Center for Independent Experts (CIE) Review of the Status of the Eastern Oyster ***(****Crassostrea virginica****)***

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1. Executive Summary of comments – Recommendations

NOAA's National Marine Fisheries Services (NMFS) received a petition to list the Eastern oyster *(Crassostrea virginica)* as either threatened or endangered under the Endangered Species Act (ESA). The Eastern oyster is an emblematic species in the US coastal states, playing a significant role at various levels: shaping coastal social communities, providing a commercial activity (public fishery & aquaculture) and recreational - amenity benefits, and as a keystone species in highly diverse and sensitive ecosystems (estuaries). This petition is likely the result of concerns regarding the partial collapse of public fisheries in several states as well as from the resulting damages to this species induced by severe hurricanes. A Biological Review Team (BRT) was convened to review the status of the Eastern oyster and produced a final report entitled 'Status of the Eastern Oyster *Crassostrea virginica*'. This work has been reviewed to provide further and complementary recommendations.

ToR 1. Status report review.

The report reviews the species' biology, provides an analysis of the ESA's five factors (habitat threats, overutilization, predation and disease, regulatory mechanisms, other manmade and natural impacts), reviews the use of aquaculture for this species, and assesses the status of the oyster population across its geographic range and evaluates, by using various approaches, the on-going conservation actions and further research needs. In spite of uncertainties regarding the oyster delineation range, the report provides extensive information based on fishery landings and also on oyster species status by using answers to questionnaires from scientists and managers from States involved in oyster fishery and management. Although commercial landings - in significant decline - represent a global indicator, it is well recognized that such information is insufficient to draw conclusions on the oyster species status. Therefore, additional information, including restoration efforts, was collected by direct interviews and from published information to provide a status report of the oyster species. Globally, the species was considered to be sufficiently resilient, with overharvesting being a minor threat to the species and recruitment sufficient to maintain population viability throughout the species range, except in a portion of the mid-Atlantic. Restoration and enhancement efforts for conservation and fishery purposes are carried out throughout the species range and are considered to be a necessary process in half of the reviewed estuaries in the mid and south Atlantic areas. Moreover, aquaculture is expanding across the species range, which further facilitates its sustainability. The species shows a wide range of survival strategies, including a strong reproductive capacity facilitating wide dispersal, and a wide tolerance to extreme environmental conditions. In spite of threats in various areas, such as disease epizootics, no threat has been considered to be so overwhelmingly dominant as to threaten the viability of the species across its range. The BRT concluded that the long-term persistence of Eastern oysters is not at risk.

The report review was based on editing the report and adding missing references from the published and 'grey' literature. Statements were reviewed and completed, and additional studies and missing scientific data were identified. Generally, the report is appropriate in reviewing all elements of the available information and the main conclusions are confirmed, notably the fact that the oyster species is not at risk now and will not be in the foreseeable future.

The oyster species shows biological characteristics leading to strong resilience, including wide adaptation to highly variable environment, large reproductive capacity, and persistent larval pelagic stage facilitating dispersion. A multifactorial approach, combining for example salinity and temperature thresholds to explain larval survival and growth rates, was suggested as an approach to better understand adaptability to highly variable environmental conditions. Additional scientific data were brought in to explain larval dispersion and retention within geographic structures, which are eventually genetically characterized in several case studies.

ToR 2.a. Are species/subspecies delineations supported by the information presented?

The issue of genetic structure of current oyster populations along the US and Central American coastlines and, more specifically, the issue of species delineation for conservation purposes and the question surrounding subspecies for *C. virginica,* was supported by appropriate information but eventually considered as a secondary issue since phenotypic differentiations of interest for conservation have not been demonstrated, nor are available with the two potential subspecies of *C. virginica*. Similarly, morphological variation obtained in the Laguna Madre oyster population was believed to be due to environmental influences rather than genetic influences, in spite of a genetically distinct population (cf p. 17). Therefore, top priority should be given to understand phenotypic/genotypic characteristics and interactions, to be able in the near future to facilitate further decisions regarding conservation options. If significant differences were to be demonstrated, it might also facilitate decisions related to the subspecies issue. Moreover, aiming for genetic variability and peculiarities at a microscale structure might likely be a more rewarding approach for identifying, by way of example, natural disease resistant strains (e.g., Lynnhaven River oyster population, Chesapeake Bay), and to sustain optimal management options for oyster stock enhancement.

ToR 2.b. Does the report include and cite the best scientific and commercial information available on the species and threats to it and its habitat?

Although not entirely comprehensive, the available information on the species and threats to it are presented in a proper way in the report. Particularly, the issue of species and/or subspecies delineations is presented in an optimal way considering large uncertainties regarding the techniques and samplings available. Moreover, it is considered as a less critical issue - due to the characteristics of the animal and its population genetics – compared to more advanced animals (e.g., killer whale). Similarly, additional up-to-date references of critical interest were unavailable at the timing of the report publication (Rose et al., 2006).

Among ESA's five factors, habitat threats were considered of critical importance, due to global environmental changes, feedback effects, and the associated uncertainties (e.g., qualitative and quantitative freshwater flows vs recruitment, species distribution).

With regard to the management options, it should be emphasized that most of the actions aim for long-term effects (reef building, minimum legal size, yield per recruit, spawning stock biomass) rather than short term actions, such as a dynamic management approach considering by way of example, environmental conditions and disease prevalence to maximize spat recruitment.

Aquaculture has several major applications in sustaining wild populations as well as in contributing to the sustainability of oyster populations through supportive breeding programs. However, the potential for selected oyster strains to contribute to the gene pool of co-occurring wild oyster populations is clearly an issue of concern to be addressed, as is the founder effect and possible genetic drift. Hatchery production might be a genetic bottleneck if supportive breeding is not properly carried out (specific requirements at the genetically effective population size). Most of the research effort is carried out through the Chesapeake Bay supportive breeding program using, by way of example, the DEBY dual disease resistant strains. Any prospect for 'genetic rehabilitation' by introgressing alleles for disease tolerance into wild populations is still speculative, although part of the scientific debate and interest. Impact of polyploids, not really discussed in the report, should also be considered: triploids are not entirely sterile and might produce gametes in specific environmental conditions (e.g., high summer temperatures).

ToR 2.c. Are the scientific conclusions sound and derived logically from the results?

The report reviewed in an appropriate way the present status of the Eastern oyster *Crassostrea virginica* across its natural range and concluded that the species is not presently at risk and will not be for the foreseeable future. I fully agree with the report's conclusion on the long term persistence of Eastern oysters. Although the report does not provide a comprehensive dataset of information, the resulting scientific conclusions are sound and derived logically from the results. Overall, the results are consistent with the conclusions.

ToR 2.d. Where available, are opposing scientific studies or theories acknowledged and discussed?

Rather than missing 'opposing scientific studies', complementary approaches could be suggested to clarify the issue, and therefore are herein proposed as research needs and gaps as an addition to the report. They concern both oyster stock assessments and genetic research at hatchery production sites to sustain appropriately supportive breeding programs. Estimates of effective population size are required, both at the hatchery and in the wild, according to various geographic sites. Ex-situ conservation, although debatable when proposed as a single management option, could be suggested in addition to in-situ management options. Both approaches require an appropriate knowledge of the populations of interest and a better understanding of the genetic population structure - therefore sampling strategy - particularly at the limits of the species distribution. Knowledge of disease resistant strains and their potential uses and impacts is required, as well as the virulence of associated diseases agents, to produce better understanding of interactions among 'environmental conditions', 'pathogens', and 'host'.

Moreover, the effects of global climate change on the oyster population status is obviously an issue to consider for the species conservation and further management options in spite of the broad distribution range of the species. This would likely facilitate overall understanding. Potentially induced changes in the distribution of exotics and disease vectors might also be of interest in a similar way for a more comprehensive view and to assess the on-going trends in *C. virginica*.

2. Background

NOAA (NMFS) received a petition to list the Eastern oyster (*Crassostrea virginica)* as either threatened or endangered under the Endangered Species Act (ESA). This petition likely reflected concerns over the near collapse of the oyster fishery in several States along the eastern US coastline and drastic climatic events (hurricanes) affecting wild and cultured oyster populations along the Gulf coastline. The key question is whether the fishery landings are a general indicator of the status of the oyster *C. virginica* population, given the lack of comprehensive spatial and temporal oyster stock assessments along the US coastline to specify the present status and population trends of this species.

Moreover, decades of fishery management have been obviously insufficient to reverse the downward trends in oyster landings, therefore prompting the managers to question the overall efficiency of management practices, and to explore complementary approaches such as reef building and sanctuaries. Adding to the complexity is the fact that oyster diseases (e.g., MSX, *Perkinsus marinus*) since their occurrence have had a significant impact on Eastern oyster populations and landings, although not leading to a fast disappearance of the species. This is to be contrasted with what happened in French waters with two *Crassostrea angulata* populations in the early 1970s, which prompted the managers to quickly introduce a new oyster species (*Crassostrea gigas*). Actually, there is no doubt that MSX and Dermo are both major driving factors that have had an impact on Eastern oyster populations, but to what extent remains unclear. For example, the natural development of disease resistant populations, resulting from a natural selection process following decades of disease-induced high mortality rates remains scientifically debatable, even though some evidence is reported in Delaware Bay oyster populations.

Moreover, the Eastern oyster is an emblematic species for the scientific community as well as environmentalist-conservation groups, considering the ecological services provided in both environmentally sensitive and highly impacted areas (estuarine & coastal areas). The oyster is usually considered a keystone species and a suitable indicator of environment health since it will thrive in a 'healthy' environment and can also be used to mitigate and reverse environmental damage in polluted estuarine and coastal areas. This is mainly due to its capacity to build three dimensional habitat (thereby providing new habitat for biodiversity improvement), as well as to develop large and extensive populations that can filter significant volumes of seawater and remove particles and bio-accumulating pollutants, and thus acting to limit eutrophication side effects.

Apart from the ecological services provided by this species, the Eastern oyster is also a centerpiece in local and coastal communities, which otherwise would not exist. Since the colonial times, the oyster has occupied a prominent role in building and propping up social communities and still represents a 'way of life' in several coastal areas (e.g., watermen in Chesapeake Bay, 'folk management' in Florida & Louisiana, etc.). Recreational and amenity benefits are also well recognized at various coastal user levels.

For all of these reasons, it makes sense to examine the present status of the Eastern oyster and future trends for those populations, and to specify further research needs for the sustainable development and conservation of this emblematic species.

3. Description of review activities

The review activities included the following:

1. Performing a literature review to verify any possible missing information, as well as to add references published in between the report completion and the present review of the report (2 days);

2. completing the published literature review by checking 'grey' literature of interest (meeting minutes of conferences and workshops) (1 day);

3. editing the report and identifying missing items (1 day);

4. discussing with additional scientists (e.g., geneticists) specific items (1 day);

5. synthesizing the information and writing the evaluation report (1 day).

4. Summary of comments

The reports contains additional comments and references directly listed using revision marks to facilitate their identification and reading.

In addition, the following comments are provided in accordance with the various chapters of the report: Species biology, analysis of the ESA's five factors, aquaculture, status of the population, conservation actions, research needs, and conclusions.

a. Species Biology

This chapter is an appropriate general overview of the *C. virginica* biology, identifying the main factors (and their values) possibly affecting oyster population. This covers all the necessary items, ranging from individual to population dynamics, from morphology, reproductive behaviour, spat settlement, and the genetic structure of the populations. The literature cited is appropriate, although other key references can be added (e.g., Mann et al. 1991).

It should be emphasized that, while all the factors are presented separately (although optimum values were not systematically provided), little information and less emphasis was placed on specifying the interactions among factors that are of critical importance to understanding the entire process. Several references were added to address the issue. By way of example, while oyster larval survival rate could be described according to single values, such as temperature, salinity, etc., multiple factor models that combine various variables are more suitable for describing and predicting survival and growth rates (e.g. Lough, 1975, Goulletquer et al. 1994; Mann et al. 1995). Similarly, the duration of the larval pelagic stage is simply presented as a two-week process, with a capacity to reach a month. Those are average figures. Actually, the pelagic phase can last more than a month depending on environmental conditions. At lower temperatures, significant survival and growth rates still can be obtained (with a final

successful metamorphosis) following a two-month larval phase, which is a critical change when considering larval dispersion and population expansion (Boudry, personal communication). These comments are made mainly to emphasize the fact that a multifactorial approach is more suitable to estimate *in situ* population trends. Similarly, oyster summer mortalities, described as occurring in sea water temperatures exceeding 30°C, are also related to oyster physiological status (e.g., active reproduction and increased energy demands). In contrast, mortality rates of 'sterile' polyploids are limited in summer. Thermal tolerances can be expanded by thermal stress, which activate oyster physiology (e.g., induced heat shock proteins – HSP). Again, the multifactorial approach should be prioritized to explain trends. With respect to growth and feeding, it should be emphasized that the high growth variability (for a similar age) is directly dependent upon carrying capacity and stocking biomass, and therefore environmental conditions. Oysters have the capacity to adapt their physiological activity and to operate a particle selection process at the gill and labial palp level, therefore explaining the production of biodeposits (e.g., pseudo feces), leading to possible siltation and subsequent environmental impacts.

Habitat preference is well described, although it should be emphasized that oyster physiology will adapt according to conditions. Dissolved oxygen saturation, between 20-100%, is 'acceptable' but at the expense of oyster physiology and behaviour. Below 60%, metabolism would adjust to those conditions, which can lead in extreme conditions to hypoxia, and anaerobiosis, a different physiological pathway mobilizing other constituents (carbohydrates are broken down to sustain metabolism).

With regard to ecology and population dynamics, I entirely agree with the statement that the species is highly resilient, therefore tolerating highly variable environmental conditions and showing a wide spatial distribution. The ecological role of oysters in the estuarine community can be reinforced by additional references using a modelling approach (e.g., Dame 1993, 1996). The report specifies the current and historic abundance (p.12) and highlights uncertainties due to the lack of reliable quantitative survey data. While it is true that biological surveys have not been carried out extensively for various reasons (p.13), statistically sound survey designs are available, as are gear efficiency comparisons (Chai, 1992a,b; Jordan et al., 2002). Therefore, the issue is at the management level, rather than a question regarding scientific feasibility. With respect to recruitment, it should be emphasized that mechanisms of dispersal and recruitment are still unclear (Epifanio, 1988). However, the local phenomenon of larval retention is generally explained by 'passive' transport induced by physical factors, by an 'active' process involving larval swimming, or by a combination of both (Deskshenieks et al., 1996). Several references can be added on that critical matter to assess oyster population status as well as in terms of management options. This is also critical to explain genetic differentiation among geographical areas.

Species delineation

This leads to the issue of genetic structure of current oyster populations along the US and Central American coastlines and, more specifically, the issue of species delineation for conservation purposes and the question surrounding subspecies for *C. virginica* (task 2a in the statement of work). First, it must be noted that, due to the oyster genetic characteristics and variability, the issue of subspecies is less critical for the Eastern oyster than a similar question would be for for a marine mammal (e.g. killer whales). The report details extensively the various methods used to obtain conclusive information regarding genetic population structure, underlining the difficulties and controversies among results. Those results have shown significant shifts concomitantly with improvements in biomolecular methods such as genetic

markers. Therefore, those recent developments explain why the conclusions are not definitively drawn on issues of subspecies and species delineation. However, the most conclusive results indicate the possibility of Atlantic/Gulf population structure with a genetic transition, while other studies report on a panmictic population (Buroker, 1983). The Atlantic/Gulf population would make sense according to biogeographical constraints between those two distinct areas.

Besides *C. virginica*, it should be noted that the whole genus *Crassostrea* is up to now still under review and that regular revisions occur. Rather than concluding directly on the existence (or not) of subspecies based upon genetic descriptors, several points can be highlighted and questioned:

1. Do we have significant phenotypic differentiations associated with the Gulf and Atlantic populations that would justify and strengthen the need for suitable conservation measures?
2. Rather than the subspecies issues, do we have more concerns around genetic peculiarities at a smaller scale (see Laguna Madre and Chesapeake Bay studies)?

Considering the first question, it is interesting to compare the *C. virginica* situation with the status of *C. angulata* versus *C. gigas* in European waters. Following a long debate on the *C. angulata* origin and speciation, and the use of various genetic markers, scientists have concluded that *C. angulata* is of Asian origin and shares a large amount of genetic structure with *C. gigas* (O. Foighil et al., 1998; Boudry et al., 1998). Actually both 'species' are so similar that they are considered as taxa. However, their phenotypic characteristics present highly significant differences in reproductive pattern, in the duration of the spawning season, and several partial spawnings versus a massive one. Sensitivity to diseases is also significantly different - *C. angulata* was eradicated by a virus while *C. gigas* was not impacted. Also, overall oyster yield is reduced for *C. angulata* compared to *C. gigas*, and metabolic activity is greater for *C. gigas*. All these differences make them valuable for conservation purposes while *C. angulata*, restrained to a small geographic area in Southern Portugal, is disappearing due to crossing with the *C. gigas* strain, cultured in similar locations. To my knowledge, such phenotypic differentiations are presently neither demonstrated nor available for the two potential subspecies of *C. virginica*. Similarly, morphological variation obtained in the Laguna Madre oyster population was believed to be due to environmental influences rather than genetic influences, in spite of it being a genetically distinct population (cf p. 17).

By contrast, a more recent study on Chesapeake Bay oyster populations by Rose et al (2006) demonstrates surprisingly a pattern of isolation by distance, meaning that efforts to restore oyster populations will have local demographic payoffs, at a scale of tributaries or regional sub-estuaries within Chesapeake Bay. This is critical in terms of conservation and restoration efforts, and it provides more insights about the less than expected larval dispersion within the Bay. Moreover, this occurs in an area where decades of management efforts (restocking programs) have extensively translocated oysters from one area to another.

Therefore, the subspecies issue is secondary in my opinion, and priority should be given to understanding phenotypic/genotypic characteristics and their inter-relationships, to facilitate further decision making about conservation options. If significant differences were to be demonstrated, it might also facilitate the decision making related to the subspecies issue. Moreover, aiming for genetic variability and peculiarities at a microscale structure might

likely be more rewarding as a means of identifying, by way of example, natural disease resistant strains (e.g., Lynnhaven River oyster population, Chesapeake Bay) and to sustain optimal management options for oyster stock enhancement.

b. Analysis of the ESA's five factors

The five factors are well covered in the report. However, additional comments can be provided:

Habitat threats

Changes in freshwater, nutrients, organic material, and silt are not the only factors that lead to eutrophication. Changes in the ratio of nutrients (N/P/Si) and a new equilibrium affect drastically the phytoplankton community structures, as well as the species successions over time. The side effects include the food availability for oysters, nutritional quality, and in specific cases, trends that favour dinoflagellate populations over diatoms, with the latter being sometimes toxic to human health (e.g. Harmful Algal Blooms, or HABs) and bioaccumulated by oyster filtering activity.

Hypoxia/anoxia is a critical factor for oyster larval survival rate, with larvae being less protected against environmental stress.

Silt is a major issue when considering spat settlement techniques and restocking programs using cultch deployment. To maximize spat settlement, cultch or/and spat collectors have to be deployed at the right time and location to guarantee maximized yield. Otherwise, silt can drastically reduce the clutch efficiency and at the same time affect the cost-efficiency of such rehabilitation programs.

With toxic wastes and pollutant inputs, recent studies have demonstrated that chemicals used in agriculture might affect oyster genetics for several successive generations. This is the case for atrazine, which can induce the loss of one to two chromosomes at the cellular level (aneuploidy). This phenomenon is directly correlated with reduced growth rates, and therefore may impact oyster populations and restoration efforts (Bouilly et al., 2003).

The increased demands on natural resources, such as for upstream water supplies, may affect the oyster recruitment as larval survival and growth rates are directly correlated with temperature-salinity combinations. By way of example, it was demonstrated that larval survivorship and spatio-temporal recruitment in Chesapeake Bay in 1985 and 1989 were mainly driven by the salinity-temperature level, even though oyster stocking biomass was at a record low (Goulletquer et al., 1994). In other words, changes in freshwater inflows may directly affect the oyster recruitment success.

Other related threats include climatic change and uncertainties about climate change feedback, including carbon and water cycles (NRC, 2003). Presently, the *C. gigas* oyster population in European waters is changing drastically with a northward pattern of recruitment. Introduced in 1970, the natural reproduction was restricted to the southwestern French Atlantic coastline. Over the last ten years the species has rapidly spread in the English Channel and is now considered as an invasive species in several countries (UK, The Netherlands, and Germany). Such changes might also affect *C. virginica* distribution and

population sustainability in the near future, and therefore should be of concern for future research.

Beside the above discussed changes, pests and exotics might emerge and become a threat for oyster populations in several areas. The Asian gastropod *Rapana veinosa* was introduced by ballast waters into the Chesapeake Bay and will likely expand its distribution along the Atlantic coastline. New exotics, introduced through ballast waters and sediments, might find favourable environmental conditions and thrive in the near future.

Overutilization of the resource

The overview of harvest should specify that increased fishing effort and spatial changes in fishing effort were systematically correlated with habitat deterioration. Both concomitant actions have led to the present situation (Rothschild et al., 1994).

The commercial utilization by region should specify that restoration and repletion efforts in Chesapeake Bay, Maryland were carried out to sustain extensive public fisheries rather than as an aquaculture approach.

Predation and disease

The diseases listed in the status report, mainly MSX, Dermo and Malpeque agent, emphasized the commonly known World Organization for Animal Health (OIE) disease list (compulsory declaration when observed). OIE members have strict regulations on the export of diseased oysters and zoning for those diseases. The threats are therefore controlled by limiting transfers of contaminated oysters among States. However, three concerns should be listed: 1. oyster transfers are not restricted within a State; 2. management options regarding emerging and unlisted diseases (e.g., Herpes virus), 3. management of oyster populations and transfers when abnormal mortality events occur (e.g., contingency plans). Information is lacking at that level.

Moreover, accidental introductions and control of the vectors of introduction (e.g., ballast water) are of critical importance. The case of MSX introduced into the Canadian Maritimes is a clear demonstration of the problem, as well as the occurrence of *Bonamia sp.* in the candidate species for introduction (*C. ariakensis)* in North Carolina. The lack of epizootic events in the Gulf region, in spite of a prevalence of *Haplosporidium nelsoni*, is of importance to understanding host-parasite-environment relationships – a multifactorial approach – including virulence factors for *H. nelsoni* and *C. virginica* susceptibility to this protozoan in this region, for which there may be a genetic basis (refer to the above genetic structure comment).

Regulatory mechanisms

This chapter is well covered in the report. The main questions and comments are related to harvest/fishery management issues. Obviously, oyster stock assessments are too time consuming and too expensive to be carried out for a dynamic management plan. Therefore, most of the fishery regulations are based on traditional tools (e.g., minimum legal size, quotas, fishing gear, etc.) and based on traditional fishery models (such as stocking biomass, CPUE, Yield Per Recruit, and others). However, this approach has historically given no consideration to environmental conditions or induced effects (disease prevalence-salinity relationships), which obviously affect drastically spat recruitment and population dynamics. Therefore, a dynamic management strategy based upon freshwater inflow by way of example (see comments on habitat threats) has not been feasible.

With respect to the conflicting information on management practices carried out to improve bottom suitability as oyster habitat or for spat settlement improvement, it should be noted that the conflicting information results from taking a public fishery management approach rather than a conservation approach (habitat improvement) or an aquaculture approach, which would aim to maximize spat recruitment by using spat collectors, deployed in less numbers but with the right timing and location.

Other Natural and Man made impacts

The issue of the Asian *C. ariakensis* oyster introduction into Chesapeake Bay waters is well covered in the report with the on-going three-year research program (completed by the end of 2007). However, it should be noted that abnormal mortality rate events, resulting from *Bonamia sp.* infections, have occurred at least in North Carolina, which therefore raises questions about the future of the project.

With regard to HAB, the summary of known toxic species is appropriate. Although not comprehensive, it should be emphasized that sophisticated surveillance and monitoring programs also aim to limit impacts of unknown toxicity vectors by using integrated bioindicators. By way of example, the 'mouse test', which can be listed in the status report, aims to detect an abnormal toxicity for human consumption whatever the origin.

Although hurricanes are well covered, no (possible) relationship with climate change trends is specified herein. It would make sense to add a paragraph on that issue describing the effects of potential seawater acidification, temperature increase, erosion, changes in tide cycle and levels, and climate change feedback.

c. Aquaculture

Clearly this chapter was written from the perspective of a fishery approach rather than by an aquaculture scientist. The word 'domestication' in the first sentence is not appropriate for the purpose. In contrast to agriculture activity, no genetic improvement of oysters has been achieved since the Roman Empire, nor has a selected strain of oysters ever been obtained. Rather, oyster aquaculture is not strictly specified as a domestication process but rather a process where human intervention and work is predominantly to optimize the rearing cycle as well as induce a specific oyster shape. Actually, oyster farmers are catching spat in the wild using a natural process, but are doing so on a yearly basis without genetic improvement. Even though hatchery techniques and control of reproduction, have been developed over the last 50-60 years, this sector is still in its 'infancy'. In shellfish research and industry, the word 'domestication' was used only very recently, when genetic knowledge reached such a level that understanding of the processes let the scientists begin considering it as a possibility, although a lot of questions remain to be worked out. Similarly, the wording of 'aquaculture' versus 'resource enhancement' might be discussed. As a shellfish scientist, I would list the practice of resource enhancement as 'extensive aquaculture' in contrast to 'intensive aquaculture' wherein artificial food is provided to grow animals. It may be a semantic perspective, but it also reveals the way aquaculture is carried out.

As specified in the report, I fully agree with the two problems listed as needing to be solved for effective management: social, with the recognition of ownership, and the technical aspect of improving seed recruitment. Actually both are related.

The potential for selected oyster strains to contribute to the gene pool of co-occurring wild oyster populations is clearly an issue of concern to be addressed through research, even though Rose et al. (2006) reported the isolation by distance, therefore meaning that the potential impact will likely be local. Most up to date research effort is carried out through the Chesapeake Bay supportive breeding program using the DEBY dual disease resistant strains (Allen & Hilbish, 2003; Hare et al., 2006, Ragone Calvo et al., 2003). Shellfish are different than finfish and are potentially riskier. Shellfish fecundity can be of an order of magnitude greater than finfish fecundity, as is their mutational load. Founder effects and genetic drift should also be considered. Similarly, heterosis, a well documented phenomenon in oysters (Launey & Hedgecock, 2001), might provide a compromise in terms of genetic bottlenecks while providing heterotic benefits in the form of seed oyster survivorship and/or fecundity. Therefore, hatchery production might be a genetic bottleneck if supportive breeding is not properly carried out (specific requirements at the genetically effective population size) (Allen and Hillbish, 2000). Moreover, any prospect for 'genetic rehabilitation by introgressing alleles for disease tolerance into wild populations is still speculative.

Besides the selected strains, impacts of polyploids are not presented in this report: actually, 'reproductive isolation by culture of triploids', as ascertained in the report, is not guaranteed (NRC, 2004). Triploids are not entirely sterile and might produce gametes in specific environmental conditions (e.g., high summer temperatures). Recommendations on that matter have prompted French scientists to develop a "biovigilance" (biosecurity) monitoring network in traditional wild spat recruitment bays because 30% of French production is triploid based. Although tetraploids are fertile, they are usually in a quarantine controlled system. However, US regulation on that matter is not specified in the report.

d. Status of the population

The information related to quantitative stock assessments and preliminary stock assessments is appropriate and correctly specifies the present status of the on-going assessments and various complementary approaches. However, as stated in the report, I fully agree that this type of information is insufficient to correctly evaluate the status of the species. The use of a questionnaire to assess the species status is of interest and complementary, although this approach is mainly qualitative. Shortcomings of the questionnaire include a lack of responses from some regions as well as from independent experts, and the fact that responses are not balanced by a weighing factor among estuaries, states, and oyster population importance. Nevertheless, the answers are highly significant and reveal consistency in the respondents' views of oyster populations in the estuaries.

With regard to domestication and farming of oysters, the listed 'ancillary benefit of moderating harvest pressure on natural populations' might be optimistic, since fishing pressure is mainly driven by the supply and demand balance (at a time of a limited supply), operation costs and direct cost-efficiency.

e. Research needs

This part of the report is short and straightforward, and the information is appropriate. Additional gaps in knowledge and further needs include the following:

1. The report focuses entirely on *in-situ* conservation. A strategy based on cryopreservation is proposed as a complementary approach assuming an appropriate knowledge of what kind of populations must be targeted and appropriate technology is available. It therefore implies: (a) appropriate knowledge of populations of interest and a better understanding of genetic population structure, as well as (b) a sampling strategy.
2. More research should be done on the 'effective population size' both at the hatchery and in the wild according to various geographic sites. Similarly, appropriate knowledge of specific sites of interest is required, and more specifically at the limits of the species distribution.
3. Knowledge of disease resistance strains and their potential uses and impacts is required. By way of example, the genetic basis of the disease resistance strains (DEBY) is largely unknown.
4. With global climate change effects, a modelling approach using various population dynamics models and climatic forecasts, including feedbacks, would facilitate understanding likely changes in species distribution. Potential changes in the distribution of exotics might be of interest in a similar way.
5. Knowledge of the virulence of associated disease agents is needed for a better understanding of interactions between 'environmental conditions' and 'pathogen' and 'host', as this remains a critical issue.
6. Delineation of oyster habitat should be carried out using a statistically sound sampling strategy.
7. A biomonitoring network of 'sentinel' sites at the federal level, across the full geographic species range, might be of interest to assess on-going trends of the *C. virginica* species.

f. Conclusions

I fully agree with the final statement that concludes that the long term persistence of Eastern oysters throughout their range is not at risk now or in the foreseeable future.

5. Conclusions – Recommendations

The report reviewed the present status of the Eastern oyster *Crassostrea virginica* across its natural range and appropriately concluded that the species is not presently at risk and will not be for the foreseeable future. Although the report does not provide a comprehensive dataset of information, the resulting scientific conclusions are sound and derived logically from the results. Overall, the results presented are valid and support the conclusions. The best available information on the species and threats to it are presented appropriately. Particularly, the issue of species and/or subspecies delineations is presented in an optimal way considering large uncertainties regarding the techniques and samplings available. Moreover, it is considered as a less critical issue - due to the characteristics of the animal and its population genetics – compared to more advanced animals (e.g., killer whale). Therefore, the subspecies issue is considered as a secondary issue compared to understanding the phenotypic/genotypic characteristics, as well as their inter-relationships, which is needed to be able in the near future to facilitate further decisions regarding conservation options. If significant differences were to be demonstrated, it might facilitate also the decision making related to the subspecies issue. Moreover, aiming for genetic variability and peculiarities at a microscale structure

might likely be a more rewarding approach for identifying, for example, resistant strains of natural diseases (e.g., Lynnhaven River oyster population, Chesapeake Bay), and to identify optimal management options for oyster stock enhancement.

Several research needs have been listed in addition to the actions proposed in the report. These concern both oyster stock assessments and genetic research at hatchery production sites to sustain appropriately supportive breeding programs. Estimates of effective population size are required, both at the hatchery and in the wild, according to various geographic sites. Ex-situ conservation should be considered in addition to in-situ management options. Both approaches require an appropriate knowledge of the populations of interest and a better understanding of the genetic population structure, particularly at the limits of the species distribution. Knowledge on disease resistant strains and their potential uses and impacts is required, as well as the virulence of associated diseases agents, to develop a better understanding of interactions among 'environmental conditions', 'pathogens', and 'host'. The effects of global climate changes on the oyster population status, by using a modelling approach, would likely facilitate global understanding. Potential changes in the distribution of exotics might be of interest in a similar way. Eventually, a biomonitoring network of 'sentinel' sites at the Federal level, across the full geographic species range, might be of interest to assess the on-going trends in the *C. virginica* species.

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Appendix 1: Area of expertise

First of all, it appears important to emphasize and specify my scientific background to review the 'Status of the Eastern oyster (*Crassostrea virginica*) final report'. Actually, I have been working on various shellfish issues over the last 25 years, and specifically on oyster fishery management and aquaculture. My PhD work focused on an ecosystem approach to optimize clam aquaculture and on interactions between clam populations and environmental conditions, including a modelling approach. My work was completed after more than 3 years of research on issues regarding the *C. virginica* fishery & aquaculture management in Maryland, Chesapeake Bay, USA at the Chesapeake Biological Laboratory, Solomons Island, MD. Several of my publications resulted from this work, including the relationship between spatio-temporal environmental variability and oyster spat settlement in Chesapeake Bay. My European aquaculture background combined with the local fishery management approach was the main interest to review the way the oyster issue is carried out in the USA, and particularly in Chesapeake Bay. At that time, I was directly involved in several meetings at the State and Federal levels regarding the rehabilitation of the oyster fishery in the East coast as well as discussing the approach of introducing an alternative species (*Crassostrea gigas*) to obtain an oyster fishery rebound as was done in the 1970s in European waters. Since then, I've been in contact with the US shellfish scientific community on numerous occasions, including the National Shellfisheries Association annual conferences, and more recently as an expert panel member for the National Research Council, National Academies of Science, Washington DC, and co-writer of the report 'Non Native oysters in Chesapeake Bay', (NRC, 2004). This expertise focused on assessing various scenarios for the future of *C. virginica* oyster management and the alternative of introducing reproductive or/and sterile *C. ariakensis* (Asian oyster) in Chesapeake Bay. Moreover, over the last 3 years I was on the executive committee of the 'Oyster Genome Consortium' project headed by Dr. D. Hedgecok, Univ. of California, USA. As a scientist and science manager, I have been in charge of an IFREMER (French research Institute for Sea Exploitation) French research laboratory dealing with various shellfish issues in the Marennes Oleron Bay, the largest European oyster producing area with over 100,000 metric tons of stocking biomass, and a pilot area for developing research studies on shellfish ecosystems. Moreover, I was in charge of the national IFREMER laboratory that specialized on the genetics and pathology of shellfish, including one of the 2 shellfish research hatcheries in France (also certified as the European Reference Laboratory for Shellfish Diseases, and an OIE Reference laboratory for two oyster diseases (protozoan *Marteilia* sp., and *Bonamia* sp.). Therefore, I am more than aware of the concepts regarding those research themes. More recently, I have taken responsibility for a national research program on aquaculture sustainability (including shellfish) and for the national coordination of 'Biodiversity' issues at the IFREMER level. I have published more than 50 publications and book chapters on shellfish issues from ecosystem management and ICZM to shellfish genomic issues.

Appendix 2: Copy of the Statement of Work

Consulting Agreement between University of Miami and Phillippe Goulletquer
STATEMENT OF WORK

Background

In January 2005, NOAA's National Marine Fisheries Service (NOAA Fisheries Service) was petitioned to list eastern oyster (*Crassostrea virginica*) under the Endangered Species Act (ESA). As required, NOAA Fisheries Service reviewed the petition and made a positive 90-day finding determining that the information in the petition and otherwise available to the agency indicated that the petitioned action may be warranted. As a result of the positive finding, the agency was required to conduct a review of the status of the species to determine if listing under the ESA is warranted.

NOAA Fisheries Service organized a biological review team (BRT) consisting of federal and state biologists to assemble the facts. In so doing, the team was instructed to organize and review the best available scientific and commercial information on eastern oysters and to then present its factual findings to the agency in a status review report. The report did not need to be based on consensus – opposing individual viewpoints were welcomed as long as the viewpoints were sound and based in science. Further, the report was not to contain any listing advice or to reach any ESA listing conclusions – such synthesis and analysis is solely within the agency's purview.

On Wednesday, October 19, 2005, NOAA Fisheries Service received a letter from the petitioner dated October 13, 2005 requesting the recall of the eastern oyster petition. In his letter, the petitioner indicated that his request to withdraw the petition was due to the public and industry's confusion over the petition and listing process. NOAA Fisheries Service accepted this request and ceased evaluation of the petition. However, a considerable amount of effort had been expended by the BRT at the point at which the withdrawal of the petition occurred. Also, the completed status review report is the most timely and comprehensive resource document for this species. As such, NOAA Fisheries Service determined that because the report is a useful tool in guiding future management decisions, the BRT should complete the status review report.

NOAA Fisheries Service is required to use the best available scientific and commercial data in making determinations and decisions under the ESA. The first question that must be addressed is what the appropriate species delineation is for consideration of conservation status. The ESA defines an endangered species as "any species which is in danger of extinction throughout all or a significant portion of its range," and a threatened species as "any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range." A species may be determined to be threatened or endangered due to any one of the following factors:

(1) the present or threatened destruction, modification, or curtailment of its habitat or range;
(2) overutilization for commercial, recreational, scientific or educational purpose;
(3) disease or predation;
(4) the inadequacy of existing regulatory mechanisms; and

(5) other natural or manmade factors affecting its continued existence.

The scientific and commercial information contained in the status review should contain essential factual elements upon which the agency could have based its ESA determination. Accordingly, it is critical that the status review contain the best available information on the species and the threats, that all relevant information is identified and included, and that all scientific findings be both reasonable, and supported by valid information contained in the document. As such, the agency requires a peer review that focuses on the factual support and scientific methodology upon which the status review report is based.

Reviewer Responsibilities

The Center for Independent Experts shall provide three reviewers. Each reviewer's duties shall not exceed a maximum of seven days to read the status review report and, as needed, the scientific papers referenced therein. Each reviewer shall produce an individual written report, with emphasis on his/her area(s) of expertise. See Annex I for additional details on the contents and organization of the reviewer's reports. No consensus opinion (or report) will be required.

There are several primary issues related to this species that must be addressed. Reviewers with the following expertise are required to ensure the best available information has been utilized.

- Life history and population dynamics of eastern oysters
- Eastern oyster genetic, physiological, behavioral, and/or morphological variation throughout the species' range
- Eastern oyster habitat requirements
- Harvest
- Predation and disease
- Regulatory mechanisms for managing the species
- Other natural or manmade impacts affecting eastern oysters
- Aquaculture
- Conservation actions including restoration efforts and recovery activities

Each reviewer will be supplied with the status review report prepared by the biological review team. Any of the reports and papers cited in the status review report will be made available to the reviewers upon their request.

Specific Reviewer Tasks and Schedule

1. Read and review the status review report.
2. Specifically address the following points (at a minimum):
    a. Are species and/or subspecies delineations supported by the information presented?

b. Does the report include and cite the best scientific and commercial information available on the species and threats to it and its habitat?
c. Are the scientific conclusions sound and derived logically from the results?
d. Where available, are opposing scientific studies or theories acknowledged and discussed?

3. No later than October 2, 2006, each reviewer shall submit a written report of comments and conclusions[1]. Each report shall be sent to Dr. David Die, via email at ddie@rsmas.miami.edu, and to Mr. Manoj Shivlani, via email at mshivlani@rsmas.miami.edu.

Submission and Acceptance of CIE Reports

The CIE shall provide the final reviewer reports to the NOAA Fisheries Service COTR, Dr. Stephen K. Brown at Stephen.K.Brown@noaa.gov, for review and approval based on compliance with this statement of work no later than October 16, 2006. The COTR shall notify the CIE via e-mail regarding acceptance of the reports. Following the COTR's approval, the CIE shall provide pdf format copies of the reviewer reports to the COTR.

Appendix 3: Clarification on task 2a in the statement of work

Date: Fri, 22 Sep 2006 21:05:16 -0400 [23. Sep 2006 03:05:16 CEST]
De: Manoj Shivlani <mshivlani@rsmas.miami.edu>
À: Philippe.Goulletquer@ifremer.fr
Sujet: clarification of task 2a in statement of work
Partie(s): Télécharger toutes les pièces jointes (en format .zip)
En-têtes: Montrer tous les en-têtes

Parties alternatives pour cette section :
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Cliquer sur ICI pour visualiser le contenu HTML dans une autre fenêtre.

Dear Philippe,

We asked the NMFS center to clarify task 2a in the statement of work that called for reviewers to determine whether species and/or subspecies delineations are supported by the information presented. Below is the center's clarification.

There is genetic information included in the status review report which indicates that there may be two distinct subspecies of /Crassostrea virginica/. Taxonomists have not yet named subspecies for /C. virginica. However, due to problems that have arisen in other status reviews (e.g., killer whale) , we felt that it was important that the status review team include all the genetic information available for the species. The team did not make a definitive decision as to whether subspecies exist. We wanted the peer reviewers to review and provide their expert opinion on the information in the document and whether there is sufficient evidence to suggest that there is distinct population structure within this species.

I hope that this clarifies the task for the purposes of the review.

Regards,

Manoj

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