Sheet for QPX (Quahog Parasite Unknown)

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Recent events (in 2002) and related studies on Quahog Parasite Unknown (QPX) detected several patterns that have triggered both new regulations and concerns from regulators, growers and hard clam seed producers, especially in the state of Virginia. Interstate shipments of clam seed between east coat states is regulated more by some states than others. Few centralized databases within or among states are available for evaluation. Recent legislation, for example, in South Carolina has discontinued the reporting of clam aquaculture harvests to SCDNR; Virginia's Marine Resources Commission (VMRC) in August 2002 passed an emergency regulation temporarily prohibiting the importation of clam seed from South Carolina and Florida (originally all states south, including Hawaii, now Hawaii is on case by case basis). As of this update, Virginia will not accept imported clam seed, unless progeny are derived from broodstock either from Virginia or U.S. states north to Canada.

We know very little about QPX. This is a new problem facing initially, growers, but ultimately with implications for native populations. QPX in many ways parallels observations for MSX (multinucleated sphere X), an unrelated protozoan parasite of oysters, first observed in the late 1940-1950s in the northeast. Recent work supports the notion that MSX may have been introduced to the east coast via shellfish transfer from the west coast (Burreson et al. 2000). Additionally, its spread into the mid- and upper portions of the Chesapeake Bay, as well as to more northern waters (e.g., Delaware Bay) was likely facilitated by the movement of infected oysters or through attempts to ameliorate loss of oysters to disease. MSX's spread to more distant northern oyster grounds (e.g., Canada) may be the result of ballast water transfers or through other unknown means. After nearly 50 years of extensive study, there are still many critical details (e.g., life cycle) that are unknown for MSX; hence, our knowledge of QPX is still in its infancy (e.g., life cycle, taxonomy and epizootiology, few papers have been published to date).

Below we summarize what is known at this time for QPX and make a series of recommendations:

1. QPX is a parasite of the hard clam, *Mercenaria mercenaria* that has been observed in both cultured and wild clam populations in Canada and the United States and has caused severe mortalities in some areas (Bower and McGladdery 2003 and references therein). Some researchers (Barber 1999, Ford et al 2002, Stokes et al., 2002,) suggest that QPX is a facultative and opportunistic pathogen, that occurs naturally in clam growing waters and can cause severe disease outbreaks when conditions are stressful (i.e., unfavorable genotype, environmental conditions, high density). Ford et al. (1997, 2002) noted that QPX seems to be most prevalent in cultured clams and clams being held in the hatchery or nursery. The pathogen has not been detected in hatchery-produced seed clams. QPX infections were generally observed in clams >1 year old and >20 mm in shell height (Ragone Calvo et al. 1998), with severe mortalities occurring in 1.5 to 2 year-old hard clams in Massachusetts (Smolowitz et al. 1998). MacCallum and McGladdery (2000) noted that although some of the infected clams from the Vernon River, Prince Edward Island (PEI), Canada site were small (18-25 mm shell height), they had been in the field

for one year and were approximately 1.5 years old. They noted that due to colder conditions in the Gulf of St. Lawrence, the small QPX infected clams (<15) examined by Whyte et al. (1994) could have been the same age as larger clams from more southern areas (i.e. Virginia and Massachusetts). The parasite is generally found in high salinity waters (Ragone Calvo et al. 1998). Smolowitz et al. (1998) reported that clams with QPX infections grew more slowly and had a lower condition index than uninfected *M. mercenaria.* Sublethal effects include reduced shelf life and indirect increased mortality due to weakened response to predators (see recent review by Kraueter 2001).

2. Significant mortalities from epizootics have been observed in hard clam aquaculture plantings in parts of Atlantic Canada, Massachusetts, New Jersey and Virginia. For example, at Prince Edward Island in 1989, since 1995 in Massachusetts, in 1996 and 1997 in New Jersey, and in Virginia detected in 1996 along the Eastern Shore's seaside, mortalities in 2001 and 2002 (summarized in Ragone Calvo et al. 1998, Ragone Calvo and Burreson 2002, pers. comm. Ragone Calvo). Recent mortalities in wild populations of clams to be relayed have been noted near Raritan Bay, within the New York portion (Debra Barnes, New York DEC, Shellfish Group, pers. comm.), with prevalence levels as high as 30%, and perhaps 20% mortality in clams averaging 45-55 mm shell height.

3. Virginia has conducted surveys to determine QPX in wild and cultured clams since 1996 (Ragone Calvo pers. comm.). Recent extensive mortalities at two large-scale aquaculture grow out sites in 2001-2002, again only along the Virginia's seaside (eastern shore), presumably by QPX have caused great concerns due to the growth of an extensive hard clam industry there (worth now nearly $30 million each year, exceeding blue crabs and oysters). Over 500 million clams are currently under crop insurance in VA (VA Growers newsletter, 2002). Some newspapers have even referred to QPX as the "Black Plague" of clams (Cape Cod Times, 2001). However, no die-offs or instances of QPX have been observed in the Chesapeake Bay proper or south of Virginia, despite numerous relaying from the VA seaside to Chesapeake Bay proper. Higher salinities due to drought conditions may influence the pattern in the near future.

4. The parasite's occurrence has been to date limited to near-market to market-sized clams (perhaps1½ to 3 years old). QPX has not been detected in hatchery- produced seed (size range from 1-25 mm, but primarily <15 mm, Ford et al. 1997), but detection methods may not be sensitive enough causing false negatives? Note that the definition of 'seed'and associated sizes vary among researchers, so one needs to define size explicitly. Infections in individuals as small as 15-30 mm have been observed (Whyte et al. 1994). Stress from poor husbandry, high salinities (>25-30 ppt), low temperatures or high field planting densities may exacerbate the problem, although for Massachusetts, QPX was observed at sites with excellent husbandry and grow-out conditions (Ragone Calvo pers. comm. from Smolowitz). Infections in field clams were not detected for at least 1 year (Ford et al. 1997) and often not till year 2 (Ford 2001).

5. QPX appears to be associated with high salinity coastal areas (e.g., Virginia), but many estuarine areas south of Virginia are typically within the range of these higher salinity regimes during drought and non-drought periods and currently or have had recent hard clam industries. Relaying may exacerbate the problem. The lack of detection of QPX south of Virginia (e.g., NC, SC, GA, FL) is unclear. It may be attributed to the lack of

reported high mortalities in clam populations or the lack of sampling for diagnostic purposes.

6. Work in the last few years (initially anecdotal, now supported by directed research) suggests that particular populations (strains) derived from clam brood stock south of Virginia (e.g., South Carolina and Florida vs. Virginia, Massachusetts, and New Jersey populations) may be more susceptible to QPX in common-garden (side by side) field trials (scientists from Virginia, Massachusetts, New Jersey, see overview by Ford et al. 2002, Ragone Calvo and Burreson 2002). The Ragone Calvo and Burreson 2002 preliminary report deals with only the Virginia portion of the larger study.

   a. Growth and condition results were very similar.

   b. Mortality rates were different, with South Carolina and Florida derived populations having higher mortality rates vs. the other three more northern populations.

   c. Infections appear to be acquired in the field. QPX was detected approximately 10 months after deployment, with clams imported from Florida having the highest prevalence. After 19 months, detectable prevalence of QPX ranged from 2-10% (former Massachusetts, latter South Carolina), increasing to 21-29% for the Florida and South Carolina imported clam populations vs. 0-10% in the northern stocks after 30 months.

   d. Note, that there can also be a lack of correspondence between prevalence and mortality (e.g., Smolowitz, WHOI), such that low incidence still results in high mortality.

   e. Overall, the results support the conclusion that the two southern populations employed had poorer survival, presumably due to QPX. It should be noted however, that the 80% - 90% of the 'SC' broodstock utilized from Atlantic Clam Farms (or ACF currently) were Florida-derived" animals and not pure SC strains, with the other 10% - 20% from Massachusetts or Virginia (C. Battey, Island Fresh Seafood, SC).

TAXONOMIC AFFILIATION

Molecular studies seem to suggest that QPX is a member of the phylum Labyrinthulomycota in the thraustochytrid phylogenetic group (Maas et. al 1999, Ragan et. al 2000, Stokes, et. al, 2002), but it has not been definitely concluded that all QPX-like organisms are the same species (Ragone Calvo et al. 1998, Smolowitz et al. 1998, Stokes et al. 2002). Some members of the phylum Labyrinthulomycota are associated with sediments, vascular plants, benthic algae and marine/estuarine detritus (Stokes et al. 2002 and references therein).

GEOGRAPHIC DISTRIBUTION AND TIMELINE

Late 1950s	first observed in wild clams in New Brunswick, Canada, where it caused observable mortalities (Drinnan and Henderson 1963).
1970s	identified in Barnegat Bay, NJ
1980s	Prince Edward Island (PEI), Canada

Since then, QPX or QPX-like organisms have been found in Massachusetts, New Jersey, New York, Virginia's Eastern Shore and Canada. (Ford et al. 2002 and references therein).

DIAGNOSTIC TECHNIQUES

Gross Pathology

Smolowitz et al. (1998, 2001) noticed chipping of the valve margins in some diseased clams and occasionally yellow nodules and mantle swelling. These clinical signs of QPX infection were not observed in infected *M. mercenaria* by Ragone Calvo et al. (1998), Ford et al. (2002), or MacCallum and McGladdery (2000).

Histology

Standard paraffin histological techniques revealed that QPX infection prevalence and intensity are generally higher in the mantle and gills (Ragone Calvo et al., 1998; Smolowitz et al., 1998; MacCallum and McGladdery, 2000, Ford et al., 2002), although infections were noted in other tissues (i.e., foot, kidney). Several parasite life stages are generally observed, sometimes surrounded by a cell free mucoid-like region (Ragone Calvo et al. 1998, Smolowitz et al. 1998). Smolowitz et al. (1998) described a mucofilamentous net often surrounding the parasite. Ragone Calvo et al. (1998) also noted hemocyte infiltration and phagocytosis as a host response to the parasite.

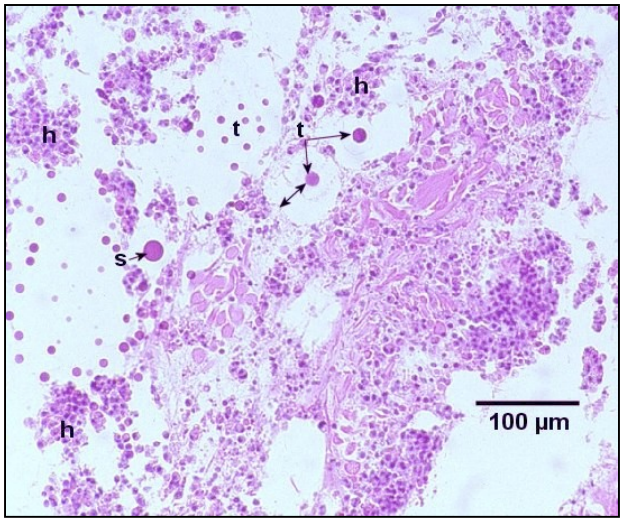

Figure 1. Accumulation of hemocytes (h) near the surface of mucoid-like material produced by thalli (trophozoites, t) and a sporangium (s) of QPX within the mantle of *Mercenaria mercenaria*. The thickness of the mucoid-like material around one thallus is indicated by a double-headed arrow. Hematoxylin and eosin stain. (Photo from: Bower, S.M. and S.E. McGladdery 2003)

MANAGEMENT STRATEGIES

Despite the lack of detection of QPX in 'seed' (Anderson et al. 2002, Ford 1997, Ragone Calvo 2002), our knowledge is too limited to use this as the basis for allowing hard clam importation without regulatory consideration, especially given past errors with oysters and shrimp. Recommendations from current researchers agree that caution related to seed origin and associated management strategies need to be considered. Concerns also need to be raised here in South Carolina regarding the`` importation of clam seed from hatcheries, nurseries (especially when water untreated) or previously deployed field-derived young clams or broodstock from areas from Canada to Virginia to minimize the potential for introduction of QPX into Atlantic and Gulf coasts states south of Virginia. An updated South Carolina importation policy

(February 2004), requiring additional information on applications for importation of indigenous molluscan shellfish has been put in place to minimize potential risks. In future updates, results, will include South Carolina's export data.

Although QPX has not been observed in South Carolina, we need to be aware that local infections may become evident in the future. Clam producers and harvesters should alert the DNR of any abnormalities or unusual mortalities they witness. Signs that may indicate a potential problem could be unusual amounts of sand in the mantle (indicating a weakened state), lesions on the soft tissue, swelling of the mantle, and chipping of the shell margins.

Since little is known about QPX disease, defining clear management strategies to mitigate its impact are unclear. Interactions between researchers, regulators and industry are essential, as well as incorporating monitoring programs into management plans (Woods Hole Oceanographic Institution Sea Grant: 1998-2004 Strategic Plan). As noted in the "Two if by Sea" MIT and WHOI newsletter (Vol. 6 No. 3), long-term monitoring programs are extremely beneficial and can offer "an early warning to growers and managers."

The stresses associated with high field density clam planting and QPX has been proposed as a possible factor in QPX infections (Whyte et al. 1994; Ragone Calvo et al. 1998; Smolowitz et al. 1998; MacCallum and McGladdery 2000). Barber (1999) suggested that disease outbreaks could be minimized if clams are planted at reasonable densities (50-75/ft.^{2} for 15 mm seed) in areas that are known for good growth.

Utilizing local seed stock only, since strains of *M. mercenaria* seem to vary in susceptibility to QPX disease, (Ford et al. 2002, Ragone Calvo and Burreson 2002, Bower and McGladdery 2003 and references therein), should be considered by clam growers.

Most recently, The New York State Department of Environmental Conservation (NYDEC) cancelled their 2003 Raritan Bay Shellfish Transplant Program due to QPX being found in wild hard clam populations there (http://www.dec.state.ny.us).

RESEARCH EFFORTS

Laboratory studies by Smolowitz et al. (2001) demonstrated that QPX was transmitted between clams (one-year old) within three months.

PCR assays and DNA probes (Ragone Calvo et al. 1998, Ragan et. al 2000, Stokes et al. 2002) as well as culturing techniques (Kleinschuser et al 1998, Brothers et al. 2000, Smolowitz et al, 2001 and others) are being researched.

Funding agencies are supporting research to understand and control the spread of QPX. Developing and testing strains of clams that will be resistant to the QPX parasite are ongoing efforts that will prove useful.

Researchers are studying the immunological response and defense mechanisms of clam host to the pathogenic parasite that causes QPX disease (see Anderson and Hall @ www.cbl.umces.edu.). Anderson et al. (2003) suggests that the mucoid secretion surrounding QPX cells in clam tissues may play a role as a virulence factor.

SOUTH CAROLINA CLAM DISEASE SURVEY

In May 2003, the small-scale study was initiated with SC clam producers from Cape Romain in Charleston County and Port Royal Sound in Beaufort County. Maricultured clams (n=25) from Cape Romain were harvested from the Cape Romain Harbor while maricultured (n=25) and wild (n= 25) clams from Port Royal were obtained from the Beaufort River.

Figure 2. Maricultured clams (n = 25) opened for histopathology.

Clam margins were carefully examined for chipped valves. All clam valves were free of any noticeable abnormalities.

Figure 3. Unopened maricultured clams with no shell abnormalities.

Gross lesions (e.g. mantle swelling, yellow nodules) were not observed when mantle tissues were examined.

Figure 4. Opened maricultured clam with no gross lesions.

Two cross-sections were dissected from each clam, representing sections from the anterior and posterior regions, and preserved in Davidson's Fixative. Fixed tissues (N =150) were processed following standard paraffin histopathological techniques. Microtomed tissue

sections (5-7 µm) were stained with Harris hematoxylin and eosin (HHE) then examined microscopically for QPX infection.

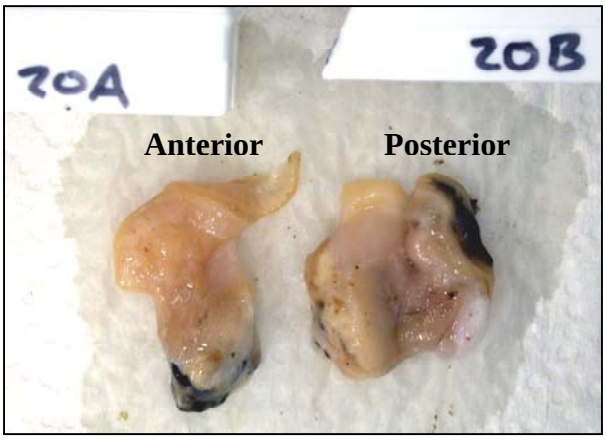

Figure 5. Anterior and posterior cross-sections dissected from each clam for histopathology. Tissue sections (N= 225) were examined under light microscopy. QPX cells were not identified in any of the tissue sections from either site (see Table 1).

Table 1. Prevalence and intensity of QPX disease in wild and maricultured hard clams, *Mercenaria mercenaria*, from two sites in South Carolina sampled in 2003.

Clam Producer	Location/ County	Date	Shell Height (mm)	Clams (tissues) Examined	QPX Prevalence
Perry Hall (Wildstock)	Parris Island/Beaufort	May 14	47-74	25 (3 sections/clam)	0%
Perry Hall (Cultured)	Parris Island/Beaufort	May 14	48-68	25 (3 sections/clam)	0%
Bill Livingston (Cultured)	Cape Romain (Charleston)	May 15	44-58	25 (3 sections/clam)	0%

RECOMMENDATIONS TO SCDNR

On October 1, 2002 W. Anderson, L. Coen, Y. Bobo and D. Richardson met to discuss the feasibility of surveying SC clam populations for QPX as a result of recent findings and related management decisions in Virginia. The following recommendations were presented for consideration to the Directors, the coordinating and permitting committees:

1. As of August 2002, emergency legislation in VA has banned the importation of SC and FL seed clams for at least 180 days because of Virginia political issues and a recent study indicating that seed from southern states may be more susceptible to developing QPX disease. In light of the fact that QPX has not been documented in SC clam populations, it would be expedient for SCMRD to become familiar with the diagnostic techniques to examine and identify QPX in the event of a future occurrence here. The MRD shellfish histology lab currently has the expertise, facilities and equipment needed to process tissues for histopathology and disease

evaluation. MRD lab personnel would communicate with researchers in VA to maximize their diagnostic training.

2. As we are aware, QPX is the first-known disease attributed to clam mortality. There have been no reported cases of disease-related mortality in SC. Although it is of immediate concern to clam growers in VA, seed producers and users in SC are concerned with the potential for introduction into our waters. Currently, SC has 36 clam (all *Mercenaria*) mariculture permit holders (5 pending) who would primarily be affected in the event of an epizootic here. While many clam mariculture permit holders have insurance to mitigate loses to their crops in the event of mortalities, the DNR would be involved in the process to assist with damage estimates and cause of mortalities concerning clam insurance.
3. We recommended an update of the MRD importation policy to incorporate concerns about QPX and other related diseases/problems. The updated importation policy will be forwarded with a "QPX notification letter" from OFM (the most recent update occurred in February 2004).
4. To become familiar with QPX, we recommended conducting a small-scale survey of cultured and wild clam stocks in South Carolina (see above). Such an effort would give us some baseline information for SC clam populations and provide familiarity with QPX histopathology. These producers would represent geographically different areas of dense clam grow-out within the state with other variables, such as New Jersey brood-stock and a range of grow-out salinities. This survey was not intended to illustrate the seasonal pattern of QPX; but rather intended to help us determine the presence (or absence) of the disease in a few SC areas and become familiar with its diagnosis.

Finally, the pilot study examining a small number of hard clams (see Table 1, both cultured and wild) found no QPX, suggesting that we need to be extremely careful to insure that QPX is not introduced into the state's waters via an interstate shipment. Current OIE (http://www.oie.int/eng/en_index.htm) guidelines recommend prohibiting the movement of organisms from diseased areas into areas where the disease is unknown or potentially not native. We suggest that the Department maintain sufficient vigilance given past events.

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The OIE is an intergovernmental organization created by the International Agreement of 25 January 1924, signed by 28 countries. In December 2003, the OIE totalled 165 Member Countries.

http://www.oie.int/eng/en_index.htm