Background for Division of Fish & Wildlife Import Policy & Disease Testing Standards for Shellfish Aquaculture in Delaware's Inland Bays

Introduction

It is the duty of the Department, "To attempt to prevent and control the spread of shellfish-borne diseases among both shellfish aquaculture products as well as wild shellfish…" (7 Del. C.§2002(5)). The Department is also given authority over import of shellfish for aquaculture, which is important for two reasons. First, it is a long standing responsibility of the Department to protect wild shellfish stocks and their commercial and recreational fisheries from imported disease challenges. Secondly, aquaculture operations involve the periodic importation of seed, brood stock and larvae from a variety of sources. In addition, shellfish in aquaculture trays are generally at much higher densities than those in wild populations. Under these conditions, an imported disease may to spread from lease to lease, potentially affecting many growers. Disease testing will help to protect growers from the potential hazards of the constant importation of seed stock, although disease testing alone can only minimize, not eliminate disease.

In addition to authorizations in the State law, USACE Nationwide Permit 48 contains the condition "Any introduced shellfish must be certified under Delaware standards as being disease and parasite free."

As currently defined in regulations, shellfish aquaculture in Delaware's Inland Bays is limited to two species, the American oyster *Crassostrea virginica*, and the hard clam *Mercenaria mercenaria*. Hard clam aquaculture is only authorized in Little Assawoman Bay, an area distinctly separated from the wild hard clam fishery in Indian River and Rehoboth Bays. Little Assawoman Bay has a low population of natural hard clams, and is classified as a non-productive resource area by the State Shellfish and Recreational Water Program (Bott and Wong 2011). Oyster aquaculture is authorized in Indian River and Rehoboth bays, but well separated from the natural seed beds and oyster dredge fishery in Delaware Bay. These geographic separations are an important and effective mechanism for accomplishing the first goal, protecting wild stocks and their fisheries.

To achieve the second goal, minimizing the importation of disease with clam and oyster seed, in order to help protect other aquaculture lease holders, disease testing of seed stock, prior to introduction, is a necessary and prudent requirement. Because of the robust health enjoyed by the Inland Bays' natural hard clam stocks, and to protect the investment of other growers, care must be taken to ensure, to the maximum extent possible, the disease-free status of imported seed stocks. Importing shellfish stock from outside of the state (as is required by aquaculture) poses a new risk to both native and cultured stock, as multiple and novel strains of pathogens could be introduced. Several studies have documented that different strains of the same pathogen exist (Reece et al. 2001, Dahl et al. 2008). The virulence of these strains can

vary (Dahl et al. 2008), as can the impact of pathogens on the shellfish exposed to them (Kraeuter et al. 2011). Delaware is geographically positioned in the midst of a mix of known dermo strains. Reece et al. (2001) found multiple genotypes of dermo in nearest neighbors New Jersey, Maryland, and Virginia. And, native clams from the mid-Atlantic region have shown a higher susceptibility to QPX (Dahl et al. 2008) than clams from northern states. Testing for presence or absence of pathogens will help safeguard shellfish in the Inland Bays.

Disease testing requirements

1. Each batch (same seed lot, same producer and held in the same environment) of hard clam or oyster seed, larvae or broodstock brought into Delaware for aquaculture purposes must undergo two types of disease testing by a Division approved lab (Appendix). A representative sample of the seed, larvae or broodstock to be imported must be histologically processed, producing microscope slides showing all major tissue types. Slides must be read by an invertebrate pathologist. All findings of pathogens (MSX, SSO, "Dermo" ROD, QPX and others) or commensals found within the tissue shall be reported. In addition, a thioglycollate culture (RTFM) or qPCR analysis of tissue from a representative sample of the seed, larvae or broodstock to be imported (both oysters and hard clams) must be performed for the presence of *Perkinsus marinus* (Dermo). A dated and detailed pathology report shall be submitted to the Division with the application. Only batches of shellfish seed, brood stock or larvae found to have zero prevalence of disease shall be approved for importation and introduction for aquaculture purposes.

2. Number of individuals to be tested
   A minimum of sixty (60) individual seed clams, seed oysters, larvae or brood stock must be processed for histology and minimum of sixty (60) must be examined using qPCR analysis or thioglycollate culture.

   Rationale: The number of individuals required to be tested varies from 30-60 in neighboring jurisdictions. If the infection level is very low, sampling a greater number of seed is necessary for detection purposes. The larger the number tested, the more expensive the test, so a balanced approach is needed. We must select a number large enough to provide detection of low level infections and low enough that we are not creating a financial hardship for the grower. According to Ossiander and Wedemeyer (1973), a sample size of 60 gives us 95% scientific confidence that the disease state of the population is below the 5% prevalence level. If we wanted to be 95% confident that the disease prevalence level was below 2%, 150 individuals would have to be sampled, so the 5% prevalence level (60 individuals) was selected.

3. Duration of disease-free certification

The dated pathology report will be considered a characterization of disease state of the shellfish seed, larvae or broodstock population for 45 days from the time the seed, larvae or broodstock was sampled (removed from ambient water) for disease testing. Seed, larvae or broodstock for importation must be removed from ambient water at the hatchery, nursery etc. for transport to Delaware within 45 days or less. Beyond 45 days, retesting of the population will be necessary.

Rationale: Dermo is a disease of concern. It is often undetectable in cold weather, but can increase in prevalence and intensity as the weather warms. Juvenile oysters, even those from a Specific-Pathogen-Free hatchery, can rapidly acquire infection in dermo disease-enzootic waters (McCollough et al. 2007). For this reason, the lag time between disease testing and transfer of the shellfish must be minimized. Maine, with a very short planting season and growing season requires only annual testing for each hatchery. Clearly that would not adequately characterize the disease state of shellfish coming into Delaware where there is a much longer growing season and where planting might go on most of the year. A lag time of a week or less would be ideal to most accurately assess disease state; however, this would be a hardship on the hatchery and testing facilities. Forty-five days is a balanced approach which should reduce hardship for grower or hatchery and still provide reasonable information about disease state.

Literature Cited

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Appendix. Delaware Division of Fish & Wildlife Approved* Shellfish Testing Laboratories

- Rutgers Haskin Shellfish Lab
- VIMS Shellfish Pathology Lab
- Roger Williams University
- Kennebec River Biosciences
- State of Connecticut, Department of Agriculture
- Stony Brook University Shellfish Lab

* If you wish to use a laboratory that is not listed, please contact the Division of Fish & Wildlife to discuss the request.