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by

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Rationale:

The native oyster species, ***Crassostrea virginica***, stocks in the Chespeake Bay have been depleted in part by the action of two diseases. These are ***Haplosporidium nelsoni***, commonly known as MSX, and ***Perkinsus marinus***, commonly known as Dermo. Despite the fact that these diseases have been present for approximately three decades and have been contributing to mortality throughout that period, the lack of natural recovery of the native oyster resource in the face of continuing disease pressure indicates that the native oyster has failed to develop a high level of natural resistance to this combination of diseases. If or when such resistance will develop is unknown. The focus of investigations reported upon today are the questions of whether or not non native oyster species have resistance to the endemic diseases and, if so, then can such species be used either directly or in a hybrid form with the native oyster to rebuild the Chesapeake Bay oyster resource?

Approach:

The Japanese or Pacific oyster, ***Crassostrea gigas***, is a closely related species to the native ***C. virginica*** and is grown in many locations throughout the world including the west coast of the United States. Over 50% of the oysters eaten in the United States are ***C. gigas*** produced in the Pacific North West or imported from the Orient. ***C. gigas*** has been introduced into several locations globally to rebuild oyster resources devasted by endemic diseases with positive results. In not one of these instances has there been devastation of the newly introduced species by the endemic disease. These observations suggest that ***C. gigas*** may have high natural resistance to a number of diseases to which other, closely related oysters succumb.

Our research is presently examining the resistance of the following oyster "strains" to ***Perkinsus marinus*** (Dermo):

native ***C. virginica***

native ***C. virginica*** in which the chromosome number has been increased by 50% from two chromosome sets per cell to three sets per cell. This is termed ***tri***ploid (3n) in contrast to normal ***di***ploid (2n).

C. gigas (diploid) grown from the egg under quarantine conditions in the V.I.M.S. oyster hatchery at Gloucester Point. Quarantine conditions follow guidelines of the International Council for the Exploration of the Seas (I.C.E.S.).

C. gigas with increased chromosome number (triploid).

Hybrids of the two species:

C. virginica (mother) x ***C. gigas*** (father)

C. gigas (mother) x ***C. virginica*** (father)

We have chosen to investigate resistance to ***Perkinsus*** because we can transmit this disease in the laboratory under controlled conditions. This disease challenge can therefore be effected without releasing the non native oysters or the hybrid oysters into the natural environment. If the oysters

all die then the study of *C. gigas* as an alternative source of genetic material can be terminated without introduction; however, if the non native or hybrid oysters show superior resistance to *Perkinsus* then a test of resistance to MSX will require exposure in the natural environment because we *cannot* transmit MSX from oyster to oyster in the laboratory. No such MSX exposures have been made so far and will not be without prior knowledge and permission of the Virginia Marine Resources Commission.

Results to date:

Eight dozen *C. gigas* adults were obtained from a west coast hatchery in February of this year and transferred directly to a quarantine system (I.C.E.S. specification) in the V.I.M.S. hatchery. Native oysters, *C. virginica*, for comparative studies were collected from Mobjack Bay. Between March 23 and May 2 of this year these parental oysters were spawned and the various "strains" described above cultured through the larval stages. After spawning all *C. gigas* adults were destroyed in accordance with I.C.E.S. guidelines.

After culture of the larval stages in the controlled hatchery condition (effluent chlorinated) the settled oysters (spat or juveniles) were maintained in controlled systems at V.I.M.S. The total number of competent to settle larvae from these spawnings and the numbers of spat surviving as of mid August are as follows:

"Strain"	Larvae	Spat	%triploid
C. virginica (2n) :	450,000	3,565	
C. virginica (3n) :	505,000	2,760	96%
C. gigas (2n) :	1,000,000	1,852	
C. gigas (3n) :	750,000	5,683	75%
Hybrid, *C. virginica* mother :	1,100,000	75,123	
Hybrid, *C. gigas* mother :	70,000	380	

Note that the number of larvae is dependent on the number of oysters originally spawned. Induction of triploidy is not always 100%, hence the percentages given for the 3n "strains". We also tried to induce triploidy in the hybrids but these crosses appear resistant to the current induction method.

The above described oysters are currently being used as source stock for disease challenge experiments with *Perkinsus* at V.I.M.S. Gloucester Point campus. In addition to the above animals seventeen dozen (204) juvenile *C. gigas* were obtained from a west coast hatchery in June of this year and transferred to the Wachapreague laboratory of the Virginia Institute of Marine Science. These animals were also were also maintained under I.C.E.S. quarantine conditions for the duration of their stay at Wachapreague where they were also examined for their resistance to *Perkinsus*.

Disease challenge of juvenile *C. gigas* with *Perkinsus* at Wachapreague.

Half of the *C. gigas* were maintained in an environment to which *Perkinsus* infective particles were regularly added. Twenty five native oysters were included in the same tank for comparative purposes. The other half of the *C. gigas* were maintained in an otherwise identical environment, together with twenty five native oysters, but in the absence of *Perkinsus* infective particles. After three months of continued disease challenge one native oyster had died and the remainder had moderate or heavy infections. By comparison no *C. gigas* died and a subsample of 25 oysters showed only very light infections. No control (no disease challenge) animals died. We are continuing to dose the surviving *C. gigas* with *Perkinsus* infective particles and still see only light infections. These observations suggest that *C. gigas* may become infected but to a much lesser extent than the native oyster under the experimental conditions.

Disease challenge of all families with *Perkinsus* at Gloucester Point.

This study is in progress using oysters cultured at the V.I.M.S. hatchery as described in the earlier table. A sample from each strain is being maintained in a closed system in an environment conducive to development of *Perkinsus*. The system is regularly dosed with *Perkinsus* infective spores. Current prevalence of infection is as follows:

C. virginica (2n) :	30.4%	
C. virginica (3n) :	16.0%	
C. gigas (2n) :	40.0%	(mostly light infections)
C. gigas (3n) :	12.0%	
Hybrid, *C. virginica* mother :	33.3%	
Hybrid, *C. gigas* mother :	48.0%	

It is important to note that these values are the percentage of the total that are infected. They do not illustrate the level of infection in each individual. The infections in the *C. gigas* are generally light (supporting, at least so far, the observations of the Wachapreague experiment). Also of interest is the comparatively low prevalence in the triploid strains. As mentioned earlier, this is a continuing experiment and these data may change as the experiment progresses. In addition, a replicate of this experiment using a higher food dose per animal is planned to begin shortly. In due course we plan to report on all of these experiments to the Commission.

Future Directions.

It is critical to underscore at this time that these are early experiments in a prolonged series. There are a number of significant *IF'S*. If these experiments proceed and all the non native species or hybrids thereof die under *Perkinsus* challenge then clearly this source of genetic material offers little hope. There are many strains of *C. gigas* and even other *Crassostrea* species worthy of examination in this instance but let us

think a little more positively. If the non native species and/or hybrids demonstrate resistance comparable to or better than the native species then the task remains to test resistance to MSX. This must be effected in the natural environment and such work would be proposed for the the V.I.M.S. campus at Gloucester Point. A request for permission to effect such a study is not appropriate at this time: however, should our studies support such a request it would be appropriate to start such a challenge no later than May 1 of 1990. A request for permission to effect such a study should therefore be made no later than the March 1990 meeting of the Commission. Such a study is not without some risk. Possible spawning of the experimental stock cannot be controlled. An extensive discussion of this possibility is beyond the scope of this document but I do not believe the impact would be significant. The possibility of introduction of an associated disease exists but experience with I.C.E.S. procedures elsewhere indicates that this is mininscule. Given the present maintainance regime of the "strains" at V.I.M.S., where water control dictates food limitation, it is probable that disease, if present, would have already manifested itself. No substantial mortalities have been observed. No evidence of disease organisms has been seen in histological sections of sampled animals.

Summary

To date our observations suggest that ***C. gigas*** may be infected by ***Perkinsus*** but not to the extent experienced by the native oyster under identical conditions. Continued exposure may result in greater intensity of infection and this is under study. Triploid oysters appear to suffer lower prevalence of disease than diploid oysters under the tested conditions. Should continuing experiments confirm superior resistance in either the non native species or the tested hybrids then field exposures to test resistance to MSX will be required. A further summary of work will be given to the Commission in the Spring of 1990. A request for permission to effect MSX testing will be made at that time only if it can be supported by the total of all experimental observations made to that time.