Statement of expectations for research and development on non-native oyster species in Chesapeake Bay

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January 24, 2003

Since 1995, VIMS has been conducting field tests of non-native oysters in Chesapeake Bay. Our tests continue to this day, and similar tests are likely in the future. We have developed a standard set of guidelines (Allen and Burreson 2002) for conducting these tests, which incorporate biosecurity measures to prevent accidental release of non-natives or associated pests or pathogens in the Bay. Below we summarize the main features of these guidelines that we feel will ensure adequate biosecurity in research or development projects with non-native oysters.

Documentation
All projects are accompanied by a rationale and justification of the work proposed.

- Goals & Objective(s) – A statement of the purpose (goal) for the research and the information that defines the end-point of the project (objective).

- Stocks / strain origin – Description of geographic origin and breeding history of the oysters to be used in the project.

- Scope – Statement about numbers of test groups and the number of oysters per test group, replications, and the size (age) of oysters to be used.

- Location(s) – Whereabouts of the test sites and principal party(ies) charged with monitoring stocks and overall site conditions.

- Duration – Defined and well justified schedule for project termination, i.e., amount of time required to realize project objectives.

- Monitoring – The method for monitoring the population for reversion with indication of termination criteria.

Biosecurity before deployment
Biosecurity before deployment is concerned with the handling of brood stock, larvae and seed before seed are deployed in a field test. The principle concerns and expectations for handling non-natives are listed below.

- Level-1 (import) quarantine – Newly acquired fertile brood stock imported from other parts of the country or world are held in complete isolation from contact with other water sources and with adequate sterilization of effluent waters.

- Level-2 quarantine for certified brood stocks – F_1 or greater brood stock that have been disease certified are held in a quarantine system that is capable of eliminating the discharge of gametes or larvae back into Bay waters.

- Hatchery – Gametes, larvae, and non-reproductive spat and post-set (0.5 - 2 mm) animals are handled in the hatchery, sometimes in large numbers. Generally this is accomplished in static tanks of various sizes with daily feeding of cultured algae, requiring complete changes of water at regular intervals. Water used in the hatchery is treated in such a way as to eliminate the discharge of gametes, larvae or post-set spat back into the Bay waters.

- Nursery – Post-set (> 2 mm up to plantable size), non-reproductive juvenile animals are held in flow-through upwelling systems that provide the animals with natural algal food resulting in continuous production of effluent water. Discharge water is screened and filtered to prevent the discharge of seed back into the Bay waters.

Biosecurity in the field

Biosecurity in the field entails designing studies to minimize the possibility of an unintended introduction. The steps we exercise and our expectations for biosecurity are listed below.

- F_1 or greater progeny for the field – All stocks used for testing in the field will be free of exotic diseases. To ensure this, only F_1 (or greater) progeny will be used and then, only after certification of the population for absence of pathology.

- Use of non-reproductive oysters – Only triploid (sterile) oysters will be deployed in the field, excluding diploids by all means possible. Triploids are obtained from either of two methods: chemical or genetic.

  Chemical triploids are made in the hatchery at the time of fertilization of the egg. The developing egg is forced to retain an extra polar body (containing the third set of chromosomes) by administration of some agent, typically a chemical one. The treatment for triploidy is applied to millions of eggs at the same time, resulting in larvae that are mostly, but not all, triploid. Therefore, in order to use chemical triploids for field tests, each individual must be certified before deployment. Typically, it is only possible to deploy on the order of thousands of chemical triploids because of the burden of certification.

  Genetic triploids are produced by mating tetraploid male oysters with female diploid ones. It is expected that all progeny from such matings are triploid. In general this is true, but there are a few ways that populations of <100% triploids might result. First, if sperm from a diploid accidentally got into the spawning process, a few percent of the offspring could be diploid. Second, if a tetraploid male used for spawning is imperfect, that is, produces some haploid gametes along with diploid ones, some diploids could result. Third, if there is mixing of larvae among batches accidentally, a foul up that we take extraordinary precautions to avoid, some

diploids might contaminate the natural triploids. Recognizing the potential pitfalls of 100% triploid production, we have instituted several key checkpoints for each "natural triploid" spawn.

1. Sperm from the tetraploid male will be tested for DNA content by flow cytometry (FCM). Only sperm with no evidence of haploid cells will be used for spawning.
2. Larvae will be tested by FCM after 2 and 4 days in culture to see if there are any diploids present. If so, larvae will be discarded and the spawn repeated.
3. Larvae will be tested again by FCM at eyed stage to ensure that no accidental mixing has occurred during the larval cycle.
4. Before deployment, 200 spat will be sampled randomly from the natural triploid group and tested for ploidy by FCM. The group will be certified "ready for deployment" if every single spat sampled is triploid.

- Monitoring for reversion – Over the last several years it has become clear that triploids of *Crassostrea* species manifest chromosome set instability. That is, triploids tend to lose chromosomes over time. The result of this loss is that the oyster, instead of having only triploid cells, will have some percent of its cells triploid and the rest diploid. An oyster with part triploid and part diploid cells is called a mosaic. The rate of chromosome loss (called reversion) seems to vary according to where they are grown and whether the triploids are "chemical" or "natural," but the process is generally slow. Typically, the rate of reversion in chemical triploids is 2-6 times higher than that in genetic triploids. Once an oyster begins to lose chromosomes, the process continues over time so that the percentage of triploid cells goes down and the percentage of diploid cells goes up. The problem with reversion is not loss of chromosomes *per se* but the possibility that mosaics might regain their reproductive capacity.

  The eventual restoration of reproductive capacity in mosaics is not a given. In other words, mosaics seem to occur with regularity but restoration of reproductive capacity in mosaics seems to either not occur or to occur very late in life. Despite the apparent lack of reproductive potential, mosaics are a concern. We recommend adequate assessment of ploidy status, with due diligence established in accordance with the nature and duration of the proposed field deployment.

- Mode of deployment – Only sites that provide security from poaching and vandalism will be used. Oysters will be deployed in containers always, and secondarily secured in place as a precaution against weather related loss. Containerization minimizes loss of animals and assures complete removal of product for storm-related emergencies or harvest.

- Unusual weather-related precautions – Each project will have written “hurricane” plans on file and these plans will be distributed and explained to all participants. In the event of storms, oysters will be secured or relocated in accordance with local conditions.

Oversight

Oversight for compliance to all the terms of non-native projects ultimately rests with VMRC, with an advisory role from VIMS. Oversight should include exercise of due diligence for ploidy assessment (if required), verification of inventories on a regular basis (including spot checks by enforcement), and adequate reporting to document the progress of the project.

References

Allen, Jr., S.K. and E.M. Burreson. 2002. Standing Policy for Non-native Oyster Research in Virginia. Virginia Institute of Marine Science Press, pp. 17.