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Congressional testimony – “Non-native Oysters in the Chesapeake Bay”

for

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Scope of activity under open water release of triploid (sterile) C. ariakensis

Thesis of this testimony

Recently the National Research Council (NRC) of the National Academy of Sciences released their report “Non-native oysters in the Chesapeake Bay.” In it was a thorough analysis of existing data for C. ariakensis and recommendations for specific research needs. The report also evaluated three management options for C. ariakensis given the breadth and quality of existing research on this species. The three options were (1) no use of non-native oysters, (2) open water aquaculture of triploid oysters, and (3) introduction of reproductive diploid oysters.

Of these three choices, the report concluded that “[T]he risks of proceeding with triploid aquaculture in a responsible manner, using best management practices, are low relative to some of the risks posed under the other management options.”

They went on to indicate that contained aquaculture of triploid C. ariakensis provided an opportunity to further evaluate the risk of introducing non-natives by serving as a proxy for the reproductive form of the oyster. Contained aquaculture of triploids also allows exploration of the potential for extensive triploid-based aquaculture.

My testimony focuses on the scope of use for triploid C. ariakensis in the Chesapeake Bay. That is, within the recommendation to deploy triploids only, there is a wide scope of possible activity with varying levels of attendant risks. In general, the more valuable the information sought for research or aquaculture, the larger the risks, even using triploids. At the current level of risk aversion in the community (i.e., extremely risk averse), the level of useful information is potentially low for both research and aquaculture.

Statement of conflict of interest

I share co-authorship of a patent on tetraploid technology obtained in my previous appointment at Rutgers University. The patent was obtained because of the broad utility of tetraploids in shellfish aquaculture and before its application to the current situation (i.e., before the development and application of tetraploidy to C. ariakensis) in Chesapeake Bay.

Brief background on triploidy in C. ariakensis

Field research on the Asian (Suminoe) oyster, C. ariakensis, began in 1998 at the Virginia Institute of Marine Science (VIMS) in response to a resolution from the Virginia Legislature to initiate investigations on alternative species. All field trials have employed sterile triploids. Initial research indicated promising performance in C. ariakensis in a variety of salinities for growth and disease resistance (Calvo et al., 2001). Research on this species is still ongoing at VIMS. With harvests of C. virginica at record lows, there is intense pressure to submit to the introduction of this non-native species. VIMS, and specifically the Aquaculture Genetics and Breeding Technology Center (ABC), has been working on options for the use of C. ariakensis in a non-reproductive form: We have developed the technology for creating 100% sterile triploids in anticipation C. ariakensis might be useful in research, grown in commercial aquaculture, or both.

Triploid aquaculture is enabled by the development of tetraploid oysters (Guo and Allen 1994b). Tetraploids have four sets of chromosomes. Since the complement of chromosomes in a tetraploid is divisible by two, which is essentially what meiosis accomplishes during gamete formation, tetraploids are fertile. Moreover, gametes produced from tetraploids contain two sets of chromosomes. (Normal reproduction in diploids yields gametes with a single set of chromosomes.) Therefore, one highly efficient method of making triploid oysters is to breed tetraploids with diploids in the hatchery (Guo et al. 1996). Triploids created in this way are referred to as genetic triploids. The major manifestation of triploidy in oysters is the disruption of normal reproductive physiology, rendering triploids functionally sterile (Allen, 1986; Allen and Downing, 1990; Guo and Allen, 1994a; Erskine, 2003).

Despite the effectiveness of creating triploids using tetraploids, the process is not perfect. There are three aspects of the biology of triploids that engender some risk for establishing reproductive populations.

Fertility – Triploids produce gametes but are generally considered sterile

Reproductive potential of triploid Crassostrea gigas has been studied extensively for a number of reasons, ranging from documentation of their sterility for commercial purposes (Allen and Downing, 1990) to estimation of their reproductive capacity for population control (Guo and Allen, 1994a). Estimation of reproductive likelihood in triploid oysters was not quite as simple as the case for fish (cf. Allen et al., 1986). Triploid Pacific oysters do in fact make significant numbers of eggs and sperm (Allen and Downing, 1990). However, it is fair to say – based on the principals of meiosis generally and the information we have specifically from Pacific oysters – that triploidy will be similarly effective as a reproductive control measure for C. ariakensis.

Our analysis of reproductive potential of triploid Pacific oyster revealed that although gametes from triploids were fully capable of fertilization, aneuploid progeny resulted (Guo and Allen, 1994a). When triploids were crossed with themselves, the ploidy of resulting embryos was 2.88n on average, that is, hypotriploid. Survival of fertilized eggs to metamorphosis and settlement was only about 0.0085%. More recent data showed that triploid males are about 1000 fold less fecund than diploid males; triploid females about 20 times less fecund. So, although triploid oysters are not sterile in terms of gamete production, their reproductive potential is extremely low, by all practical measures, 0.

Fidelity – "100%" triploids

Until recently, the production of spawns of 100% triploids seemed all but impossible. This is because the state of the art for making triploids involved an induction procedure in which the newly fertilized egg is poisoned with an antibiotic, usually cytochalasin B (CB), to cause the failure of cytokinesis during the elimination of the second polar body (PB2) (Allen et al., 1989). The chromosome contained in the polar body contributes the third chromosome set to the embryo. Because the treatment (whether CB or anything else) has to be coordinated with the elimination of the second polar body and because PB2 elimination in a population of newly fertilized eggs is subject to inherent variation, some eggs escape treatment and remain diploid. This imprecision gives rise to broods of oysters with varying proportions of triploids. For perfect biological containment, pure triploid populations are necessary.

In summer of 1993, Dr. Ximing Guo and I were successful in creating the first viable tetraploid bivalves, specifically C. gigas (Guo and Allen, 1994b). Tetraploids, crossed to diploids, are very effective in producing large numbers of pure triploids. Fecundity of tetraploid females seems relatively high, only slightly lower than diploids (Guo et al., 1996). Fecundity of males is sufficient to fertilize about 50 million eggs with a single 2-3" male. Survival of 4n x 2n crosses (both reciprocals) in the larval stage were at least as high as the diploid controls, and two orders of magnitude higher than triploids produced by standard induction procedures. These initial data indicate that it is feasible to create 100% triploids using a tetraploid breeding population.

Since this work on C. gigas, subsequent work at VIMS has shown that the production of triploids is not exactly 100%. In a 2000 year class of "100%" triploids for industry trials in Virginia, 4 diploid (normally reproductive) oysters were found among about 3400 examined (0.12%). Two spawns in 2003 indicated 4 diploids among 3000 (0.13%) and 2 diploids among 3004 examined (0.07%). Thus as a general rule, we can say that "100%" triploid spawns to date are actually about 99.9% triploid. While this is still very good, say, compared to induced triploids, it is not perfect. Furthermore, when even a very low probability of diploid occurrence is multiplied by large numbers of oysters – e.g., 1,000,00 or 100,000,000 – substantial numbers of normal diploids can obtain (see below).

Proportion of diploids among triploids
Number of triploids deployed
Expected number of diploids

0.1%
10,000

10

0.1%
1,000,000
1000

0.1%
10,000,000
10,000

0.1%
100,000,000
100,000

Stability – reversion and mosaics

Certified triploid C, gigas were deployed in Delaware and Chesapeake Bays in 1993. After about 9 months of exposure, we found a relatively high proportion of mosaics – that is, oysters with both diploid and triploid cells in the somatic tissue – among our triploid oysters. The occurrence of mosaics themselves is not particularly surprising since the triploid induction process (then based on induction) effectively poisons newly dividing embryos. Abnormal progeny, such as mosaic individuals with two cell types, might be expected as a matter of course.

The surprising result was that the frequency of mosaics in several triploid populations increased over time, suggesting that some triploids have a tendency to lose chromosome sets. We have called this process reversion.

The classic definition of mosaicism is the presence of two or more cell types in the same organism. In our case, it is the presence of triploid and some other cell type(s) within the same oyster. This other cell type is generally diploid, although (i) whether or not the “diploid” cells contain balanced sets of chromosomes is unknown; (ii) there can be more than one other cell type, as has been recently found in our lab among tetraploid oysters; and (iii) some mosaic conditions, like that found in the gonad of triploids, is natural because of the process of meiosis. The presence of mosaics among triploid populations is generally unappreciated for two major reasons. First, it requires some level of sophistication in ploidy analysis, for example, flow cytometry (FCM), to find mosaics. With FCM, the frequency distribution histograms of mosaics appear as distinct ploidy types, usually triploid and something else. The second reason mosaics have gone unnoticed is that they generally occur in very low frequency (e.g., ~5%), although if sample size is large enough they always seem to be found.

In recent evaluations of populations of triploids, both induced and genetic, shows that the process of reversion is quite slow, taking a year or so to begin affecting the population (Zhou, 2002). The process is progressive, however, such that populations of triploids left for longer periods of time produce more and more mosaics. The frequency of mosaics ranges from 2-5% in the first year, perhaps reaching about 10% by year three. The frequency of reversion in genetic triploids is about 1/3 that of induced triploids. The salient risk in the process of reversion is whether or not the “unstable” triploids will eventually yield reproducing oysters. To date, there has been no evidence that normal reproduction occurs in mosaics. This risk is especially low in animals less than or equal to typical market size (~3”) (Chandler et al, 1999).

Application of triploidy to recommendations by the National Research Council report

Application to research

Full assessment of the biological and ecological characters of C. ariakensis for the purpose of evaluating the risk of introduction is clearly a difficult task. It is made all the more difficult by the Catch-22 of intentional introductions: You can’t know the true impact of an introduction until you have actually made it; you can’t make an introduction until you can predict the environmental impact. In the case of shellfish introductions of the past, a full evaluation – at least an ecological one – was absent. Introduction was primarily based on economic considerations. For the most part, and as reviewed in the NRC report, these introductions became economically important and generally ecologically innocuous.

But we are in a different era now, one more cognizant of the downside of introduced and non-native species. We are also in a different era of technology, vis a vis shellfish genetics, which allows us to take an intermediate course between “no introduction” and “complete introduction.” That intermediate course invokes the use of triploids as a tool for ecological and economic evaluation of non-native introduction before it is irreversible.

The table below summarizes the major research recommendations made by NRC and suggested approaches for their empirical determination. More than half of the issues that need attention can be addressed by using sterile (triploid) progeny in the field as a proxy for diploids. Answers to research in other categories require some aspect of reproductive biology to be fully operational, such as reproductive output in various environments or recruitment dynamics. Other research can be limited to laboratory work, with the rather large caveat that lab studies cannot always be extrapolated to relevance in the field. And some research, like evaluation of population genetics of the species, is completely doable in the lab.

Category	Research Recommendation	How, where accomplished
Biology		
	Effects of temperature, salinity	Triploids in field
	Growth rate	Triploids in field
	Reproductive cycle	Laboratory* Triploids in field
	Larval behavior	Laboratory
	Settlement patterns	Laboratory*
	Settlement in different hydrodynamic regimes	Impossible
	Size specific post settlement mortality	Triploids in field
	Susceptibility to parasites	Triploids in field
	Susceptibility to pathogens	Triploids in field
	Susceptibility to predators	Triploids in field

Category
Research Recommendation
How, where accomplished

Ecology

Interspecific competition
Laboratory

Triploids in field

Reef building capacity
Triploids in field

Genetic, phenotypic diversity

Geographic population structure
Laboratory

Phenotype
Triploids in field

Biology of other related species
Laboratory§

Integrated response to environmental change
Triploids in field

Larval dispersion

Circulation model of Chesapeake Bay
Laboratory

Economic, social impacts

Public versus private
Process too dynamic

Economic, socio-cultural study
Process too dynamic

Economic feasibility
Triploids in field

Model of impacts
Process too dynamic

Management options

(1) Prohibit non-natives
¾

(2) Open water triploid

Susceptibility to Bonamia
Laboratory,

Triploids in field

3n biology – fertility, fidelity, fertility
Triploids in field

Model triploid reproductive potential
Triploids in field

(3) Diploid introduction

Survival and reproduction
See Biology above

Reproduction
See Biology above

Reef building capacity
See Ecology above

Marketability
Triploids in field

* – research can be accomplished in lab, but extrapolation to relevance in the field is difficult.

§ – author does not agree with this recommendation, unless it is limited to literature search, not empirical studies.

The NRC report clearly indicated that adoption of a careful approach to open water triploid aquaculture should be considered an interim action to provide researchers an opportunity to obtain critical data on non-native oysters for risk assessment. I'm not sure that the report envisioned the full potential of triploid experiments for this purpose. It seems to me that they framed the recommendation for open water aquaculture on the "inclusion of parallel ecological experiments designed to generate information critical to evaluating the risk that triploid aquaculture will eventually produce a diploid population." More directed ecological research, not necessarily resembling or associated with commercial aquaculture, is possible. That is, there is a range of experiments that could be designed using triploids that have no relationship to how triploids may be grown in commercial aquaculture.

Envision an experiment designed to test the ecological function of a C. ariakensis reef, for example. Hatchery produced spat on cultch could be produced and placed into one or more estuaries, with or without

C. virginica interspersed, and community structure examined over the course of several years. New year classes of triploids could be “recruited” to the reef by subsequent hatchery spawns and deployment, all the while obviating colonization, or at least decreasing its risk to diminutive levels for the sake of gaining the information. Such creative experiments using triploid, not diploid, C. ariakensis could be enormously instructive.

While research with triploids is highly promising as an alternative to diploid studies, it is not risk free. (The risk of reproduction among triploids was briefly discussed above.) At the present time, however, it is my opinion that the regulatory environment is too risk averse to entertain anything other than highly restrictive trials. Perhaps that stems in part from the NSC report’s admonition that “stringent regulations will be necessary to ensure [emphasis added] that aquaculture of triploid C. ariakensis does not result in the establishment of a self-reproducing population . . .” Ensure is a powerful word.

Application to aquaculture

In fact, the NRC report used a number of descriptors to describe the scope of aquaculture recommended by the panel: they include “ensure,” “contained,” “confined,” “careful,” “responsible,” and “open water.” Depending on interpretation, these terms could entail different levels of risk (see below). How do we define that level? What is reasonable? What is acceptable?

high

none

x 100?

x 1000?

x 10000?

NO non- natives

“ ensure”

“ contained”

“ confined”

“ careful”

“ responsible”

“ open water”

“ diploids in Bay”

PROBABILITY OF SELF-SUSTAINING POPULATION

For the industry aquaculture trials recently approved in Virginia, the level of permissibility has been to “ensure” – ensure that aquaculture does not result in a self-sustaining population. In addition to the conditions placed on the growers themselves, which includes double containment of oysters, bonding, and additional investments, there has been a host of other conditions placed on the trial that can only be satisfied with stringent sampling regimes accomplished by researchers, in this case VIMS. At this phase in the evolution of C. ariakensis trials, these provisions seem appropriate. However, it is probably unreasonable to think that this level of restriction on aquaculture can yield meaningful economic data, other than marketing information. That is to say, the expense to growers for raising oysters greatly exceeds what might be expected with lesser restriction. With highly restrictive aquaculture, it will be impossible to show economies of scale that would accrue if there were, for example, no restrictions. In short, it will be difficult to realize the considerable economic potential of this species.

No one expects “no restrictions,” for the time being, but there seem to have been some expectations in the NRC report for limited success in aquaculture. They listed some of the benefits of open water aquaculture as determining viability of aquaculture, aquaculture employment, and retention of fishery benefits to the Bay. So, which of the descriptors (i.e., what levels of risk) apply to these expectations and how open water can open water aquaculture be?

As in research, there are tremendous opportunities to learn of the economic potential of aquaculture by a slightly less risk averse environment. For example, deployment on-bottom with triploids that could be dredged at market size would yield information on the viability of this species to standard practices in use for C. virginica. It would yield information on the heartiness of this species for fisheries use, anticipating the possibility of a diploid introduction for fisheries purposes. After all, there is a general assumption that the introduction of this oyster will provide a similar fishery to C. virginica. On bottom trials could indicate the feasibility of extensive, repletion aquaculture – already practiced by the state of Maryland – of triploids. An on-bottom trial might yield information on density dependent growth. More interestingly, trials of this sort, carefully (is this what the NRC report meant by this word?) integrated with scientists, could yield fishery, aquaculture and ecological data simultaneously – but not without some risk.

The “H-bomb” effect

It seems that one of the tacit assumptions among those who enthusiastically oppose non-native trials is what I call the “H-bomb view” of risk. There seems to be a feeling that any reproduction at all stemming from open water aquaculture is the “big one,” the final consequence. But in fact, reproduction episodes stemming from triploid trials (or for that matter, open water triploid aquaculture) will be much more gradual and are not necessarily cataclysmic. What might happen if there was some reproduction as a consequence of research or commercial aquaculture?

For one thing, recruitment likely would be severely hampered by impediments to colonization (the NRC reports calls it “barriers to successful introduction”) such as, water quality, lack of substrate, sedimentation, habitat loss, and suitability of C. ariakensis to Chesapeake Bay. If populations did establish, what is likely to be their size, considering that triploids were used and security was breached by a potentially very small number of individuals? Would not the very process of “escape” give rise to research opportunities? Are reproduction episodes truly un-eradicable? Could eradication be favored with careful placement of these trials in specific estuaries? If eradication was “ensured,” could small populations of diploids be used to gather data?

Integration of research and commercial trials

I bring up these issues because the need to understand the risks and benefits – for fisheries and aquaculture – probably is going to involve the need for more aggressive trials yielding critical data in a timely fashion. Perhaps it is time to pay serious attention to well-integrated programs.

Currently, VIMS is embarked on a unique collaboration with the industry, the Army Corps of Engineers, Virginia’s Center for Innovative Technology and the Virginia Marine Resources Commission in a comprehensive trial of about 1,000,000 triploid C. ariakensis. In short, scientific evaluation of reproduction, disease incidence, reversion, comparative growth (with triploid C. virginica), and economic potential have been coupled with the commercial scale trials of triploids. I have suggested some other avenues of “integrated” research above. It would be helpful to encourage such programs, as well as finding mechanisms to enable interstate collaboration among Virginia, Maryland and North Carolina, by providing resources and allowing reasonable levels of risk.

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