effects (e.g. increased disease resistance)?

2.2.1 General Information

Genetic interaction between introduced aquaculture stocks and wild, native populations of hard clam species is a consideration when assessing aquaculture operations. Changes in genetic composition of wild hard clam populations have the potential to affect the fitness and resilience of these populations, though this concept is poorly studied in hard clam genetic literature (Arnold, et al., 2009).

2.2.2 Inter and Intra Species Hybridization

Arnold, et al., 2004 and Arnold, et al., 2009 studied the effects on the native *Mercenaria campechiensis* (southern quahog) by aquaculture introduction of *Mercenaria mercenaria* (northern quahog). In both studies, hybridization occurred between the wild *M. campechiensis* and introduced *M. mercenaria*. Genotypes from before the introduction of aquaculture were predominately *M. campechiensis*, but individuals born after aquaculture introduction showed much more presence of the hybrid and *M. mercenaria genotypes*.

In addition to showing hybridization between two different hard clam species, Arnold et al. 2009 also showed the introgression of cultured *M. mercenaria* alleles into wild populations of *M. mercenaria* by observing the presence of the “notata” allele in wild populations. The “notata” allele is expressed as an obvious physical marking, and this therefore is commonly selected for by aquaculturists to help differentiate between cultured and wild clams. Less than 2% of wild clams exhibit the “notata” coloration in areas where aquaculture has not yet been introduced. However, in locations with aquaculture operations, the “notata” markings are commonly found in wild populations of *M. mercenaria*, indicating an exchange of genes between cultured and wild *M. mercenaria* populations. The authors state that while this is the most apparent indicator of genetic exchange, there are most likely other, less obvious changes to the wild species’ genome (Arnold, et al., 2009).

Metzner-Roop, 1994, looked at the dispersal of genes from transplanted Massachusetts clams in South Carolina waters, 3 years after the shutdown of a local aquaculture operation. Metzner-Roop, 1994, cited Dillon et al. 1988 to generate their hypothesis that presence of the foreign genome due to aquaculture can dilute the native population beyond recognition. However, Dillon and Manzi (1990) compared nursery stocks of hard clams (selected for fast growth) to corresponding wild populations. Neither the cultured nor the wild populations exhibited a loss in heterozygosity (two different alleles at a specific locus; one of the first genetic "parameters" typically evaluated in natural population studies). Genetic drive was apparent, suggesting that crosses could result in genetically different lines. Metzner-Roop (1994) found no significant presence of Massachusetts alleles in the current South Carolina population. It is noted that the “notata” allele may have been selected against by natural pressures, such as predation, which allowed for the low prevalence of this gene 9 years after initial exposure.

There is evidence from other fisheries studies showing that genetic exchange will occur with the introduction of aquaculture. Diana, 2009 discusses the potential negative genetic interactions between naturalized and cultured fishes. Most of this discussion focuses on exchange between two different species, or escape of cultured individuals into the wild and becoming invasive. It is noted that avoiding negative impacts of invasive species or genotypes is to culture species within their native, or common, range (Diana, 2009).

2.2.3 Effects of Hybridization on Fitness

While several studies have shown some genetic interaction as a result of aquaculture introduction (Arnold et al. 2004; Arnold et al. 2009; Diana, 2009; Dillon, 1990), there is little information in the literature regarding the effects these genetic changes have on species fitness.

In the Gulf of Mexico, for example, the introduction of *M. mercenaria* due to aquaculture results in hybridization with the native species *M. campechiensis*. Hybrid individuals were shown to have no more susceptibility to the most prevalent debilitating disease in the Gulf of Mexico, gonadal neoplasia, than their pure-species counterparts (Arnold et al., 2009).

In some cases, aquaculturists breed their individuals to exhibit certain traits such as the “notata” allele, size, and infertility (to prevent inter-breeding) (Diana, 2009). While these traits may be detrimental in some way to wild populations, none of the papers reviewed discussed specific applications of this concept to hard clam aquaculture.

2.3 Uncertainty Analysis

The uncertainty analysis associated with literature searches primarily looks at two variables, effectiveness of the literature search and the accuracy of the literature reviews. The literature search for this effort implemented relatively general search strategies. The strategies provided for a broad capturing of related records. Based upon the limited number of records identified with this type of search strategy, it is suggestive that there is limited number of records publicly available on the search questions. The search also captured less than 25 percent records that were not directly applicable, indicating acceptable search resolution. Finally, during the review of the records, there was a high frequency of inter-record referencing indicating a relatively small number of studies and researchers.

Regarding review accuracy, senior personnel selectively reviewed the information summarized by the primary reviewers. The accuracy was high and there was agreement between similar records. A similar literature review was performed by the South Carolina Department of Natural Resources – Marine Research Division between 2002 and 2004. The findings of this review were in high agreement with that presented herein.

3 Interpretation of Findings

The interpretation of findings is intended to provide an object interpretation of the research presented. It is not intended to represent any personal or political views. The interpretation of findings also provides insight relative to how the gathered information applies to the two issues identified by CIB for shellfish aquaculture in the Inland Bays.

3.1 Question 1

What is the state of science regarding hard clam aquaculture and the spread of hard clam diseases in both wild and farmed populations, during low-level and epizootic occurrences, and within estuarine systems?

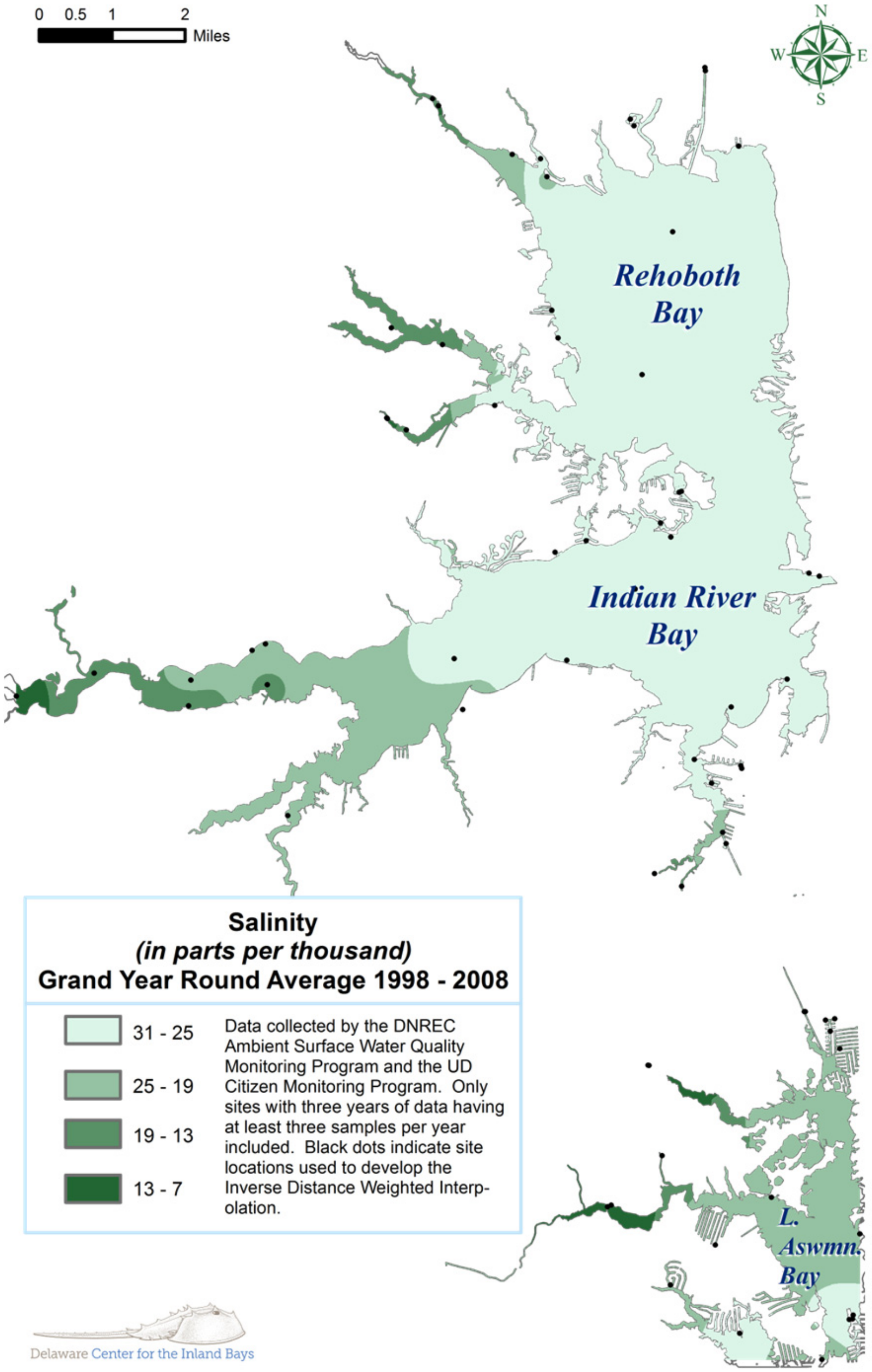
The literature review for this question included targeting the primary hard clam disease, QPX; etiology; the baseline hard clam habitat requirements; and regional trends and related studies. QPX pathogenicity is significantly influenced by salinity and temperature. Comparing minimal salinity survival requirements (≈ 20 ppt) and the salinity range for optimal pathogenicity (≈ 30 to 34 ppt) identified in literature to that which occurs in the Delaware Inland Bays (Figure 1 – Salinity map for the Inland Bays), the following considerations were identified:

> QPX is capable of existing in most areas of Delaware's Inland Bays.

> Freshwater inputs and precipitation, in certain portions of the Inland Bays, have the potential to prevent QPX occurrence. The likelihood of QPX displaying high or moderate pathogenicity is greatly reduced due to insufficient salinity.

The effects of water temperature on QPX pathogenicity is more complicated because QPX prefers cooler waters, becomes more pathogenic as water warms between 13 and 21 degrees Celsius, but does not exhibit pathogenicity at temperatures above 27degrees Celsius . However, as water warms, especially from 21 degrees Celsius and greater, the ability of the hard clam to fight QPX infection also increases. As such, water temperature in the Inland Bays may be at optimal temperatures for QPX to infect hard clams during 35 to 55 percent of the year.

The Inland Bays are not far from the southern extent of the QPX disease. The literature suggests that the strains expected to be within or nearby the Inland Bays are of lesser pathogenicity than more northern strains. Conversely, the literature clearly indicates that the hard clam's natural resistance to QPX increases from being poorly resistant in the south (e.g. South Carolina and Florida) to highly resistant in the north (e.g., Massachusetts). The Inland Bays are located in a region that has been generally grouped with the resistant genotypes, but also recognized as the least resistant in that group. Investigations published in 2007 by Ulrich et al. reported that QPX was not detected in wild hard clams collected in the Delaware Inland Bays. Although consistent with the above noted, the findings of Ulrich et al. are not conclusive regarding the presence of QPX in wild hard clams or in other media within the Inland Bays.



Data collected by the DNREC Ambient Surface Water Quality Monitoring Program and the UD Citizen Monitoring Program. Only sites with three years of data having at least three samples per year included. Black dots indicate site locations used to develop the Inverse Distance Weighted Interpolation.

Figure 1 **Delaware Inland Bays Salinity Map**

Virtually all aquaculture hard clams come from hatchery brood stock. Where the seed stock originates is significant to the success of the farmed clam survival. QPX epizootics in cultured hard clams have abated following the voluntary (NJ) and mandatory (VA) restrictions on importing clam seed produced from SC and FL brood stocks. Likewise in MA, QPX-associated mortalities of cultured hard clams have decreased because the practice of importing seed from out-of-area stocks has ceased. In MA, significant losses in aquaculture operations still occur despite the use of local clam seed, which proved to be highly resistant to QPX (Calvo, et al., 2007). "….Hard clam culturists should consider the geographic origin of clam seed an important component of their QPX disease avoidance/management strategies. In particular, southern stocks should not be used to produce seed to be grown in the area where QPX is enzootic." (Calvo, et al., 2007).

In summary, the literature review indicated the following key points:

> The Delaware Inland Bays do not provide optimal conditions for QPX pathogenicity;

> Hatchery populations used in aquaculture do not transport the disease;

> The implementation of proper restrictions to control the source of brood stock would appear to serve to further reduce the probability of QPX outbreaks; and

> Although the majority of records reviewed focused on farmed stock survival and health relative to QPX, there was no direct evidence suggesting farmed hard clam stock causes disease outbreaks in wild hard clam populations.

3.2 Question 2

What is the state of science regarding the potential for impacting wild genetic hard clam stock through introduction of farmed hard clam populations during aquaculture operations? Furthermore, if there are documented genetic effects, did they result in negative effects (e.g., reduce fitness) or positive effects (e.g. increased disease resistance)?

The available literature on impacts to wild hard clam stock through the introduction of farmed hard clam populations is limited. Literature has indicated that hybridization between similar clam species may and has occurred in other regions. The literature is inconsistent regarding the competition, resilience, or reproduction of hybrid populations. Some of the records provide hypotheses or predictions on how these hybrid populations could affect wild populations, but there was no consistency on this topic.

Regarding intra-species variation regionally and between farmed and wild hard clam stock, there is evidence that "foreign" genomes can affect local wild stock genomes. In certain cases, slight visual changes, such as "notata," have occurred in both intra and inter species mixing. However, none of the records have demonstrated significant impacts to heterogenic characteristics of the wild hard clam stock. The general trend is that local wild species seem to have maximized resistance to the local strain(s) of QPX. As such, the local stock remains the fittest and most capable of fighting off QPX infection. The literature also recommends obtaining brood stock using local/regional seed sources to further reduce genetic impacts.

3.3 New Questions

The majority of the literature reviewed evaluated hard clam susceptibility to QPX, regional variation of QPX pathogenicity, tolerances variation of hard clams relative to their state of origin. The literature search did not define consistent or specific infection source or transport. That is, hard calms were placed in an environment (lab or natural) for a period of time and were then tested for the disease. The presence, symptoms, and severity were often reported, but not the mechanism of infection. How does QPX spread during outbreaks? Where does it come from?

Most literature brushes over this very difficult question because there is no one correct answer. As noted, QPX is generally believed to be an opportunistic disease and can exist in many strains living in sediment, invertebrates, plants, shellfish, etc. QPX outbreaks tend to occur during periods of hard calm stress, such as extreme temperatures or salinity, high densities, exposure to pollution, etc. Because of this complexity of strains and stressors, each region/system has the potential of having its own highly unique etiology. The presence/absence form of study is practical.

Lastly, the vast majority of reported QPX-related mortality and outbreaks are related to aquaculture operations. However, outbreaks have occasionally been reported with wild populations. Studies have consistently indicated that the disease is not imported hard clam hatcheries, therefore, it is likely present prior to commencement of aquaculture activity. We often do not know what causes the outbreaks or where the infectious strain of QPX originated. We also do not know whether or not wild hard clam mortality is occurring more often than reported, because wild populations of hard calms are not monitored as extensively as aquaculture or hatchery populations.

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APPENDIX

A

LISTING OF DIALOGUE DATABASES SCREENED

Appendix A
Listing of Dialogue Databases Screened

ALLENVIR list (59 files)

File Name

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   2: INSPEC_1898-2013/Feb W1
   5: Biosis Previews(R)_1926-2013/Feb W2
   6: NTIS_1964-2013/Feb W1
   8: Ei Compendex(R)_1884-2013/Feb W2
  10: AGRICOLA_70-2013/Feb
  24: CSA Life Sciences Abstracts_1966-2013/Jan
  28: Oceanic Abstracts_1966-2013/Jan
  29: Meteorology & Geoastrophysical Abstracts_1966-2013/Jan
  34: SciSearch(R) Cited Ref Sci_1990-2013/Feb W2
  35: Dissertation Abs Online_1861-2012/Dec
  40: ENVIROLINE(R)_1975-2008/MAY
  41: Pollution Abstracts_1966-2013/Jan
  44: Aquatic Science & Fisheries Abstracts_1966-2013/Jan
  49: PAIS Int._1976-2013/Jan
  50: CAB Abstracts_1972-2013/Feb W2
  51: FSTA(R)1969-2013/Feb W2
  53: FOODLINE(R): Science_1972-2013/Feb 14
  58: GeoArchive_1974-2013/Jan
  60: ANTE: Abstracts in New Tech & Engineer_1966-2013/Mar
  61: Civil Engineering Abstracts._1966-2013/Feb
  64: Environmental Engineering Abstracts_1966-2013/Feb
  65: Inside Conferences_1993-2013/Feb 14
  72: EMBASE_1993-2013/Feb 11
  73: EMBASE_1974-2013/Feb 11
  74: Int.Pharm.Abs_1970-2013/Feb B1
  76: Environmental Sciences_1966-2013/Dec
  78: Aqualine_1966-2013/Jan
  87: TULSA (Petroleum Abs)_1965-2013/Feb W2
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89: GeoRef_1785-2013/Jan B2
96: FLUIDEX_1972-2013/Feb
98: General Sci Abs_1984-2011/Nov
99: Wilson Appl. Sci & Tech Abs_1983-2011/Nov
103: Energy SciTec_1974-2013/Jan B2
108: Aerospace and High Technology Database_1962-2013/Mar
110: WasteInfo_1974-2002/Jul
117: Water Resources Abstracts_1966-2013/Jan
118: ICONDA-Intl Construction_1976-2013/Jan
134: Earthquake Engineering Abstracts_1966-2013/Feb
144: Pascal_1973-2013/Feb W1
152: Medline(R)Publisher_2010-2013/2013/Feb 14
155: MEDLINE(R)_1950-2013/Feb 13
156: ToxFile_1965-2013/Dec W2
162: Global Health_1983-2013/Feb W2
172: EMBASE Alert_2013/Feb 08
181: Adverse Reaction Database_2008/Q3
203: AGRIS_1974-2013/Jan
266: FEDRIP_2013/Dec
292: GEOBASE(TM)_1980-2013/Feb W2
317: Chemical Safety NewsBase_1981-2013/Jan
332: Material Safety Data Sheets__2012Q2
336: RTECS_2013/Q1
354: Ei EnCompassLit(TM)_1965-2013/Jan W4
369: NEW SCIENTIST_1994-2010/JAN W5

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APPENDIX B

LITERATURE REVIEWED

Appendix B
Literature Reviewed

Literature Review but found not to be applicable is gray-toned.

Abgrall, M.-J., S. Bastien-Daigle, G. Miron, and M. Ouellette. 2010. “Potential interactions between populations of Softshell Clams (*Mya arenaria*) and Eastern Oysters (*Crassostrea virginica*) in temperate estuaries, a literature review.” *Canadian Technical Report of Fisheries and Aquatic Sciences 2892*. (2010). 88 p.

Adamkewicz, L., S. R. Taub, and J. R. Wall. 1984. “Genetics of the Clam *Mercenaria mercenaria* I. Mendelian Inheritance of Allozyme Variation.” *Biochemical Genetics* 22.3/4 (1984): 215 – 219.

Araki, Hitoshi., C. Schmid. 2010. “Is hatchery stocking a help or harm? Evidence, limitation and future directions in ecological and genetic surveys.” *Aquaculture*. 308 (2010): 2-11

Arnold, William S., S. L. Waters, J. S. Fajans, S. C. Peters, and T. M. Bert. 2004. “Influence of congeneric aquaculture on hard clam (*Mercenaria* spp.) population genetic structure*.” Aquaculture International.* 12 (2004): 139-160.

Arnold, W. S., S. P. Geiger, S. P. Stephenson. 2009. “*Mercenaria mercenaria* introductions into Florida, US, water: duration, not size of introduction, influences genetic outcomes*.” Aquatic Biology*. 5 (2009): 49-62.

Baker, S., D. Petty, R. Francis-Floyd, R. Yanong, L. Sturmer. 2012. “Introduction to Infectious Diseases in Hard Clams.” University of Florida (2012).

Bergquist, D. C., D. Heuberger, L.N. Sturmer, and S. M. Baker. 2008. “Continuous water quality monitoring for the hard clam industry in Florida, USA.” *Environmental Monitoring Assessment.* 2008.

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 (2000): 200-201.

Calvo, L. M., J. G. Walker, E. M. Burreson. 1998. “Prevalence and distribution of QPX, Quahog Parasite Unknown, in hard clams Mercenaria mercenaria in Virginia, USA.” *Diseases of Aquatic Organisms.* 33 (1998): 209-219.

Calvo, L.M. and E.M. Burreson. 2002. “QPX Susceptibility in Hard Clams Varies with Geographic Origin of Brood Stock.” *Virginia Sea Grant Marine Resource Advisory*. 74(2002): 3-7.

Calvo, L.M., S. E. Ford, J. N. Kraeuter, D. F. Leavitt, 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.” Journal of Shellfish Research*. 26(2007): 109-119.

Carnegie, R.B. 2008. “Diseases of Concern in Molluscan Aquaculture.” *Environmental Monitoring Assessment*. 2008.

Coen, L., M. Y. Bobo, D. Richardson, W. D. Anderson. 2004. “Sheet for QPX (Quahog Parasite Unknown).” South Carolina Department of Natural Resources – Marine Resource Division, November 2002. Last Revised February 2004.

Dahl, S. F., B. Allam. 2007. “Laboratory Transmission Studies of QPX Disease in the Northern Quahog (=Hard Clam): Development of an Infection Procedure*.” Journal of Shellfish Research*, 26.2 (2007): 383-389.

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

Dahl, S. F., J. Thiel, and B. Allam. 2010. “Field Performance and QPX Disease Progress in Cultured and Wild-Type Strains of *Mercenaria mercenaria* in New York Waters.” *Journal of Shellfish Research* 29.1 (2010): 83-90.

Dahl, S. F., M. Perrigault, Q. Liu, J. L. Collier, D. A. Barnes, B. Allam. 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 (2011): 314-321.

Diana, James S. 2009. “Aquaculture Production and Biodiversity Conservation.” *BioScience*. 2009.

Dillon, R.T., John J.M. 1990. “Outcrossed Lines of the Hard Clam *Mercenaria mercenaria.” NOAA Technical Report, NMFS.* 92 (1990).

Dillon, R.T., J.J. Manzi. 1990. “Outcrossed Lines of the Hard Clam *Mercenaria mercenaria.” NOAA Technical Report, NMFS.* 92 (1990).

Dillon, R. T. and J. J. Manzi. 1992. “Population Genetics of the Hard Clam, *Mercenaria mercenaria,* at the Northern Limit of Its Range.” *Can. J. Fish. Aquat. Sci*. 49 (1992): 2574 – 2578.

Dove, A.D.M, P. B. Bower, R. M. Cerrato. 2004. “Histological Analysis of an Outbreak of QPX Disease in Wild Hard Clams Mercenaria mercenaria in New York*”.* Journal of Aquatic Animal Health. 16 (2004): 246-250.

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 (2002): 23-28.

Kraeuter, J. N., S. Ford, D. Bushek, E. Scarpa, W. C. Walton, D. C. Murphy, G. Flimlin, and G. Mathis. 2011. “Evaluation of Three Northern Quahog (=Hard Clam) *Mercenaria mercenaria* (Linnaeus) strains grown in Massachusetts and New Jersey for QPX-resistance.” *Journal of shellfish Research* 30.3 (2011): 805 – 812.

Kreeger. D., P. Cole, D. Bushek, J. Kraueter, and J. Adkins. 2011. Partnership for the Delaware Estuary (PDE). “Marine Bivalve Shellfish Conservation Priorities for the Delaware Estuary’.. PDE Report #11-03. (2011): 54 pp.

Lyons, M. M., R. Smolowitz, M. Gomez-Chiarri, and J. E. Ward. 2007. “Epizootiology of Quahog Parasite Unknown (QPX) Disease in Northern Quahogs (=Hard Clams) Mercenaria mercenaria*.”* Journal of Shellfish Research. Vol 26, No 2: 371-381, 2007.

Metzner-Roop K. L. 1994. “The Effect of Aquaculture on the Genetics of Natural Populations of the Hard Clam *Mercenaria Mercenaria* (L).” *Journal of Shellfish Research*, 13.2 (1994): 487 - 491.

Perrigault, M., and B. Allam. 2009. “Cytotoxicity of quahog parasite unknown (QPX) toward hard clam (*Mercenaria mercenaria*) haemocytes and interactions between different pathogen isolates and host strains.” *Parasitology* 136 (2009): 1281 – 1289.

Perrigault, M., A. Tanguy, and B. Allam. 2009. “Identification and expression of differentially expressed genes in the hard clam, *Mercenaria mercenaria*, in response to quahog parasite unknown (QPX).” BMC Genomics. 377.10 (2009).

Perrigault, M., S. F. Dahl, E. P. Espinosa, L Gambino, and B. Allam. 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 (2011): 314-321.

Piferrer, F., A. Beaumont, J.C. Falguiere, M. Flajshans, P. Haffray, and L. Colmbo. 2009. “Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment.” *Aquaculture* 293.3-4 (2009): 125-156.

Roegner, G. C., and R. Mann. 1991. Hard Clam *Mercenaria mercenaria*. Hard clam. pp. 5.1-5.17. In: Funderburk, S., J. Mihursky, S. Jordan, and D. Riley (eds.) Habitat requirements for Chesapeake Bay Living Resources, 2nd edition. Living Resources Subcommittee, Chesapeake Bay Program. Annapolis, MD.

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.” *Journal of Invertebrate Pathology* 71 (1998): 9 – 25.

Stokes, N. A., L. M. Ragone Calvo, K. S. Reece, and E. M. Burreson. 2002. “Molecular diagnostics, field validation, and phylogenetic analysis of Quahog Parasite Unknown (QPX), a pathogen of the hard clam *Mercenaria mercenaria*.” *Disease of Aquatic Organisms* 52 (2002): 233-247.

Ulrich, P. N., J. W. Ewart, and A. G. Marsh. 2007. “ Prevalence of Perinsus marinus (dermo), Haplospridium nelson (MSX), and QPX in bivalves of Delaware’s inland bays and quantitative, high –throughput diagnosis of dermo by QPCR.” *Journal of Eukaryotic Microbiology*. 54(6) (Nov-Dec. 2007): 520-526.

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APPENDIX

C

FULL RECORD COPIES

Appendix C
Full Record Copies

Full Record Copies to be provided on CD and in the Final.