PROJECT COMPLETION REPORT

97-4 "Investigation into the Occurrence, Distribution and Severity of QPX in Feral and Cultured Hard Clamsand Development of Management Methods for Infected Leases "

Termination Report Period: June 1, 1997 through June 30, 2000

NRAC Total Funding: $99,954 (June 1, 1997 through May 31, 1999)
(No-Cost Ext'n through May 31, 2000)

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REASON FOR TERMINATION: Project completed.

PROJECT OBJECTIVES:

1. Determine the infective cycle of Quahog Parasite Unknown (QPX) in cultured quahogs, *Mercenaria mercenaria*, obtained form hatcheries and planted in experimental plots in infected lease sites.
2. Determine whether QPX can cause infections in quahogs in locations distant from infected leases, but within the same bay; and if infections occur, is the capacity for infection different than that occurring within an intensely cultured lease in which the parasite may be artificially propagated.
3. Determine how ambient conditions (especially temperature) and managed conditions (especially density) of the planted quahogs, affect the occurrence and severity of QPX infections in areas with and without a known history of QPX infections.
4. Investigate possible management methods to determine if they have an affect on the occurrence and tissue localization of QPX infections in clams planted in leases with a history of QPX infection.
5. Compare levels of QPX infectiveness between hatchery reared varieties of quahogs with a high proportion of "notata" parentage, and a cultured variety of quahog without the distinctive "notata" shell phenotype and with a high proportion of wild parentage.
6. Monitor other feral and cultured populations of hard clams form the presence of QPX in order to determine the spatial distribution of QPX in the northeastern U.S.

7. Disseminate the results of this study by a presentation at a scientific meeting, by holding a regional informational meeting, and by production of a management fact sheet that will be available both as a pamphlet and as information on NRAC's www home page.

ANTICIPATED BENEFITS:

Information concerning the temperature, sediment type or tidal locations important in development of the disease in clams will help aquaculturist in choosing future lease locations.
Determination of the effects of planting density on development of the disease in clams will help culturists in managing their infected leases.
Determination of the resistance, or lack of resistance in inbred vs. out breed clams will provide information about possible seed stock to use in infected leases.
Determinations of disease pressure in the leases vs. unleased bottom within Provincetown Bay will help predict where and how prevalent the clam disease is in infected bays with established leases. This information can be used in both Provincetown and Duxbury and in other potentially infected areas to determine future leasing potentials.
Examination of possible treatment methods for infected leases will provide management information.
Evaluation of samples of clams from Duxbury and Provincetown as well as other locations on the northeast coast of the U.S. will help determine the prevalence of QPX.

PRINCIPAL ACCOMPLISHMENTS:

Objective 1:

Summary of findings. Infections due to QPX were very rarely identified in the experimental clams over the duration of the experiment so comparisons between stains and locations were not possible. Moon snail and crab pressure was heavy and resulted in high initial mortalities in the plots with loss of several plots (Fig. 1). Growth was slow as compared to animals planted in Duxbury (see Condition Index data, Fig. 2).

Objective 2:

Only once was a sample positive for QPX so comparisons between stains and locations were not possible.

Objective 3:

Salinities measured between 30 and 32 ppt in both the Provincetown and Duxbury sites. Temperatures in both the Provincetown and Duxbury sites have indicated wide temperature swings during the daily and weekly tides.

Rutgers Based Density Research:

Statistical analysis:
Even though QPX in the means by density table looks like a trend indicating a QPX increase with increasing density this is not significant due to the high variance between samples (Figure 4).

The most interesting result is that the QPX level is more nearly similar to the samples from the spring of 1997 when the clams were first tested (WP 0.8). This sample was taken from clams that had already experienced a loss of approximately 30% in February 1997.

The surviving clams collected in May were slightly larger (42 mm) as opposed to those collected the preceding November (37 mm) indicating either some growth or loss of smaller clams due to the extensive mortality. The condition index (dry weight of meat/shell volume) of the clams remaining alive in the spring of 1998 (0.0135) was somewhat lower than that recorded in November (0.0182).

Objective 4:

At a site in an infected Provincetown lease the sediment was hydraulically turned, and then limed, 2 weeks before planting. Results of the lime treated plots in Provincetown were similar to those seen in objective 1 and 2. Only once were positive samples found (Fig. 1) and any group of clams.

The data collected from Duxbury showed very different results. By April, 2000, heavy mortality was noted in the Duxbury plots when samples were take. Mortality was identified in the core samples of 68% in plot B1 and 87% in plot B2. Interestingly, the number of animals positive for QPX decreased at that sampling with grossly visible nodules and swelling present in 15% of clams from B1 and 34% of clams from B2; and in 20 and 54% respectively of the clams from each plot examined histologically.

Objective 6:

Feral and cultured clams sampled from 6-1-97 to 6-31-99 as part of this study are listed in Figure 4. All

samples have been collected and examined. Samples were collected from wild and cultured clams in Duxbury, Provincetown, Chatham, and Barnstable, MA. Positive findings of QPX were identified in wild and cultured animals from Duxbury, MA and in wild clams from Chatham, MA. All other samples were negative for QPX infections, including wild clams from Provincetown Harbor. Interestingly,

However, QPX was not identified in the wild clams collected from an area of low salinity (Black River) in Duxbury. This information indicates that QPX may not proliferate well in low salinities areas in water bodies where it does exist and suggests that establishing leases in low salinity parts of an infected harbor or bay may help prevent or control the disease in aquacultured animals in infected waters.

Samples were also collected from locations in Connecticut (Long Island Sound), New York (north and south shores of Long Island) and New Jersey (Atlantic coastal bays) in the fall/winter and spring of 1997, 1998, and 1999. Only one individual among all the Connecticut and New York samples was detected with QPX. This was a lightly infected wild clam, collected in June 1998, from a Long Island Sound bed. In New Jersey, two groups of clams were sampled: one was produced from a local (New Jersey) stock; the other originated from South Carolina broodstock of the same age (2-3 years old). QPX was detected in all four South Carolina samples at prevalences of 34 to 84%. One group of clams that were only one year old was already 40% infected. Growers reported heavy mortalities in several year classes of South Carolina clams. Comparable samples of the New Jersey clams had prevalences of 0 to 17%.

Objective 7:

Information detailing the work done has been distributed to the Massachusetts aquaculturists involved in the project. Aquaculturist and the Provincetown shellfish warden have been monitoring the experimental plots since they have been planted. No storms or other major weather related problems have been encountered since planting.

IMPACTS:

A grant entitled "Provincetown's Quahog ReCLAMation Project" was funded by the Cape Cod Economic Council in March of 1997. It attempted to develop QPX resistant clams using wild clam stock collected from Provincetown Bay. In order to determine if seed clams produced from the wild stock have any "resistance" to QPX, they were planted in duplicate plots adjacent to the plots in the 3 locations in Provincetown Harbor established as part of the NRAC grant. Sampling of the wild seed clams with both cores and histological examinations have occurred at the same times as the seed clams planted as part of the NRAC study. This complimentary study continued to the end of the NRAC study. Results from the NRAC study were to be used as a yard stick against which to measure any "resistance" that wild clam seed may have shown. Unfortunately because of the lack of disease development in Provincetown, this was not accomplished. However, the fact that the disease has lessened significantly in Provincetown may be good news in itself and might indicate that for unknown reasons, an infected area may again become usable.

The NRAC study has helped to provide further understanding and information about QPX to both the culturists and shellfish wardens of Provincetown and Duxbury, MA.

RECOMMENDED FOLLOWUP ACTIVITIES:

Clams should continue to be monitored in these areas in sentinel plots and in aquaculture plots. The number of clams planted in Provincetown has dramatically decreased with most culturists leaving the area. However, a few culturists remain and the town has begun to plant clams again. The general feeling is that the disease crisis may have passed. However, if clam aquaculture begins again in the area, we could see a resurgence of the disease. Needed additional work is the identification of QPX infection in other bivalves co-cultured with infected clams, and the identification of passive carriers and or environmental reservoirs of the QPX organism. Information on the methods of disease transmission (infective form and environmental parameters) are needed.

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED:

Publication in Print:

Moss,P., S. Kleinschuster, M. Dykstra, R.Smolowitz and J. Parent. 1999. Molecular characterization of QPX (quahaug parasite unknown), a pathogen of *Mercenaria mercenaria*. J. Shellfish Res. 18: 561-567.

Presentations:

Kraeuter, J. Effects of planting density and depth on QPX infections in hard clams. 18th Milford Aquaculture Seminar, February 23-25, 1998.

S. Kleinschuster, R. Smolowitz and J. Parent. In *Vitro* Culture and Life Cycle of QPX. 18th Milford Aquaculture Seminar, Feb. 23-25, 1998.

Kraeuter, J.N., S.E. Ford, R. Smolowitz, D. Leavitt and L.M. Ragone-Calvo. QPX a protistan parasite of hard clams (*Mercenaria mercenaria*) and its importance to rehabilitation efforts. International Conference on Shellfish Restoration. Hilton Head, SC, Nov. 18-21, 1998.

Smolowitz, R. QPX, a protozoan parasite of hard clams. Third International Symposium on Aquatic Animal Health, Baltimore, MD, August 30 to Sept. 3, 1998.

Smolowitz, R., E. Marks, C. Brothers, D. Leavitt and B. Lancaster. Results of QPX field studies. National Shellfisheries Association, Seattle Washington, March 19-23, 2000.

Smolowitz, R., E. Marks, C. Brothers, D. Leavitt and B. Lancaster. Recent results from field and laboratory studies of QPX. National Shellfisheries Association., Milford Aquaculture Seminar, Milford CT. Feb. 2000.

Smolowitz, R. Molluscan Disease Workshop sponsored by Barnstable County (SEMAC) , Cape Cod Museum of Natural History, Feb. 2000.

PART II

TECHNICIAL ANALYSIS AND SUMMARY

Objective 1:

A sub-sample of seed clams were obtained both from Bay Farm (BF) and Aquacultural Research Corporation (ARC) in August of 1997. Samples were histologically examined for any abnormality including QPX and were negative. In October, 1997, seed clams were received from both nurseries and were planted in a grided, duplicate 10 x 10 ft. plots (50 clams/sq. ft.) in an infected Provincetown lease. Samples of 25 animal from each of the paired 10 x 10 ft. plots were collected in May and November, 1998, May and October of 1999 and April of 2000. They were evaluated grossly and histologically for QPX and any other disease problem.

Core samples were collected in December, 1997, May and November, 1998, May and October, 1999, and April, 2000. Mortality and Condition index data resulting from core sample and histological samples have been accomplished on all samples collected (by Dr. Dale Leavitt).

Summary of findings. Infections due to QPX were very rarely identified in the experimental clams over the duration of the experiment (Fig. 1). The reasons for the decrease of disease prevalence in clams planted in Provincetown leases are not known but possible theories might involve: the dramatic decease in clams cultured in the Harbor due to the loss of culturists and cultured clams in the area which would decrease the available clams to be infected or to act as reserivors (wild clams are rare in the area and may not provide a large enough reserve to perpetuate the disease) or alternately a change in environmental conditions or availability of infection organisms may have occurred. Moon snail and crab pressure was heavy and resulted in high initial mortalities in the plots with loss of several plots (Fig. 1). Growth was slow as compared to animals planted in Duxbury (see Condition Index data, Fig. 2)

Objective 2:

Seed clams from both nurseries were also planted in grided, duplicate 10 x 10 ft. plots (50 clams/sq. ft.) in a location never before leased in Provincetown Harbor that was out of the water flow from infected leases. Core and histological samples of from each of the paired 10 x 10 plots were collected in May and November, 1998, May and October of 1999 and April of 2000. All animals have been evaluated grossly and histologically. Only once was a sample positive for QPX. The lack of QPX disease in the plots might be due to the plot positioning (away from infected plots) or to the general lack of QPX infection in the Harbor during the study years (see objective 1).

Objective 3:

Temperature and tidal cycle meters were deployed in the experimental sites in Provincetown and Duxbury, MA. Additionally both Mr. Jackett (Provincetown Shellfish Warden) and Mr. Bennett (aquaculturist) recorded temperatures as backup and collected water samples for salinity. Also, cultured QPX grows poorly below 28 ppt. Salinities measured between 30 and 32 ppt in both the Provincetown and Duxbury sites. Data gathered from this work and work in Virginia indicates that QPX prefers salinities of 30 ppt. Temperatures in both the Provincetown and Duxbury sites have indicated wide temperature swings during the daily and weekly tides (Table1 and 2), but also on an hour to hour basis in the Bay. The effect of temperature variation on the occurrence of

the disease is not known, but recent experiments with cultured QPX showed that the organism prefers room temperatures and dies above 30° C.

Rutgers Based Density Research:

Cultured clams from the 1995 year class were collected from a QPX infected location that was subtidally just below the experimental plots. Clams at the time of planting showed 44% infected with QPX. Clams were planted in densities of 20, 40 and 60 animals/ sq. ft. In replicates of 3 in 1 m^2 randomized plots. Figure 3. Means by density. Density 1 is low, density 3 is high. Percent survival was used in this data as opposed to percent mortality.

Statistical analysis:

Reporting was based on the combined data for each plot (mean of the means) rather than individual data (this eliminates any bias due to larger numbers of individuals available in some plots). Tests were run on the following parameters: Number live, Number dead, Number missing, Length, Width, Thickness, Computed volume, Dry meat weight, Ash Free dry meat weight, Dry shell weight, Ash free dry shell weight, Dry meat weight/dry shell weight, Dry meat weight/shell volume, % mortality (arcsine transformed) and Weighted QPX rank. The General Linear Model (SAS) test was utilized to separated the experimental design into components based on rows, columns and density. There were no differences in rows or columns for any parameter indicating that position was in the bed was not important. There were no differences in density for any parameter except number of dead. Even though QPX in the means by density table looks like a trend indicating a QPX increase with increasing density this is not significant due to the high variance between samples (Figure 4). Examination of the mean QPX data for individual plots indicates this trend is due to a relatively few plots, and there are a significant number of plots with 0 QPX.

The significant difference in the number of dead was because the experimental design had a large enough initial difference that we were able to overcome the high variance in the final results. The lack of a significant difference in mortality and number of live resulted from the very high mortality in many plots. This mortality reduced the number live to nearly the same level in many plots.

The most interesting result is that the QPX level is more nearly similar to the samples from the spring of 1997 when the clams were first tested (WP 0.8). This sample was taken from clams that had already experienced a loss of approximately 30% in February 1997. The question that remains is whether this drop in parasite level is due to loss of heavily infected clams or overwinter loss of the parasites or a combination of the two.

The surviving clams collected in May were slightly larger (42 mm) as opposed to those collected the preceding November (37 mm) indicating either some growth or loss of smaller clams due to the extensive mortality. The condition index (dry weight of meat/shell volume) of the clams remaining alive in the spring of 1998 (0.0135) was somewhat lower than that recorded in November (0.0182). The loss in condition may be due to a comparison between intertidal and subtidal beds, loss of condition during the winter or loss of condition due to the disease.

Objective 4:

At a site in an infected Provincetown lease the sediment was hydraulically turned, and then limed, 2 weeks before planting. Seed clams from both hatcheries were then planted (50 clams/sq. ft.) in grided, duplicate 10 x 10 ft. plots. Duplicate 10 x 10 ft. plots planted with ARC seed only (50 clams/ sq. ft.) were located in an area of a Duxbury lease that has laid fallow since a severe disease outbreak in August through December of 1995. Samples of 25 animal from each of the paired 10 x 10 ft. plots were collected in May and November, 1998, May and October of 1999 and April of 2000. All have been evaluated grossly and histologically. Core samples and resulting condition index data ere collected at the sample periods also. Results of the lime treated plots in Provincetown were similar to those seen in objective 1 and 2. Only once were positive samples found (Fig. 1) and any group of clams.

The data collected from Duxbury showed very different results. In May and November, 1998 samples, QPX was detected microscopically at low levels (4-8% of animals examined). Then in May of 1999, QPX was identified in 4% of clams in plot B1 and 28%, in plot B2 at Duxbury. No nodules or swelling were identified grossly in the clam mantles at that time. By Oct., 1999, both plots B1 and B2 were heavily infected with QPX histologically (60 and 76%) and both plots contained high numbers of individuals with nodules and swellings in the mantles (32 and 44%). Mortality counts were not done on these animals, but unusual mortality was not identified by the samplers. In April, 2000, heavy mortality was noted in the plots when samples were take. Mortality was identified in the core samples of

68 and 87% respectively. Interestingly, the number of animals positive for QPX decreased at that sampling with grossly visible nodules and swelling present in 15% of clams from B1 and 34% of clams from B2; and in 20 and 54% respectively of the clams from each plot examined histologically.
Such findings suggest:

1. QPX mortality lags behind the highest infection rate by a few months. This infection vs. mortality pattern is seen in other bivalve infections, such as dermo infections of oysters.

2. Once mortalities begin, the total number of animals showing infection both grossly and microscopically may decrease from the initial high. These post mortality levels of disease were similar to those identified in the initial investigations of aquacultured clams in Provincetown in which clams had already showed mortalities from QPX infections.

3. Lower winter temperatures may slow the disease down and prevent significant mortalities till the following spring brings warmer temperatures again.

4. Larger/older clams (Duxbury clams were much larger than their counterpart in Provincetown) developed the disease faster and with more severity than smaller/younger clams. Possible reasons for this are:

5. Larger clams were exposed to more infectious particles due to the larger volume of infected water flowing through the mantle cavity.

6. The number of infectious particles increased with increasing number of infected and dying animals in the plot (other ongoing experiments have shown that QPX is directly infective) so over time, the exposure to infectious QPX particles would be greater.

7. Poor growth was not related to increased susceptibility to QPX in this study.

8. Letting the land lie fallow did not have any effect on the severity of the disease or resulting mortality from the disease in Duxbury animals.

Because of grower preferences, only mixed parentage animals (80% notata/20% wild, ARC) clams were planted in Duxbury and clam strains was not possible. However, this data does show that QPX is still present in Duxbury and is capable of continuing to effect cultured clams populations there. Additionally, significant QPX infections have been identified in wild clam populations in Duxbury. Wild clams, which are abundant in Duxbury, might serve as a reservoir since hard clam aquaculture has ceased in Duxbury with the onset of QPX. Interestingly, QPX was not identified in the wild clams collected from an area of low salinity (Black River) in Duxbury. This information again indicates that QPX may not proliferate well at low salinities areas in water bodies where it does exist and suggests that establishing leases in low salinity parts of an infected harbor or bay may help prevent or control the disease in aquacultured animals in infected waters.

Objective 5:

Planted at each location in Provincetown, MA (total of 3) were 2 plots of seed clams with 80% notata and 20% wild parentage and 2 plots of seed clams with 100% notata parentage as described above. Additionally, with the help of Cape Cod Economic Council, 2 plots of 100% wild parentage seed (spawned from wild clams collected from Provincetown Harbor) (WP) were also planted at each of the three Provincetown locations. Comparisons of the rate of disease development and ultimate disease severity was to be accomplished at end of the study.

Since only very rarely were positive animals identified in Provincetown plots, no comparison could be made between these three groups of clams as related to disease resistance. Comparison of condition index data showed no significant differences in growth between the three strains of clams over the sampling time period. Growth tended to be plot specific and not strain specific. The factors affecting this are unknown since plots were often closely positioned.

Objective 6:

Feral and cultured clams sampled from 6-1-97 to 6-31-99 as part of this study are listed in Figure 4. All samples have been collected and examined. Samples were collected from wild and cultured clams in Duxbury, Provincetown, Chatham, and Barnstable, MA. Positive findings of QPX were identified in wild and cultured animals from Duxbury, MA and in wild clams from Chatham, MA. All other samples were negative for QPX infections, including wild clams from Provincetown Harbor. Such findings indicate that QPX is either not present in other locations in MA, or more likely, has not yet caused significant mortality.

Samples were also collected from locations in Connecticut (Long Island Sound), New York (north and south shores of Long Island) and New Jersey (Atlantic coastal bays) in the fall/winter and spring of 1997, 1998, and 1999. The clams, which, with one exception, were at least two years old and included both wild and cultured stocks, were examined by tissue section histology for the presence and intensity of QPX. Only one individual among all the Connecticut and New York samples was detected with QPX. This was a lightly infected wild clam, collected in June 1998, from a Long Island Sound bed. In New Jersey, two groups of clams were sampled: one was produced from a local (New Jersey) stock; the other originated from South Carolina broodstock of the same age (2-3 years old). QPX was detected in all four South Carolina samples at prevalences of 34 to 84%. One group of clams that were only one year old was already 40% infected. Growers reported heavy mortalities in several year classes of South Carolina clams. Comparable samples of the New Jersey clams had prevalences of 0 to 17%. In at least two locations, the New Jersey and South Carolina clams were planted close to each. Despite the proximity of heavily infected South Carolina clams, the final sample of New Jersey clams (June 1999) in the most intensively planted site (Dry Bay) had no histologically detectable QPX and no unusual mortalities were reported by the growers.

Objective 7:

Information detailing the work done has been distributed to the Massachusetts aquaculturists involved in the project. Aquaculturist and the Provincetown shellfish warden have been monitoring the experimental plots since they have been planted. No storms or other major weather related problems have been encountered since planting.

The Full Report with all the data, tables and appendices is available at the NRAC office upon request.