Aquaculture of Triploid *Crassostrea ariakensis* in Chesapeake Bay

A Symposium Report

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Maryland Sea Grant College Program
0112 Skinner Hall
University of Maryland System
College Park, Maryland 20742
www.mdsg.umd.edu

Aquaculture of Triploid *Crassostrea ariakensis* in Chesapeake Bay

A Symposium Report

By Eric Hallerman

Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University

Merrill Leffler
Maryland Sea Grant College, University System of Maryland

Sally Mills

Virginia Sea Grant, Virginia Institute of Marine Science

Standish Allen, jr

Aquaculture Genetics and Breeding Technology Center
Virginia Institute of Marine Science

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Non-Native Oyster Introductions and the Chesapeake Bay

The Eastern oyster, *Crassostrea virginica*, was for three centuries the object of a major fishery in the Chesapeake Bay. In 1957, the protozoan parasite *Haplosporidium nelsoni* spread MSX disease southward from Delaware Bay to the lower Chesapeake Bay, devastating native populations of the eastern oyster. By the 1970s, MSX had wiped out vast tracts of oysters on Virginia's high salinity grounds. Through the 1980s, MSX was joined by a second virulent parasite, *Perkinsus marinus* (the cause of Dermo disease), which also began killing oysters. Dermo was thought to be limited to salinities greater than 15 parts per thousand (ppt); however, by the 1980s, it began to appear in lower salinity waters of 10 to 12 ppt. Together, both diseases have frustrated restoration and aquaculture efforts and have brought the Bay oyster fishery to near ruin, especially in Virginia. The immense loss of oysters and their capacity for filtering algae is likely to have contributed to the decline of water quality in the Bay. In addition, the entire industry is in jeopardy, as much of the infrastructure for processing and packing oysters could fold for lack of product.

During the late 1980s, several baywide workshops were convened in the mid-Atlantic region to explore the options for countering the impacts of disease. These workshops, which brought together resource managers, policy makers, scientists, commercial fishermen, aquaculturists and concerned citizens, focused on specific topics, among them, research needs to combat MSX and Dermo, socio-economics issues related to the oyster industry, and the ecological and genetic implications of introducing non-native oyster species. Another workshop focused on the implications of introducing *Crassostrea gigas*, then the major non-native oyster candidate (Synopsis of the Oyster Ecology Workshop 1991; Leffler and Greer 1991). The goals were to: (1) evaluate how *C. gigas* introductions affected ecosystems around the world; (2) provide an overview of the ecological factors that would affect the growth of *C. gigas* in the Bay; and (3) assess the ecological risks and benefits of its introduction in mid-Atlantic waters. The symposium reported on here occurred 10 years later, almost to the day. Clearly, interest in the use of non-native oysters persists.

In 1995, the Virginia General Assembly took a more directive approach to non-native oyster research. It charged the Virginia Institute of Marine Science (VIMS) to submit and initiate a strategic plan for evaluating non-native species; VIMS submitted the plan in 1996. Field studies began with *C. gigas*. Native to Japan, it had been imported to the U.S. west coast early in the century, where it has since been the mainstay of the industry that depends entirely on hatchery production of oyster seed. *C. gigas* is also the basis of oyster production industries on every continent except Antarctica.

In 1997, laboratory and field trials were undertaken with *C. gigas;* overall, these oysters exhibited unremarkable performance in growth, disease tolerance and taste acceptability compared to the native oyster (Calvo et al. 1999). VIMS researchers then began investigations of the Suminoe oyster; also a species from Asia, *Crassostrea ariakens*is was first brought to the United States by growers in the Pacific Northwest. Production has been limited, largely because the region's higher salinities make hatchery culture inconvenient and more costly than for *C. gigas*. VIMS undertook field comparisons between infertile (i.e., triploid) *C. ariakensis* and the Bay's native oyster, *C.virginica*, between June 1998 and September 1999. Focusing on survival, growth and disease susceptibility, the results demonstrated that *C. ariakensis* was faster growing, reached market size in about a year (compared with the two to four years for *C. virginica*), and tolerated MSX and Dermo disease; furthermore, it was largely indistinguishable in taste from native oysters.

These findings suggest that hatchery-reared *C. ariakensis* holds promise for rebuilding the commercial oyster industry in Virginia and Maryland through aquaculture production. Furthermore, use of sterile triploids in aquaculture could greatly reduce reproductive potential. At the same time, there are concerns, if not strong reservations, over employing non-native or exotic species, when there are so many instances of their ecological impacts.

In an attempt to broadly inform Chesapeake Bay stakeholders on these issues, a consortium of institutions sponsored the Symposium on Aquaculture of Triploid *C. ariakensis* in Chesapeake Bay as a means for: (1) exchanging information on research findings and practical experience in Virginia (there have been no studies on *C. ariakensis* in Maryland) and (2) considering critical issues regarding research needs, policy and regulatory matters, and the rehabilitation of the oyster industry.

The symposium was attended by federal and state agency managers, non-governmental organization (NGO) representatives, scientists, watermen, aquaculturists and concerned citizens from Virginia, Maryland, North and South Carolina, Delaware and New Jersey. The agenda included presentations and discussions related to the biology of *C. ariakensis*, disease concerns, research and industry field trial results, production of triploids, a predictive risk model, strategies for minimizing environmental risk, and public attitudes about non-indigenous species. These presentations, together with poster sessions on key technical aspects of *C. ariakensis* research, provided a baseline of shared knowledge for three stakeholder discussion groups that represented industry, scientific, and policy and regulatory interests.

Based on the presentations, each of these discussion groups was asked to consider major concerns and issues related to the aquaculture of triploid *C. ariakensis* in the Chesapeake Bay. This report summarizes the outcomes of the group deliberations and key points in the discussions that followed. It also provides a synopsis of each of the presentations.

Industry, Science, Policy and Regulation: Concerns and Issues Related to Crassostrea ariakensis

The symposium organizers provided the three stakeholder groups with questions related to their particular interests as a means of catalyzing discussion of key concerns and issues (see Appendix 1). The groups were not constrained to considering only these questions.

While the group discussions were directed to focus on the aquaculture of triploid *C. ariakensis*, each group independently found that it is difficult to consider the risks of aquaculture of triploid (infertile) *C. ariakensis* as separate from the risks of diploid (fertile) *C. ariakensis*. That is, there was consensus that triploid aquaculture would inevitably lead to some introduction of reproductive individuals in the Bay, with unknown outcomes for population growth. Because VIMS data and industry field trial findings to date indicate that *C. ariakensis* has superior resistance to MSX and Dermo disease, can reach harvest size much more quickly than the native oyster, and appears to be indistinguishable in taste, concern was raised over the potential for unauthorized release of diploid broodstock into Bay waters.

The industry group urged scientists to begin studying the environmental and ecological impacts of diploids in the wild immediately. Scientists recommended that ecological risks of introducing diploids must be assessed over a broader geographical region than just the Chesapeake because an introduced species could potentially spread throughout the Atlantic and Gulf Coasts. Researchers in North Carolina apparently are beginning to work with field plantings of triploid *C. ariakensis*, and so all east coast states must be included in any discussion of a possible introduction.

Industry Discussion Group

The industry participants reported a sense of urgency about the need to move forward rapidly because the very survival of many commercial operations is at stake. The group's key conclusions are:

- If allowed to proceed with production of triploid *C. ariakensis*, the Virginia industry expects to produce one million "natural" triploids in the first year, and could saturate the regional market demand of 270 million oysters within five years.

- If 100% natural triploid oysters (see summary of Triploid Strategy and Risks) are unavailable, the industry would be willing to use chemically induced triploids.

- Virginia growers would be willing take reasonable measures to confine triploid oysters and to undergo a training course on how to grow biosecure oysters. Course completion could be tied to the granting of a permit for growing oysters.

- Industry members would be willing to open their production records on purchase, movement, harvest, and sale of triploid *C. ariakensis* product to outside scrutiny, but are not willing to share access to customer lists and financial information. Virginia growers recognize the Virginia Marine Resources Commission as the responsible agency for managing and mitigating any risks, with the Virginia Institute of Marine Science serving an advisory role.

- Virginia producers expressed solidarity with Maryland producers, who also are interested in *C. ariakensis*, but currently face a regulatory climate that opposes introduction of triploid *C. ariakensis*. Maryland producers are specifically interested in a diploid introduction and might oppose the introduction of a triploid without a concurrent plan for a diploid oyster. Their concern is that a triploid-only introduction in Virginia waters would threaten their competitive stance in the marketplace and allow Virginia to “get ahead” of Maryland. The sentiment, “Give us both or nothing,” was repeated several times.

- Industry strongly encouraged an undertaking to develop a disease-free diploid stock by January 2003. The industry reasoning includes the importance of taking the long view, especially that getting oysters back on the bottom in natural oyster bars is the only viable solution for getting the industry, including processing houses, back on track. The Washington State oyster industry, which is based primarily on hatchery production, was discussed as a model of the approach and scale needed in the Chesapeake Bay.

- Although most industry participants agreed with the views summarized here, a minority favored immediate introduction of diploid *C. ariakensis*.

Science Discussion Group

In considering risks associated with the introduction of a non-native species, there was consensus that some risks are indeed unknowable and, hence, complete certainty regarding the risks involved may never be acheived. However, a more complete understanding of the biology and ecology of *C. ariakensis* would help identify these risks and perhaps assuage some of these concerns. While all risks can be avoided by not permitting the introduction of *C. ariakensis*, there also are risks associated with doing nothing. These include the lack of economic benefit associated with having a viable commercial oyster species as well as ongoing degradation of habitat and water quality in the Bay and other locations on the east coast that lack major oyster populations.

Adverse Risks

Potential hazards associated with the introduction of *C. ariakensis* were ranked from highest to lowest. Some of these hazards follow from the consensus viewpoint that the use of triploid oysters for aquaculture purposes will inevitably lead to the introduction of diploid oysters through the three mechanisms below, listed in order of severity.

- Illegal Introductions. *Uncontrolled illegal introductions of diploid oysters using methods not in compliance with ICES (International Council for the Exploration of the Sea) protocols for purposes of establishing naturally reproducing oyster populations*. *C. ariakensis* is being reared in a limited manner in at least one commercial oyster hatchery in the Pacific Northwest, using broodstock that were introduced many years ago during shipment of Japanese oyster seed. Theoretically, these oysters could be raised commercially and hence would be widely available in the seafood market in addition to being available from native stocks in China. The possibility of an illegal introduction argues for public education about this problem because it is by far the highest risk, worst-case scenario for the Bay. It also emphasizes the importance of an organized, officially controlled introduction (assuming one proceeds) under ICES protocols.

- Catastrophe. *Possibility of storms and other events destroying aquaculture biosecurity measures that results in the loss of triploid oysters to the wild*. There is a high probability that aquacultured triploid oysters lost through disaster, and left unharvested, could revert to diploidy and gain fertile status as they grow older. This may lead to reproductively competent oysters in the Bay (see summary on Triploid Strategy and Risks for discussion of “reversion”).

- Biological/Biosecurity. *Variability in the degree of triploidy (see summary on Triploid Strategy and Risks) and level of harvest success in recapturing aquaculture-planted triploid oysters (e.g., there is a risk of triploid reversion to diploidy) are hazards whose risks can be minimized in a contained aquaculture system*. These risks can be reasonably well quantified using the risk model (see summary on Risk Model).

Another hazard of introducing *C. ariakensis* is disease transmission or opportunity.

- Disease Risk. *Possibility of introducing an unknown or unidentified disease in oyster broodstock*. This risk can be minimized by performing introductions under the ICES protocols, as has been done with all work on this species by VIMS. Unfortunately, there remains some risk that viruses may be transferred even when using ICES protocols. As Dr. Eugene Burreson pointed out (see summary on Disease), more testing for viruses in *C. ariakensis* broodstock using the latest molecular techniques will provide a higher level of assurance, although even that approach cannot guarantee that all viruses will be found. A study of diseases in the natural range of *C. ariakensis* in China would enable us to become cognizant of any potential diseases and parasites that this species might harbor. Such analysis also should include this species in the Pacific Northwest. Also unknown is whether *C. ariakensis*, as a new species in the Bay community, will prove a host for a disease not yet problematic.

Potential Scenarios from C. ariakensis *Introduction*

- Competition of *C. ariakensis* and *C. virginica*. Either a triploid that reverts to diploidy or an introduced diploid will have limited opportunity to compete with native *C. virginica* oysters in Virginia simply because of the decimated populations in Virginia's higher salinity waters. In Maryland, however, with lower salinity waters and less disease pressure, there is still hope of restoring the native species, *C. virginica*. (The ongoing attempts to breed and select eastern oysters that can better tolerate MSX and Dermo is an ongoing strategy to help restore oyster stocks [Restoring Oysters to U.S. Coastal Waters 1999]. Some researchers pointed out, however, that such selective breeding is altering the native species genetically, such that it may be a "native" oyster in name only.) Thus, potential interspecific competition between these two species in Maryland, and in other states along the east coast of North America, is a concern. It also is possible that there might be synergies between the two species (e.g., the recently completed field trials showed that eastern oyster larvae set and grew on the shells of triploid *C. ariakensis*). Certainly, the use of *C. ariakensis* for aquaculture could reduce fishing pressure on dwindling stocks of eastern oysters. In any event, research is needed to explore some of these possible interactions. Triploid *C. ariakensis* that are maintained under appropriate biosecurity measures could be used to explore some of these possible interactions in both the laboratory and field. Any such experimental work should be subject to rigorous oversight to ensure that experimental design and biosecurity concerns are met. Probably the greatest area of ignorance that needs to be addressed is how well diploid *C. ariakensis* will reproduce, recruit and survive under natural conditions in east coast waters. Studies of the west coast stocks may help to answer some of these questions. Even after more research, the ultimate outcomes of any introduction can never be predicted with certainty.

- Return to Benthic-Dominated Ecosystem. While there was general agreement that more oysters could restore the Chesapeake Bay to a benthic-dominated system, participants disagreed about the merits of such ecosystem-level changes. If established, widespread oyster populations likely would promote water quality and habitat benefits, including declines in gelatinous zooplankton as predicted by ecosystem-level models; however, a benthic-dominated system has the potential for reduced finfish populations. This scenario is highly speculative and should be explored among a wider range of scientists than was present in this workgroup.

- *C. ariakensis* a Nuisance Species? It is possible that *C. ariakensis* could so establish itself in the bay that oyster populations would become overcrowded, potentially resulting in small, commercially unacceptable oysters. Such high production could drive down market values. There were suggestions that if *C. ariakensis* became extremely prolific, it could turn into a costly nuisance, for example, fouling boat bottoms and water intake pipes; however, this argument is based on problems associated with such accidentally introduced species as the gypsy moth and zebra mussel that have escaped the natural biological controls imposed by predators in their native range. Because *C. ariakensis* is so similar in size, shell characteristics, reproductive mode, etc., to the native *C. virginica*, the suite of current predators (from humans, cownose rays, and blue crabs to the ubiquitous polyclad flatworms, *Stylochus ellipticus*) likely would control the abundance of *C. ariakensis* as was done historically for *C. virginica*.

Need for Risk/Benefit Analysis

There is a need to undertake a risk/benefit analysis that emphasizes an understanding of the economic and ecological consequences of having *C. ariakensis* introduced into the east coasts of North America. This analysis must take into account the ecological, social, and economic implications to Atlantic Coast stakeholders. For example, some groups such as watermen and processors would likely embrace an increase in oysters, whether they be *C. virginica* or another species, while others may object to a non-native oyster on the Atlantic Coast.

Research Support

Because of the identification of a number of research issues, it was recommended that the National Sea Grant Program's Oyster Disease Research Program (ODRP) be broadened to support research on non-native oyster species. The ODRP website is www.nsgo.seagrant.org/research/oysterdisease.

Regulatory and Policy Discussion Group

There was consensus on the need for additional resources and technical expertise for expanding the capability of looking at a "considered opinion" on the commercial use of triploid non-native oysters in the Chesapeake Bay. The group considered what is reasonable to ask and how much must be known in order to make an informed decision on whether and how to go forward with aquaculture of *C. ariakensis*. Key information needs for reaching defensible decisions include the following:

- More knowledge of basic species biology of *C. ariakensis*.

- More simulation modeling, including modeling of the economic dimension. There is a need to validate the risk model's predictions through field observations and experiments.

- A better sense of user need conflicts, for example, the effects of *C. ariakensis* culture on: (1) blue crabs, finfish fisheries, and boating in the Chesapeake Bay and (2) Atlantic Ocean and possibly even Gulf Coast ecosystems.

- A better sense of possible benefits to ecological function by restoration of filtering capacity using *C. ariakensis*.

- A better sense of the ecological consequences of introduction of the diploid *C. ariakensis*.

Biosecurity

Biosecurity concerns could be addressed by incorporating risk minimization strategies into the permitting process. The group discussed the possibility of including a bonding requirement in the permit. It also had an inclusive view of who should be included among the responsible parties in managing and mitigating any risks, including not just state agencies, but also federal agencies and the permittees themselves.

Maryland Opposition to Introduction of Non-Native Species

Although most participants agreed with the viewpoint of proceeding with caution, Maryland agency representatives oppose the introduction of a non-native species in any form.

Concluding Discussion: Summary of Key Issues Concerning Crassostrea ariakensis

In the wide-ranging discussion following the group reports, a number of key themes emerged on the potential benefits and risks, and on appropriate future actions regarding the aquaculture of *C. ariakensis* in the Chesapeake Bay.

Key Potential Benefits of *C. ariakensis*

- Expansion of employment and income with a restored shellfish industry.
- Increased state revenues from a vigorous industry.
- Improved ecological function in the Chesapeake Bay with increased numbers of filter-feeding bivalves.

Key Potential Hazards of *C. ariakensis*

- Hazard of unsanctioned "hooligan" introduction of *C. ariakensis*. These oysters would not be quarantined, posing the potential of introducing pathogens, parasites and other organisms. These oysters would be fertile diploids, lacking reproductive confinement.
- Hazard of changed ecosystem state, for example, of changing the current situation where most production is pelagic to one where most production is benthic. Such changes could reduce the productivity of certain valued fisheries.
- Any introduction of a non-native species poses unknown hazards.
- Hazard of not realizing the benefits posed should *C. ariakensis* <u>not</u> be commercially produced.
- Trojan oyster potential: Published reports establish the inviability of larvae produced by mating *C. virginica* and *C. ariakensis* (fertilization is relatively successful). Given that reproductive populations of the two species would be juxtaposed and spawn simultaneously, the newly formed embryos would serve as a gamete sink (for both species). In the case of *C. virginica*, this could represent an impediment to native restoration efforts.

Key Research and Development Needs

- Continue efforts to produce commercially viable numbers of tetraploid individuals.
- Continue research with the triploid in confined environments, especially to understand hazard pathways and probabilities.
- Give serious consideration to the possibility of introducing reproductively capable diploid *C. ariakensis*: it may be appropriate to initiate opportunity and risk assessments soon.
- Continue development of selectively bred eastern oyster stocks, although we must recognize that it would be genetically altered compared to a wild native oyster.

Summaries of Presentations on C. ariakensis

Biology of *C. ariakensis* in its Native Range, Roger Mann, Virginia Institue of Marine Science (VIMS)

Roger Mann discussed our limited knowledge of *C. ariakensis* biology in its native range and outlined limitations in the ability of scientists to predict outcomes were diploid (fertile) *C. ariakensis* introduced into the Chesapeake Bay. One reason that the *Crassostrea* species is so dominant is its ability to withstand large temperature and salinity ranges. He pointed out the need for biosecurity to guard against possible management mistakes. He described the dimensions of possible benefits and possible risks posed by introduction of triploid *C. ariakensis*, both of which were large in magnitude.

Disease Certification, Eugene Burreson, VIMS

The importance of MSX and Dermo in the decline of the Eastern oyster underlines the importance of disease certification for imported oysters. There is no information on diseases and pathogens in the native range of *C. ariakensis*; therefore, only offspring that are bred from non-native broodstock should be used to produce triploids. These protocols have been adhered to in VIMS research to date: *C. ariakensis* deployed in Virginia were the offspring of imported individuals, which eliminated many possible bacterial or protozoan disease problems. While hatchery spawning eliminates most diseases and pathogens, this is not necessarily the case for viruses. Viruses cannot be diagnosed without molluscan cell culture or electron microscopy. Overall, the risk of viral disease seems low, though it is not absent.

Field Trial Studies of *C. ariakensis* and *C. virginica*, Mark Luckenbach, VIMS

Trials of *C. ariakensis* and *C. virginica* were conducted at six sites of high, medium, or low salinities (from 36 parts per thousand (ppt) down to 8 ppt). VIMS scientists found that *C. ariakensis* grew faster than *C. virginica* at all sites; mortality of *C. ariakensis* was low at all sites, while mortality of *C. virginica* was high; prevalence and intensity of Dermo were low in *C. ariakensis* and very high in *C. virginica* (Calvo et al., 2001).

In preliminary experiments that compared the growth of post-set juveniles in quarantined conditions, VIMS scientists found that *C. virginica* was somewhat more competitive than *C. ariakensis*, gauged by increase in shell height and weight. Under these conditions, *C. virginica* grew faster and had higher survival; *C. ariakensis* had poorer survival and grew more slowly when *C. virginica* were present. It is important to note that these findings were based only on juveniles and did not account for the effect of oyster diseases. Thus, says Mark Luckenbach, extrapolation to field conditions is difficult.

Industry Trials of *C. ariakensis*, James Wesson, Virginia Marine Resources Commission

Field trials by members of the Virginia Seafood Council were conducted at six sites at three salinity ranges. Trials begun in August 2000 showed low mortality, rapid growth, and excellent yield of shucked meat of *C. ariakensis*. Some *C. ariakensis* deployed in trials begun in June 2001 had reached 5 to 6 inches in shell length by October. The oysters grew well in a variety of confinements, including corrals, floating cages, and sunken trays. Consumer acceptance was very high. Hence, there is great interest in the commercial sector for producing *C. ariakensis*.

Triploid Strategy and Risks, Standish Allen, VIMS

Standish Allen discussed the general approaches to developing a triploid *C. ariakensis* aquaculture industry and possible risks posed by commercial production in Virginia waters of the Chesapeake Bay. For strain development (domestication), identification of the best-performing *C. ariakensis* stocks from native populations has begun. Stocks from western Japan, northern China, and southern China are being reared at VIMS. For developing sterile stocks, triploids are produced. Production of triploids through chemical induction is imperfect and infeasible for commercial production. Development of tetraploids for crossing with diploids is more efficient and reliable, and hence can provide the numbers of triploid seed needed for an industry. The availability of tetraploids is now the limiting factor for stepped-up commerical production. Risk factors associated with the triploid aquaculture of *C. ariakensis* include:

- Reliability of triploidy induction (i.e., the possible occurrence of diploids among triploids even using tetraploid crosses).

- Sterility of triploids (the possible production of euploid gametes among the many aneuploid gametes with unmatched chromosomes).

- Stability of triploids (triploidy can be unstable in a certain proportion of the population and over time, this instability yields individuals that have dipliod and triploid cells simultaneously, called mosaics).

- Fertility of mosaic individuals (mosaics may recover reproductive capability given time, i.e., years, although this phenomenon has not been clearly demonstrated in studies so far).

Risk Model, Jim Berkson and Jodi Dew, Virginia Polytechnic Institute and State University

Interactions of scientific uncertainties and management options must be considered to reach an informed decision on whether and how to go forward with commercial production of triploid *C. ariakensis*. In this context, it is useful to develop and use a simulation model as a tool for approaching a range of "What if?" questions. Jim Berkson and Jodi Dew presented a risk assessment model for considering issues posed by culture of triploid *C. ariakensis*. The model was developed in Visual Basic because of its user-friendly interface and is available from Virginia Sea Grant (Dew et al. 2001). Simulation of population growth under specified ecological conditions and management strategies was used to evaluate the likelihood of a population becoming self-sustaining at a given site. Mortality, growth, and reproductively effective reversion rates were modeled as stochastic processes. Because knowledge is lacking for *C. ariakensis*, an existing model for fertility in *C. virginica* was used. The model predicted that the likelihood of a self-sustaining population becoming established increases as salinity increases, as minimum harvest size increases, as certainty of harvest decreases, and as population density in the culture system increases.

Strategies for Minimizing the Risk of *C. ariakensis* Aquaculture, Standish Allen, VIMS

In the context of the predictions of the risk model, Standish Allen presented a strategy for commercialization of triploid *C. ariakensis* production while minimizing potential risks posed to the Chesapeake Bay ecosystem. A seed-stock production enterprise would need a more elaborate structure than that in the shellfish production industry currently. A "technology" group would be involved in broodstock development, especially in development of a stock of tetraploid males and domesticated lines of diploids, used together for triploid production. Aquaculture operations would involve the following:

- Oversight and training, diligence with confining larvae, and certification and tracking of batches of seed-stock.
- Nursery stage would entail separate rearing and tracking of individual batches.
- Grow-out methods would have to be custom designed for site characteristics, notably salinity.
- Harvest would have to be complete, with accountability for complete harvest, and harvest would have to occur before spawning.

Dr. Allen also suggested co-stocking with *C. virginica* to serve as gamete traps, or as "Trojan oysters."

Attitudes about Non-Native Aquaculture, Ratana Chuenpagdee, VIMS

Because the decision of whether and how to go forward with commercial production of a non-native species is value-laden, stakeholder attitudes were considered by Ratana Chuenpagdee, who reported the results of her survey of six key constituencies. Her paired comparison-based survey showed that watermen and members of the seafood industry most highly valued possible impacts of *C. ariakensis* on human health and on other marine species. Oyster experts, scientists, environmental non-governmental organizations, and the general public most highly valued impacts on other marine species, human health, and wild oyster populations. No group was highly concerned with the costs of government oversight.

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Glossary

Aneuploid gametes. Germ cells (egg or sperm) that have more or less than a complete complement of chromosomes. Chromosomes in most living organisms come in two sets, and gametes generally only have one set. For example, a set of human chromosomes has 23 and a set of oyster chromosomes has 10.

Benthic. Those biological and chemical processes associated with the seabed.

Biosecurity. In the context of triploid *C. ariakensis*, biosecurity refers to measures taken to minimize the likelihood of stocking this species in the Bay, such as rampant reproduction or the introduction of pathogens or pests.

Broodstock. Adult organisms that yield offspring.

Cell culture. The cultivation of cell populations isolated from tissues of plants or animals, artificially in the laboratory.

Diploid. A chromosome state in which each chromosome is represented twice (2N). (In animals with sex chromosomes, the sex chromosomes are not necessarily present in two copies.)

Electron microscopy. High resolution microscopy obtained by using atomic particles to illuminate the subject. Electron microscopy often is used in detection of viruses.

Euploid gametes. Germ cells (egg or sperm) that contain an even multiple of chromosome sets, i.e., one set in gametes from diploids, two sets in gametes from tetraploids.

Gamete sink. In the context of this report, gametes of two species that combine to form inviable hybrid embryos, thereby wasting reproductive effort of both.

Gelatinous zooplankton. Invertebrate animals of the phylum Coelenterata, including jellyfish, comb jellies, hydras, sea anemones and salps.

Hazard. An undesired outcome, for instance, an accidental introduction of a new oyster disease.

Molecular techniques. Methods of examining genetic relationships using traits at the DNA level. For example, oysters can be identified to species based on unique sequences of nucleic acids that are revealed by isolating, cutting, amplifying, and visualizing DNA fragments.

Pelagic. Those biological and chemical processes associated with the water column.

Protozoan. Any single-celled member of the animal kingdom in the phylum Protozoa.

Ploidy. The number of chromosome sets.

Polyploidy. A chromosome number that is a multiple of the normal diploid number.

Reversion. In the context of triploid *C. ariakensis*, the gradual progressive loss of chromosomes from polyploid individuals, producing individuals with cells of more than one ploidy.

Risk. The probability of a hazard becoming realized when exposed to a causal agent.

Tetraploid. Having four sets of chromosomes in the nucleus.

Triploid. Having three sets of chromosomes in the nucleus.

Appendix I. Suggested Discussion Questions for Break-out Groups

Industry/Watermen Break-out Group
Discussion Points

- If allowed to proceed, what level of production can be anticipated in 1 year, 5 years, 10 years?
- What measures would you be willing to take to minimize risks, presumably at the expense of profit margins?
- Would you make your books available on purchase, movement, harvest and sale of triploid *C. ariakensis* product?
- What (more) do we need to make an informed decision on whether and how to go forward with aquaculture of triploid non-natives?
- Who/what are the responsible parties/agencies in managing and mitigating any risks?

Requested Participation

William Abbott	Ann Arseniu	Chad Ballard
Ernest Bowden	Warren Cosby	Lake Cowart
Marshall Cox Sr.	Jeffrey Crockett	G.G. Crump
Andy Drewer	Carter Fox	Tom Gallivan
Joseph Hanberry	Joseph Hicks, III	A. Lionel Jenkins
Douglas Jenkins, Sr.	Aubrey Justis	Kenny Keen
Tommy Kellum	Tommy Mason	Joshua Merritt Jr.,
Pete Nixon	Mike Pierson	Frances Porter
Margaret Ransone	Larry Simns	Kenneth Williams
George Washington		

Chair	Tom Kellum
Rapporteur	Carter Fox
Chronicler	Sally Mills

Regulatory/Policy Break-out Group
Discussion Points

- Do regulatory groups have the capability to look at a “considered opinion” on the use of triploid non-natives in the Bay?
- How would biosecurity concerns be incorporated into a permitting process?
- Other “critical issues?”
- What (more) do we need to make an informed decision on whether and how to go forward with aquaculture of triploid non-natives?
- Who/what are the responsible parties/agencies in managing and mitigating any risks?

Requested Participation

Bill Anderson	Russell Babb	Lowell Bahner
Russ Baxter	Gordon Birkett	Arthur Butt
Frank Dawson	Mike Fritz	Craig Hardy
Henry Lane Hull	Jim Joseph	Chris Judy
Andrew Manus	Mike Marshall	Laura McKay
Monaca Noble	Tom O'Connell	Rob O'Reilly
Preston Pate	William Pruitt	Eric Schwaab
Pat Stuntz	Ann Swanson	Rich Takacs
Jeff Tinsman	Dennis Treacy	Carolyn Watson
Jim Wesson	John Wolflin	John Paul Woodley

Chair	Frank Dawson
Rapporteur	Jim Joseph
Chronicler	Wanda Cohen

Science Break-out Group

Discussion Points

- What is your assessment of the current level of risk for commercial aquaculture?
- What aspects of "risk" are satisfactorily addressed and which need more research?
- Other "critical issues?"
- What (more) do we need to make an informed decision on whether and how to go forward with aquaculture of triploid non-natives?
- Who/what are the responsible parties/agencies in managing and mitigating any risks?

Requested Participation

George Abbe	Stan Allen	Jim Berkson
Sasha Bishton	Don Boesch	Gene Burreson
Bob Byrne	Lisa Calvo	Mark Camara
Ratana Chuenpagdee	Jodi Dew	Hugh Ducklow
Emmett Duffy	Bill DuPaul	EricHallerman
Steve Jordan	Fred Kern	Jonathan Kramer
Mark Luckenbach	Roger Mann	Don Meritt
Cheryl Morrison	Roger Newell	Richard Osman
Ken Paynter	Pete Peterson	Kim Reece
Bill Rickards	Greg Ruiz	Linda Schaffner
Kevin Sellner		

Chair	Roger Newell
Rapporteur	Cheryl Morrison
Chronicler	Merrill Leffler