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Report of the Working Group on Marine Shellfish Culture (WGMASC)

Portland (Maine), USA
13–15 May 2004

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1 Opening of the meeting

1.1 Place and date

The Working Group on Marine Shellfish Culture held its second meeting in Portland, Maine, USA, from 13–15 May 2004, at the University of South Maine, Science Building.

Special thanks are due to Brian Beal, who made every effort to arrange an adequate venue at the university, while he was currently working far from Portland, and provided a van for transportation.

The meeting was opened at 9:30 hrs on Friday 13 May. The Chair welcomed two new participants: Richard Langan (USA) and Francis O’Beirn (Ireland), incoming Chair of WGEIM.

1.2 Attendance

Eight people representing six countries attended the 2004 meeting of WGMASC (See detailed List of participants in Annex 1). Other members, who were unable to attend, sent apologies (S. Gollatsch and R. Wenne). Several countries among largest shellfish producers were not represented yet. WGMASC expressed concerns about this, and an effort to promote membership will be made at the next ICES Annual Science Conference in Vigo (Spain).

2 Adoption of Agenda

A general discussion was held regarding the Council’s final resolution 2F05 and the report from the last meeting of Mariculture committee in Tallinn. The Terms of Reference for 2004 (Annex 3) were discussed by the group, in order to organize the work. As was done last year, the tasks were dealt with in sub-groups, each of them dealing with a specific term of reference. Plenary sessions brought discussion among all participants. The draft agenda was discussed.

Following discussion, it was clear that all members were interested in participating in the group working on the Term of Reference D (sustainability of shellfish culture). This was scheduled to Saturday morning, before the final plenary session.

After such modifications, the agenda was formally accepted. The meeting agenda is shown in Annex 2.

3 Appointment of Rapporteurs

Volunteers took notes during each plenary session to assist the Chair. Brian Beal was appointed Rapporteur for the first Plenary Session, Thursday 13 May from 9:30 to 12:30 pm, Pauline Kamermans took over on the Friday afternoon (14 May), and David Fraser on Saturday afternoon (15 May).

4 ToR A: Provide a synthesis on the development of hatcheries, the proportion of cultured animals to wild conspecifics and the relative proportion of triploids and other selected strains produced by hatcheries

Preliminary remarks and WG comments

During its last meeting, the Working Group prepared a questionnaire to be distributed to shellfish hatcheries within ICES Member Countries. The return relates only to those countries represented in the WG, and only to commercial hatchery production. However the data set is incomplete, despite being designed to protect confidentiality of individual businesses. This is partly due to the reluctance of several hatcheries to provide relevant information.

After some discussion, the WG agreed to redesign the questionnaire and to broaden its audience to include regional organizations to which the hatcheries may be part, local, regional or state authority, and competent scientists in this field. It was also clear from the different contacts that a summary report of the questionnaire be sent to all those participating. This has yet to be defined. It has been suggested that the final product of this ToR should be presented as a report of hatcheries in ICES countries, to reveal the trends in production and needs for research and development.

To improve the contacts with hatchery managers, a new form with an improved presentation was prepared by a member and scrutinised by the group. It is intended for use under the auspices of both ICES and the scientist's affiliation. A copy is given in Annex 3.

It is stressed that the present document is draft work conducted by the WGMASC. It was initiated in 2003, and is still a work in progress. It will be expanded in future meetings to more thoroughly address the issues raised.

4.1 Rationale

Hatcheries are an essential tool for securing spat availability to the industry, and for the dissemination of genetic stability and improvement. There are several reasons why hatcheries exist. These include the need to restock wild fisheries which have been depleted, to satisfy the demand by culturists and shellfish farmers for a consistent, high quality source of seed and to produce organisms that are not normally available (introduced species or specific strains). Over the course of the last 20 years, there has been a dramatic increase in the number and the size of shellfish hatcheries (see Tables 1 and 3)

Initially, most hatchery technology was developed through publicly-funded government laboratories which were later transferred to and developed by private industry. Hatcheries developed in response to three different needs:

- 1) To complement the decline in wild fisheries that could not supply the market with the demand from local and foreign markets (i.e., *Pecten maximus*, France);
- 2) To supply spat for cultivation of a non-indigenous species; and
- 3) To diversify the sources of spat of a species naturally available and collectable; to ensure a more consistent, higher-quality supply of material for culturists to use as well as to produce specific genetic strains.

The percentage of hatchery-produced animals seeded compared to those caught from wild sources is increasing. In France, for example, approximately 15–20% of *Crassostrea gigas* spat are now produced from hatcheries, most of which are triploids.

4.2 Current status

The degree of hatchery technology varies widely among species. The reasons for these differences are driven by market demand for the end product and the consistent availability of wild-collected spat. Species such as mussels (*Mytilus edulis*) are often readily available from natural settlement and therefore, very few hatcheries have produced commercial quantities of mussel seed. However, owing to the vagaries of natural settlement of mussels, in Ireland and Norway in recent years, there is a perceived need for development of hatchery facilities for that species. Others, such as *C. gigas*, *Tapes philippinarum*, *Mercenaria mercenaria*, and *Argopecten irradians* are routinely produced in hatcheries throughout the world. While hatchery technology is well developed for these species, others such as scallops (*Pecten maximus*, *Placopecten magellanicus*) and *Ostrea edulis* have proved more difficult to rear on a routine basis.

Currently, detailed information on hatchery production is not readily available within ICES countries and as a result comparisons on gross production, trends and new developments are difficult to provide. As a result, a survey questionnaire was developed to obtain this information and distributes to hatcheries by WG members.

Some of the basic requirements of the design of hatcheries are related to the maintenance of high-quality standards and the implementation of a good biosecurity policy. Hatcheries require high-quality water supply, secure from future developments that may negatively impact it. Facilities should be designed to maintain high standards of hygiene and efficiency in all phases of production (larval rearing, algal production, etc.) with physical separation of those phases and duplication to avoid failures of any one component. A quarantine unit (strict confinement, effluent treatment) is essential in hatcheries using broodstock from non-native origins, from genetically unique forms (tetraploids), and from areas of unknown disease status. Hatcheries require staff trained in mariculture principles through hands-on learning and formal coursework. Because technology is continually advancing, there should be a requirement for on-going training and development.

Shellfish nurseries are a natural extension of the hatchery system to enable successful and cost-effective rearing of juveniles to a suitable growout size. They are usually located in relatively close proximity to the hatchery, but the feasibility of transporting competent larvae, e.g., in moist containers lacking water, has led to the technology of remote setting. This enables skilled farmers to nurse spat themselves. Nurseries require a high quality, secure water supply in areas of high primary productivity. The nursery should also be sited in areas with a low probability of introduction of pollutants, bio-fouling, pests or diseases. They may ideally be sited in an approved, disease-free area, thus allowing unrestricted movement of stocks. However, nurseries sited in restricted areas may still send their products in identical areas. In addition to a nursery site, some hatcheries will be more vertically integrated with growout (on-growing) facilities that require environmental and biological characteristics similar to the nursery.

4.3 Regulations

Establishment of a shellfish hatchery depends on national or local legislation to ensure that regional development balances with environmental sustainability, and this may vary in different countries. Specific legislation that may affect hatcheries includes classification of waters and approved zonation for disease agents. Both of these can influence the movement of shellfish for production and utilization of areas. In some cases, shellfish hatcheries, because of poor water quality or the presence of disease agents such as *Bonamia ostrea*, can be severely restricted in their development. For example, in Scotland an oyster hatchery was relocated to a more remote location because of lowering water quality standards which affected conditioning of broodstock and hatching of larvae.

Other legislation includes land-use issues where shellfish hatcheries may not be sited in regions zoned for other activities (Readers will find in the revised COP in the Introductions and Transfer of marine organisms detailed information about the risks of transferring species and methods to reduce those risks). These regulations, in conjunction with local land prices (real estate costs), will often make siting of a hatchery difficult. In other areas, hatcheries are more easily established because they occur in remote locations with low population densities. There is often a lack of education and knowledge of these regulations and, as a result, hatchery owners may circumvent these rules. The implementation of Codes of Practice will be important to increase awareness of responsibilities and bring the existing industry into compliance.

4.4 Impacts

Hatchery production, as previously stated, responds to both quantitative and qualitative needs of the shellfish industry, and, therefore, has positive impacts on this sector as well as consumption. These positive impacts will continue to grow as efficient production practices for the cultured species continue to evolve.

Hatcheries which follow operating codes of practice and biosecurity policies will typically have a low impact on the local environment with respect to water quality, and discharges from these hatcheries will generally be of a high standard. In the absence of accepted facility operating standards, there are potential risks that include the uncontrolled growth of pathogens (e.g., *Vibrio* spp.), discharges of antibiotics, chemicals, disease-agents, fouling organisms and genetically modified materials.

The main function of a shellfish hatchery is to produce seed for planting into the natural environment. The two main **biological impacts** from these activities are flooding the natural populations with potentially less genetically diverse stock and interaction (competition, predation) with other organisms, including conspecifics in the environment. The significance of this impact (genetic diluting) will be related to the proportion of cultured seed and origin of parent broodstock, in relation to wild stocks in a particular area.

Although the goal of producing cultured seed is to create a high quality, vigorous progeny, in reality, because of low numbers of broodstock used in the hatchery and inadequate rearing (culling) techniques, large numbers of poor quality (low fitness or lowered genetic variability compared with wild stocks) juveniles may be released. The long-term impact of this practice could compromise the success of wild populations by diluting the genome and introducing more undesirable traits into the overall population. A monitoring program to assess genetic variability would ensure the development of diversified broodstock.

One solution to this may be the production of sterile triploids as in the case with *C. gigas* in France and *Mytilus galloprovincialis* on the West Coast of the U.S. Currently there are two methods to produce triploid animals. One is via chemical induction and the other is crossing of tetraploids with diploid broodstock. The dangers in the former technique are that less than 100% of the animals produced are triploid while the dangers of the latter technique would be the unintentional release of tetraploids into the marine environment which could potentially interact with natural diploids

producing sterile triploids. Obviously, bio security protocols must be strictly enforced in these cases. For example, a negotiated protocol between French hatcheries (in charge of implementing quarantine facilities), the administration and scientists (ploidy surveys on natural stocks) is under discussion at the moment. Another issue with genetic selection is the development of disease-resistant strains. Although these animals may not be susceptible to local pathogens, they could act as a reservoir and pass the disease on to wild populations¹. The wild populations should be considered as a valuable source of genetic material (gene bank) and as such, adequately protected.

When introducing non-indigenous species, adverse ecological impacts would involve direct competition for space and food with wild stocks and increasing the number of species interactions. By adding a potentially large biomass of filter-feeding organisms to the environment, some resources may become depleted and limiting to other species. The degree of this impact will be related directly to the volume of the system and its relative productivity. The addition of cultured organisms usually changes the population size of shellfish predators. Depending on stocking densities and on the scale of this response, predation rates on natural populations may either increase or decrease. For example, the increase of cultured seed may deflect some predation from the wild stocks due to their relative proportion or simply encourage an increase in the population of predators by providing an increase in the supply of food. Another potential impact is the intentional introduction of an exotic species to an area through hatchery cultivation (e.g., *T. philippinarum* in France). Unintentional introductions are more numerous and have often arrived at a location with broodstock or juveniles (i.e., *Carcinus maenas*, *Urosalpinx cinerea*, *MSX*, *Crepidula fornicata*, etc.). The danger of these introductions is that the spread of new species is often unrestricted due to the lack of local biotic and abiotic controls. This highlights the need for a quarantine facility.

Although the risks of culturing unwanted organisms are relatively low in the hatchery environment due to a higher level of control, movement of cultured seed from nursery to growout areas may result in the spread of pests and diseases within and between countries (e.g., *Polydora* infestation of *C. gigas* and *P. maximus* in Scotland). Current legislation encourages trade between EU member states and, unless good legislation and high standards of biosecurity are employed, such pests and diseases are likely to spread.

As part of an ICES working group Terms of Reference on marine shellfish culture, data was sought on the production and development of shellfish hatcheries within ICES countries. A summary of returns is presented reporting on the activities and outputs of hatcheries, highlighting environmental issues and areas of priority for Research & Development. The data set is incomplete and recommendations have been made to obtain a more complete data set for the 2004–2005 survey. To achieve a comprehensive overview of existing expertise and technology, the group consider it important to expand the survey to other parts of the world where species of interest for ICES members are cultured in hatcheries, such as the Pacific coast of North America and the Mediterranean.

4.5 Summary of data collected

Data are presented mainly as anonymous tables. Some are incomplete, since some hatcheries are reluctant to give sufficient information.

4.5.1 Number of commercial shellfish hatcheries

Table 1. Number of commercial shellfish hatcheries¹.

Canada	4
United States	49
Ireland	6
United Kingdom	4
Norway	1
Denmark	1
France	6
Spain	5

¹ This does not include experimental hatcheries. It does include all commercial hatcheries.

¹ This issue should be discussed together with ICES working groups : WGAGFM and WGPDMO, respectively

4.5.2 Species reared in shellfish hatcheries

Table 2. Species reared in shellfish hatcheries. These are species produced commercially in the ICES area. Netherlands, Belgium, Germany, and Poland do not have any commercial shellfish hatcheries. No information was available about Latvia, Lithuania, Estonia, Sweden, Portugal, Finland, and Iceland at this time. The UK hatcheries also include those in the Channel Islands.

Species	Canada	UK	Ireland	USA	Denmark	France	Norway	Spain
<i>Placopecten magellanicus</i>	X			X				
<i>Argopecten irradians concentricus</i>	X			X				
<i>Mya arenaria</i>	X			X				
<i>Mercenaria mercenaria (notata)</i>	X	X		X				
<i>Ostrea edulis</i>	X	X	X	X	X	X	X	X
<i>Crassostrea virginica</i>	X			X				
<i>Chlamys islandicus</i>	X							
<i>Spisula solidissima</i>				X				
<i>Ensis directus</i>	X							
<i>Mactromeris polynema</i>	X			X				
<i>Pecten maximus</i>		X				X	X	X
<i>Crassostrea gigas</i>		X	X			X		X
<i>Tapes philippinarum</i>		X	X			X		X
<i>Tapes decussatus</i>		X						X
<i>Venerupis pullastra</i>								X
<i>Venerupis rhomboides</i>								X
<i>Haliotis tuberculata</i>			X					
<i>Haliotis discus hannai</i>			X					
<i>Strongylocentrotus droebachiensis</i>	X			X				

4.5.3 Hatchery production

Table 3. Estimated molluscan shellfish hatchery production (in millions), 2003¹.

Species	Canada	UK	Ireland	US	Denmark	France	Norway	Spain
<i>Placopecten magellanicus</i>	5			n/a				
<i>Argopecten irradians concentricus</i>	n/a			n/a				
<i>Mya arenaria</i>	n/a			5–10				
<i>Mercenaria mercenaria (notata)</i>	n/a	n/a		>100				
<i>Ostrea edulis</i>	n/a	<5	n/a	>25	0.1	<1	n/a	n/a
<i>Crassostrea virginica</i>	n/a			>100				
<i>Chlamys islandicus</i>	n/a							
<i>Spisula solidissima</i>				>10				
<i>Ensis directus</i>	n/a							
<i>Mactromeris polynema</i>	n/a			n/a				
<i>Pecten maximus</i>		<1				10	n/a	n/a
<i>Crassostrea gigas</i>		225	n/a			800		n/a
<i>Tapes philippinarum</i>		150	n/a			100		n/a
<i>Tapes decussatus</i>		20						n/a
<i>Venerupis pullastra</i>								n/a
<i>Venerupis rhomboides</i>								n/a
<i>Haliotis tuberculata</i>			n/a					
<i>Haliotis discus hannai</i>			n/a					
<i>Strongylocentrotus droebachiensis</i>	n/a			>10				

¹ Some hatcheries were reluctant to provide production estimates. Either put n/a at all or none or specify the difference. I suggest leaving n/a out.

4.5.4 Employment

Table 4. Number of commercial shellfish hatchery employees¹.

Number of commercial shellfish hatchery employees ¹	Full-time	Part-time
Canada	>4	n/a
United States	>49	n/a
Ireland	>6	n/a
United Kingdom	7	7
Norway	n/a	n/a
Denmark	4	n/a
France	12-20	n/a
Spain	>5	n/a

¹ We assumed that each commercial shellfish hatchery had at least one full time employee.

4.5.5 Technical facilities

Table 5. Summary of quarantine capacity, water source, and effluent treatment of shellfish hatcheries (numbers of hatcheries). n/a = not available.

	Quarantine Yes / No	Water Source Flow through / Recirc	Effluent Treatment Yes / No
Canada	n/a / 1	1 / :n/a	n/a
United States	n/a	n/a	n/a
Ireland	n/a	n/a	n/a
United Kingdom	1 / 3	3 / 1	1 / 3
Norway	n/a	n/a	n/a
Denmark	0 / 1	0 / 1	1 / 0
France	n/a	n/a	0/6
Spain	n/a	n/a	n/a

4.5.6 Biotechnology

Table 6. Summary of broodstock origin, selection criteria, conditioning methods, and use of triploids in hatcheries.

	Broodstock ¹	Conditioning	Triploids
	Wild /Selected	Yes : No	Yes / No
Canada	1 / 1	1 / n/a	1 / 3
United States	n/a	49 / 0	3 / n/a
Ireland	0 / 6	6 / 0	0 / 6
United Kingdom	3 / 3	4 / 0	1 / 3
Norway	n/a	n/a	n/a
Denmark	1 / 0	0 / 1	0 / 1
France	0 / 6	6 / 0	6 / 0
Spain	n/a	n/a	n/a

¹ A hatchery may hold both wild and selected broodstock, which means that the numbers within a section may be greater than the number of commercial hatcheries (see above)

4.5.7 Larval rearing and spat settlement techniques¹

Detailed systems employed and problems encountered during larval rearing and spat settlement.

Rearing systems employed include: Flow-through tank systems; static tank systems with 2-3 day 100% water changes, UV- and Ozone-sterilized water

Larval rearing problems: *Ostrea edulis* failed to breed under hatchery conditions. For example, low survival rates were found between 8-10 days post hatch. This may be due to an imbalance in the relationship between feeding techniques and seawater temperature. In addition, larvae are highly susceptible to pathogens, e.g., *Vibriosis* from cultured algae. Viruses have also been isolated from some shellfish hatcheries. During the larval stage, immediate tank disinfection is the most practical treatment of such infections. In Europe, and specifically in *Crassostrea gigas*, some hatcheries have detected oyster velar virus disease, OVVD, otherwise known as Blister disease. Toxic events often go undetected in shellfish hatcheries until there is a catastrophic failure. To circumvent such failures, there is a need to develop suitable bioassays to monitor water quality problems and to develop analytical protocols to isolate and identify causal agents allowing remediation. Preventative measures could include improved husbandry and biosecurity throughout the hatchery, including treatment of inflow water and quarantine of broodstock.

Spat settlement techniques: PVC plates are used for *Ostrea edulis*. Ground glass and crushed shell are used for substrate for settlement. Settlement may occur onto nylon screening in floating trays. Aminobutyric acid (GABA) is sometimes used to speed up metamorphosis. For some, water motion is created to stimulate metamorphosis. Remote setting is used in oysters.

Spat settlement problems: High individual variation in growth rate. Sea scallop metamorphosis and settlement is highly variable within and between years and between hatcheries.

Micro algae used for the production of larvae and/or spat

¹ Some hatcheries did not choose to respond to this question because they perceived their confidentiality may be compromised

Table 7. Micro algae used for the production of larvae and/or spat.

Species	Canada	UK	Ireland	US	Denmark	France	Norway	Spain
<i>T. isochrysis galbana</i>	Yes	Yes	Yes	Yes	Yes	Yes	n/a	n/a
<i>Chaetoceros</i> spp.	Yes	Yes	Yes	Yes	Yes	Yes	n/a	n/a
<i>Pavlova</i> spp.	Yes	No	n/a	Yes	No	Yes	n/a	n/a
<i>Thalassiosira</i> spp.	Yes	Yes	n/a	Yes	No	n/a	n/a	n/a
<i>Tetraselmis</i> spp.	Yes	Yes	Yes	Yes	Yes	Yes	n/a	n/a
<i>Nannochloropsis</i> spp	Yes	Yes	n/a	Yes	No	n/a	n/a	n/a
<i>Rhodomonas</i> spp.	Yes	No	n/a	Yes	No	n/a	n/a	n/a
<i>Dunaliella</i> spp.	Yes	No	n/a	Yes	No	n/a	n/a	n/a
<i>Skeletonema</i> spp.	n/a	No	n/a	Yes	No	Yes	n/a	n/a

4.5.8 Origin and destination of products

Table 8. Origin and Destination of hatchery-reared mollusks¹

¹ In some countries, this information is considered confidential

Canada	The single east coast hatchery to respond to the survey from Nova Scotia, ships bivalve seed to Nova Scotia, Prince Edward Island, and the north-eastern shore of New Brunswick
UK	The four hatcheries to respond to the survey, move bivalve seed within the U.K, to Ireland, South Africa, Namibia, Canada, Spain, Italy, and France
US	Interstate shipping occurs after disease pathogens have been assessed. Overseas shipments occur, but no specific data are available at this time
Ireland	Some abalone have been shipped to Spain.
Norway	n/a
Denmark	Oyster seed is shipped within Denmark and to Sweden and Germany
France	<i>C. gigas</i> is shipped to Ireland and New Caledonia.
Spain	n/a

4.6 Research & Development: Issues and concerns

Canada: There is need to improve European oyster stock through broodstock selection. Giant scallop (*Placopecten magellanicus*) culture is promising economically, but to date there is no consistency from year-to-year with larval and juvenile rearing methods. There is a lack of support and consistent funding for hatchery-based research. Water quality – should an international standard be developed? Research on recirculation is needed. Standards should be developed for national or international disease certification for seed.

UK: Broodstock selection program for *C. gigas* to improve stock using scientific methodology. A more diverse brood stock population for both *Crassostrea gigas* and *Ostrea edulis* should be maintained in hatcheries,

preferably in quarantine. Broodstock selection would be designed to avoid inbreeding, and select for parameters such as growth, disease-resistance, and shape, and care should be taken to avoid loss of important alleles for survival, such as temperature tolerance and disease resistance.

Equipment is needed to measure the success of triploidy. There is a concern that triploids may breed successfully with wild diploids and reduce wild spawning stock. There is a need to improve technology on transfer capacity to identify and explore new nursery methods. There is a need to develop a fast, sensitive and economical technique of diagnosing the presence of existing and novel diseases listed under current legislation as guided by the Office International des Epizooties (OIE). Legislation can hamper trade making the movement of oysters and clams in the current system expensive, time-consuming, and inefficient. Scientific support needed to increase communication among and between countries. Relate commercial needs to R&D. Financial support is needed to develop consistent batches of European oysters and scallops. There is a general need to support and develop innovative hatchery techniques.

USA: A concern is legislation that restricts interstate movement of shellfish. Other legislation restricts the areas where seed can be grown. There is a need to

- develop and improve larval and juvenile rearing techniques for sea scallops and other novel species (including the green sea urchin, *Strongylocentrotus droebachiensis*).
- develop and improve culture methods for microalgal production (continuous vs. batch vs. micropellets).
- increase funding for both basic and applied research relating to shellfish hatchery production.
- Provide research on the specific nutritional requirements of larval and juvenile cultured species.

Ireland: n/a

Norway: n/a

Denmark: n/a

France: Genetics and selection: prior domain, under development, in which a share of tasks must evolve between research, expertise, professional sector, individual enterprises. Breeders conditioning: not fully controlled until now. Improvements required from environmental and food management for a better control of initial energy reserves, gamete germination, and the sexual maturation process. Larval rearing: water quality, temperature and food management. The genetic effect of culling the slow growers should be better understood. Phytoplankton production: cost reduction by replacing batch method by continuous method (different levels of intensity and control from SeaCAPS method to photo bioreactor method)

Spain: n/a

4.7 Future directions

Hatcheries are playing a larger role in the production of juveniles for both intensive and extensive (stock enhancement) aquaculture operations, as they allow a better control on juvenile availability. It is predicted that production requirements will increase dramatically for many currently cultured species, either related to declining commercial stocks, or to improvement in cultural traits.

The trends in marine shellfish hatcheries appear to be toward larger, more efficient and automated systems. As scale of production increases, this will result in the creation of more small hatcheries as well as larger facilities. Hatcheries will likely diversify production toward newer, novel, higher-valued species (e.g., *Haliotis* and *Panope*) or bulk species easy to rear at moderate cost and amenable to genetic improvement, (e.g., Pacific oysters scallops and mussels).

There is an overall recognition of the importance of high quality water, well-selected broodstock and appropriate technology to improve production. Broodstock selection presently based on empirical methods should take benefit from the increasing scientific knowledge and expertise available in genetics (genetic markers, heritability of useful traits, selection protocols). There is also a need for scientific support in the field of broodstock conditioning, nutrition, larval and post-larval pathology, so as to ensure reliability of hatchery productions, and effective gains from genetic improvement.

In the future, the WG will address several of these subjects, to provide a review of the knowledge concerning the development of shellfish hatcheries and the related questions.

4.8 Conclusions

The WG members are not satisfied with the level of information they have collected to date and are planning to include all ICES countries and other countries where there is significant hatchery production. Those to be contacted will involve regional organizations representing hatcheries, provincial, state, or other regulatory agencies, or scientists working in

association with hatcheries, to participate in the survey. The group developed a revised hatchery survey questionnaire with guidance notes for completion (Annex 3) which is intended for distribution to all ICES member countries and, if possible, other interested countries.

4.9 References

Anonymous, 2003. Report of the ICES Working Group on Introductions and Transfers of Marine Organisms, Vancouver, Canada, 26–28 March 2003. Advisory Committee for Marine Environment, CM 2003/ ACME:04. 168 pp.

5 ToR B: Review literature on stress indices to identify potential diagnostic tools to detect a declining condition leading to death in cultured populations of molluscs

Preliminary remarks and WG comments

- *It was agreed by the Working Group that we would begin to review the issue of unexplained mortalities by looking potential solutions that involved diagnosing the relative “health” of the animal. To start, we would initiate a review of the existing “stress” metrics for shellfish and then begin a discussion on how these metrics might fit into a system for monitoring and diagnosing the issue of “unexplained mortalities” for aquaculture operations.*
- *The number of publications dealing with stress in shellfish was considerable (over 1000 references from the database ASFA) and therefore, the first phase would be to summarise the general techniques used with an emphasis on those that had cross-species applicability.*
- *It was noted there are a number of cross-linkages with other ICES working groups with this theme such as: Study Group to Review Ecological Quality Objectives for Eutrophication, Working Group on Biological Effects of Contaminants, Working Group on Ecosystem Effects of Fishing Activities, Working Group on Pathology and Diseases of Marine Organisms.*
- *There are linkages of this theme with the TOR on Carrying Capacity in order to develop metrics/indicators for measuring sustainability.*

5.1 Background

There is usually a good cause and effect relationship to explain many shellfish mortalities. This could be due to an attack by a predator or it may be a result of the failure of their physiological system. In the former case, particularly for macro-predators, the onset of death is usually relatively rapid for the individual and if the attack is not successful, telltale marks are often left behind. Shellfish have developed various means to combat these attacks through shell development, noxious substances, camouflage and various behavioural escape responses. In a shellfish aquaculture operation, mortalities from macro-predators are generally evident as the predators can be seen and the results of their attacks are left behind in the form of empty shells, etc

The second category of death, the failure of the physiological system of the animal, is far more subtle in nature and encompasses more categories of assault on the organism. In this case, death is not immediate, but the animal may take some time to die. The interaction with the target organism may be physical in nature such as extremes of temperature, salinity, oxygen, wave strength, sedimentation, desiccation or light (i.e., UV). The interaction may also be biological in nature and involve either starvation or disease from viruses, bacteria or protists. It could also involve parasitism as either a primary or alternate host. Both of the latter causes could also be thought of as forms of predation. The organism could also have a genetic defect that makes it impossible to grow beyond a certain point due to a deficiency that can not be overcome by the anabolic processes of the animal. A subcategory of the second type of mortality would be the interactions the organism has with human activities and products. The results of construction impacts and waste disposal of various toxic substances are examples of some of these interactions.

For a shellfish grower, the second category of death may be more problematic. In many cases, the cause of mortality may not be obvious as the farmer is left with a large number of empty shells and he was not even aware there was a problem. To compound the, the remaining shells may give very little information as to the cause and any moribund animals remaining may simply be under attack from secondary opportunistic organisms that are exploiting the effects of the original stressor. This then falls in the general category of “unexplained mortality”.

However, it should be recognised that the shellfish have existed for several hundred million years in evolutionary time and have developed successful internal defence systems to ward off the ravages of disease and physical extremes. In many cases some have evolved to live in harsh environments and have developed mechanisms to moderate the impacts such as many epibenthic intertidal species (i.e., mussels, gastropods, clams, etc). These organisms have to deal

with 30°C temperature and 30 PSU salinity swings twice a day. They have to accumulate an oxygen debt when exposed at low tide and they have to withstand the ultraviolet radiation and desiccation in the summer months and freezing in the winter. All of these characteristics require a sophisticated physiological system to deal with these impacts.

The current paradigm for disease expression revolves around the confluence of three factors: 1) the presence of the disease organism, 2) the presence of a suitable host and 3) the appropriate environmental conditions for the interaction to take place. In the absence of one of these components, the infection does not take place. The third factor could include both the external environmental conditions (temperature, etc) and the internal environment of the animal (i.e., physiological state). The physiological state can also be considered as the degree of “stress” or “condition” of the animal.

We are just beginning to understand how the shellfish deal with external stressors on their physiological system, whether they are physical or biological. Over the last 20 years, instrumentation and new techniques have developed in biological research so that we can now start to follow the series of biochemical responses that the animals use to try and maintain the stability of their systems. The level of physiological understanding reaches its peak in human health, research and diminishes as we move into other organisms. Shellfish are still early in the exploration phase. What we do know about shellfish research has been driven by very specific anthropogenic needs. The cause of loss of a food source (wild or cultured) to a disease has driven much of the research. The rationale is that by understanding the mechanisms of the infection, we can then perhaps derive a solution. As a result, more information is available for oysters than for periwinkles, for instance. The second anthropogenic need has been the need to understand what our anthropogenic activities (construction, waste disposal, etc) have had on the marine ecosystem and how this may come back to haunt us in the future. As a result, certain animals have been used as indicator organisms or sentinels to monitor for biohazards and to understand what their effects might be on humans. Mussels, clams and oysters have often been used for this purpose. For either category, the objective is to understand what the normal physiological states of the animal are and then to define thresholds based on species, season, geography, etc., so that we will know when the animal (and possibly the whole system) is stressed, what the likely cause is and hopefully, what we can do to restore balance to the system (assuming we have control).

Therefore, this review will briefly summarise some of the current methodologies that are being used to assess stress in shellfish. It is not meant to be exhaustive, incorporating every paper in the literature dealing with stress, but rather a sampling of some representative studies on the subject. An annotated bibliography is presented on page 23 of this report. This work is still in progress.

5.2 Synopsis

As the stressor begins to act on the organism, there is a defence system that is initiated by the neuroendocrine system to ward off the intrusion and to maintain physiological stability within the animal. In oysters for example, if the animal is shaken or exposed to extremes in temperature or salinity there is a release of noradrenaline and dopamine (Lacoste, *et al.*, 2001a). This happens within 5 minutes of the event and depending on the duration of the shaking, the animals will recover after 60–90 minutes. Salinity and temperatures effects lasted much longer (3 days). Once the animal is stressed calcium can be released into the haemolymph (Pekkarinen, Suoranta, 1995) and the haemocytes can migrate from the tissues to the plasma to ingest any foreign objects or substances (Oubella, *et al.*, 1993). The activity of the haemocytes can be impaired, depending on the type of stressor (i.e., toxins), in their ability to move, change shape, etc. Tributyl tin would be an example of one of these toxins (Oliver, *et al.*, 2000). Many of these responses are common among species and even phyla (Table 1).

Table 1. List of existing biochemical/cell-based techniques used to evaluate stress in shellfish from the literature. Data are from references gathered from Abstracts from Fisheries and Aquatic Sciences (ASFA) in May 2004.

Measurement	Species	Common Name	Stressor	Source
Heat Shock Proteins - HSP70	<i>Cherax quadricarinatus</i>	Australian crayfish	Temperature	(Cimino, <i>et al.</i> , 2002)
	<i>Penaeus monodon</i>	Tiger shrimp	Temperature	(Cimino, <i>et al.</i> , 2002)
	<i>Haliotis rubra</i>	Black lip abalone	Temperature	(Drew, <i>et al.</i> , 2001)
	<i>Haliotis midae</i>	S. African abalone	Temperature, ammonia	(Reddy-Lopata, <i>et al.</i> , 2000)
	<i>Crassostrea virginica</i>	American oyster	Heavy metal, PCB, temperature, disease agent	(Brown, <i>et al.</i> , 1993);(Cruz-Rodriguez, <i>et al.</i> , 2000)
	<i>Crassostrea gigas</i>	Pacific oyster	Temperature	(Shamseldin, <i>et al.</i> , 1997)
	<i>Stylophora pistillata</i>	Branching coral	Temperature	(Tom, <i>et al.</i> , 1999)
	<i>Geodia cydonium</i>	Sponge	PCB's	(Wiens, <i>et al.</i> , 1998)
	<i>Mytilus galloprovincialis</i>	Gallo mussel	Temperature, bacteria	(Tiscar, <i>et al.</i> , 1996)
	<i>Mytilus edulis</i>	Blue mussel	Temperature, cadmium	(Brown, <i>et al.</i> , 1995)
	<i>Gammarus duebeni</i> .	Amphipod	Cadmium	(Brown, <i>et al.</i> , 1995)
Metallothionein	<i>Mytilus edulis</i>	Blue mussel	Heavy metals	(Steinert, Pickwell, 1988)
	<i>Macoma balthica</i>	Baltic clam	Heavy metals	(Bordin, <i>et al.</i> , 1997)
	<i>Corbicula fluminea</i>	Asiatic clam	Heavy metals	(Baudrimont, <i>et al.</i> , 1997)
	<i>Ruditapes decussate</i>	Clam	Cadmium, heavy metals	(Bebianno, <i>et al.</i> , 1993)
	<i>Mya arenaria</i>	Soft-shell clam	Cadmium, manganese	(Blaise, <i>et al.</i> , 2002)
Neutral lipids	<i>Mercenaria mercenaria</i>	Hard-shell clam	Starvation (larvae)	(Gallager, Mann, 1984)
	<i>Crassostrea virginica</i>	American oyster	Starvation (larvae)	(Gallager, Mann, 1984)
	<i>Ostrea edulis</i>	European oyster	Starvation (larvae)	(Gallager, Mann, 1984)
	<i>Macrobrachium borellii</i>	Shrimp	Starvation	(Pollero, <i>et al.</i> , 1991)
Neuroendocrine	<i>Crassostrea gigas</i>	Pacific oyster	Shaking, temperature, salinity	(Lacoste, <i>et al.</i> , 2001a)
	<i>Eledone cirrhosa</i>	Octopus	Air exposure	(Malham, <i>et al.</i> , 2002)
Haemocytes (phagocytosis)	<i>Eledone cirrhosa</i>	Octopus	Air exposure	(Malham, <i>et al.</i> , 2002)
	<i>Crassostrea gigas</i>	Pacific oyster	Antigen,	(Lacoste, <i>et al.</i> , 2002)
	<i>Crassostrea virginica</i>	American oyster	Temperature, salinity, TBT, metals, bacteria	(Volety, <i>et al.</i> , 1999), (Tirard, <i>et al.</i> , 1997), (Oliver, <i>et al.</i> , 1995)
	<i>Panulirus cygnus</i>	Spiny lobster	Handling, starvation	(Jussila, <i>et al.</i> , 2000)
	<i>Ruditapes philippinarum</i>	Clam	Bacteria, starvation	(Oubella, <i>et al.</i> , 1993)

5.2.1 The role of physical stresses

Physical stressors often have the capability of interfering with metabolism by causing changes in cell structure and modifying biochemical constituents. One of the ways animals deal with these changes is to produce chemicals that will ameliorate the effects such as: antifreeze proteins to lower freezing points, incorporating pigments from plant sources to reduce damage from ultraviolet radiation and by producing proteins to stop protein denaturation from heat. These latter proteins are known as heat-shock proteins (HSPs) and help protect the animal from heat stresses, although they are also produced in response to other stimuli. The HSPs come in different sizes (measured in kilo Daltons) and so far have been found as 70, 55, 35 and 19kDa (Brown, *et al.*, 1993). Another form of protein protection may be found in another compound known as metallothioneins. They are commonly produced in response to exposure of the organism to heavy metals (Tanguy, Moraga, 2001) (Table 1), although some studies have found that they are not always present (Pickwell, Steinert, 1988). Intertidal species often show greater tendencies to produce such protective biochemical, likely due to the evolutionary selective forces acting on organisms living in the harsh intertidal zone.

5.2.2 The role of biological stresses

Biological stressors often act in an indirect fashion. In the case of parasites, a light load may not bother the animal at all (particularly if they have evolved in a symbiotic fashion), but at some point the physical erosion of the host and the metabolites of the parasite will be enough to cause an immune response. At that point, similar processes to physical stressors will be initiated. Haemocytes will be mobilised to deal with particulates and foreign substances (Oubella, *et al.*, 1993; Volety, *et al.*, 1999). Certain changes in the haemolymph also occurs as free amino acids and alanine can increase (Paynter, *et al.*, 1993).

5.2.3 Interactions and synergistic effects

Of course, the physical and biological factors do not act alone. They can often operate in a synergistic fashion where multiple stressors act either concurrently or sequentially to produce deleterious conditions for the animals. Exposure of animals to physical stresses can often weaken the immune response of the animal and reduce its defences for later intrusions. For example, hot summer temperatures were thought to weaken oysters in the Bay of Morlaix (France). An oyster bacterium was more plentiful and virulent at this time and was therefore able to exploit the oyster resource, unfortunately for grower, also killing it (Lacoste, *et al.*, 2001b).

5.3 Conclusions

There are a number of different methods available to evaluate the stress levels in shellfish. The earliest methods involved simple growth comparisons and morphometric indices. The problem with most techniques is that measure cumulative anabolic activities that integrate large periods of time and are therefore not particularly sensitive. Changes to an animal begin at the cellular level, get incorporated into the tissue level and then extend through organs, systems and finally to the entire organism. Therefore, early-warning systems will have to monitor changes in the animal at a lower level such as the tissue or biochemical. Similarly, environmental impact studies often study changes at the population level as the current view is that changes at an individual organism or sub-cellular level is too small (or inconsequential) to evaluate. However, when the problem of ecosystem change is viewed from the perspective of time scales, the study of population change through biodiversity indices, etc are again problematic due to their low temporal resolution. It requires significant human and fiscal resources to conduct population-based ecosystem surveys and therefore, it is unlikely that they can be conducted on a regular basis over large areas to provide a time series that can be used to make real-time management decisions.

Therefore, if we are to evaluate the mechanisms on how animals respond to certain potential stressors, it will have to be at the lowest levels. While histology will play a role, this likely means more biochemical techniques. One benefit to working at the lower levels is that many processes are consistent across phyla. Table 1 shows some of the current options and the species investigated.

Management, in the future, may be able to use these stress responses to monitor systems. Once baseline values are established, it may be possible not only to monitor the target species being grown, but also the other species associated with that particular ecosystem. Much more work needs to be done to establish the seasonal and spatial patterns that will define a healthy crop and ecosystem, but the infrastructure to make this happen is already in place in most industrialised countries with the environmental regulation agencies. The main questions to be addressed are:

- 1) What are the indicators we should use to robustly evaluate the stress on shellfish?
- 2) Where and how should the baseline data on these stress indices be generated so that various systems can be compared?
- 3) What are the cause and effect relationships of the myriad of stressors the shellfish are exposed to? Which ones are the most important? Can we generalise these effects and create diagnostic tools to allow us to model the impacts?

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Current methodologies used to assess stress in shellfish

Oysters

- (Abbe, Sanders, 1988) looked at the meat condition index in oysters in Maryland and suggested that declines in the yield indicated stress on the system, although they could not say if it was related to MSX, pollutants or other factors. They went on to suggest that monitoring the condition index could serve as an early warning system for populations of oysters threatened by MSX.
- (Bachere, *et al.*, 1991) - stress on molluscs tends to elicit a phagocytosis response. In vertebrates, this is followed by chemiluminescence. This was adapted to Pacific oysters and was used to test the effects of pollutants and antibiotics that are commonly used in aquaculture.
- (Brown, *et al.*, 1993) reported that 70, 55, 35 and 19 kDa heat shock proteins were present in haemocytes when oysters were stressed with temperature, salinity and *Perkinsus*.
- (Cheney, *et al.*, 2000) concluded in a study on the Pacific oysters in Washington that water quality factors influenced their mortality, specifically lower oxygen levels after neap tides and warmer waters.
- (Chu, *et al.*, 1997) - when oysters were exposed to PAHs adsorbed onto sediment that the plasma total lipids, phospholipids and sterols decreased in the short-term (19 days), recovered and then decreased again after 108 days. This trend did not seem to hold for oysters that were reproductively active and in spawning condition.
- (Clark, *et al.*, 2009) - oysters were more resistant to effluent from an outboard engine than mussels, although they still showed signs of physiological distress (?).
- (Crawford, *et al.*, 1993) postulated that blooms of *Mesodinium rubrum* caused increased in bacterial populations in the water column. This peak in bacterial populations caused an increase in the haemolymph lysozymes, which are an antibacterial agent in oysters.
- (Cruz-Rodriguez, *et al.*, 2000) - HSP70 was produced when oysters were exposed to contaminated sediment (cadmium and PCB) although there was no strong dose response found.
- (Fisher, Moline, 1988) - oyster haemocyte characteristics changed such as spreading to an amoeboid shape and rate of locomotion when they were exposed to acute and short-term temperature and salinity changes. This was also found to differ between habitats.
- (Friedman, *et al.*, 1997) noted that a bloom of *Gymnodinium splendens* caused in Pacific oysters in California a diffuse to acute and multi-focal inflammation surrounding digestive tubules, thickening of peritubular muscularis and connective tissue, and increased vacuolization and dilation of digestive tubules.
- (Friedman, *et al.*, 1998) - both the Eastern and Pacific oysters produced HSP70. The eastern oyster was less able to acclimate to higher temperatures.
- (Gabbott, Walker, 1971) showed that glycogen:protein ratios are not a good index of stress in oysters. They felt that high temperatures and low food levels were important stressors on the animals.
- (Gallager, Mann, 1984) stated that eastern and European oyster larvae utilise neutral lipids or TAG when under stress. Therefore, staining larvae is a good way of monitoring their health.
- (Garcia-Esquivel, Bricelj, 1998) - the larvae were able to recover inversely related to their days of starvation. [This would suggest that stress is cumulative in some cases]
- (Hamdoun, *et al.*, 2000) - while oysters were able to acquire some thermo-tolerance after a sub lethal heat shock, they may not be able to mount a rapid stress response.
- (Lacoste, *et al.*, 2001b) - isolating the bacteria responsible for summer mortalities in juveniles was *Vibrio splendidus* was very difficult in moribund oysters. Instead they used a noradrenaline response in juvenile oysters. The bacterium was virulent at higher temperatures, could not be transmitted horizontally and did not affect adults. They felt this bacterium was responsible for the summer deaths in France's Bay of Morlaix.
- (Lacoste, *et al.*, 2001a) - stressors such as extremes in temperature, salinity and shaking caused neuroendocrine responses in oysters. Increased levels of noradrenaline and dopamine were found. The response happened within 5 minutes of the stress for mechanical stress and returned to normal after 60–90 minutes after stopping. Temperature and salinity lasted much longer up to 72 hours.
- (Lacoste, *et al.*, 2002) looked at the circulating hemocytes, the migratory and phagocytic activities and reactive oxygen species production of hemocytes after a shaking stress. The immune functions were downgraded for 30–240 minutes. They go on to suggest that the influences of stress on the immune system is why we get outbreaks of disease in shellfish culture, and that checking the state of stress regularly may be important in culture or research.
- (Littlewood, Ford, 1990) - Eastern oysters that were infected with *Haplosporidium nelsoni* had normal oxygen uptake rates at ambient temperatures, but increased their oxygen uptake by 70% at higher temperatures. They conclude that external stress factors can compound the damage done by parasitism.

Current methodologies used to assess stress in shellfish

(continued)

- (McDonald, *et al.*, 1989) were interested in finding specific biochemical responses of oysters to specific stressors (chemical). They looked at benzo(a)pyrene hydroxylase (BPH) and cytochrome P-450 reductase activities. No clear results were found as oysters may have cytochrome P-450 properties not observed in some other bivalve species.
- (Okoshi, Sato-Okoshi, 1996) suggest that looking at the shell structure of bivalves can allow for a historical view of the factors that affected the animals, such as stress.
- (Oliver, *et al.*, 1995) found TBT inhibited haemocyte rate of locomotion and chemiluminescence.
- (Oliver, *et al.*, 2000) correlation was positive: $r = .934$, between canonical variables composed of haemocyte number, PI, serum protein and lysozyme for defence, and Cd, Fe, Al, Pb, Zn, Mn, Sb, Ni, and Cr for metals. This suggestion of heightened defence activities in oysters from metal-contaminated sites is consistent with previous observations. The likelihood of complex relationships between oyster immune measurements and contaminant stress suggests that single chemical exposures and univariate analyses may be inadequate or misleading.
- (Paynter, Pierce, 1992) - *Perkinsus marinus* (Dermo) caused an increase in free amino acids and alanine. Glycine levels were lower.
- (Pickwell, Steinert, 1988) - TBT did not produce heat shock proteins or metallothioneins in oysters. Serum protein did not increase over the time of exposure.
- (Rose, Heath, 1978) - temperature and chemicals could stress the motility of Eastern oyster sperm. They tested for viability with an eosin-nigrosin stain.
- (Shamseldin, *et al.*, 1997) found heat shock proteins HSP-70 produced after the Pacific oyster was exposed to sub-lethal temperatures. The thermal tolerance lasted for 10 days based on the HSPs.
- (Tirard, *et al.*, 1995) found cold shock did not produced HSPs but that heat did. The HSPs were 70, 37, 34, and 32 kDa. The 70 form was primarily produced with extreme temperature shocks.
- (Tirard, *et al.*, 1997) - oysters produced a different set of proteins when stressed by temperature or salinity. Preferential synthesis of these proteins may represent an adaptation to preserve or restore oyster cell functions under adverse conditions.
- (Volety, *et al.*, 1999) developed an assay to assess the ability of oyster's (*Crassostrea virginica*), hemocytes to kill the human pathogenic bacterium, *Vibrio parahaemolyticus*. Bacterial killing was estimated colorimetrically by the enzymatic reduction of a tetrazolium dye using a microplate reader. It estimates the haemocytes ability to eliminate microbial agents.
- (Winstead, 1995) found digestive tubule atrophy in oysters that were either starved or exposed to salinity stresses compared to controls.
- (Wright, Hetzel, 1985) used RNA/DNA ratios to compare the growth between starved and fed oysters. It correlated well with condition index.
- (Yevich, Zarroghian, 1989) - brown cells in connective tissue increased in both size and colour at contaminated field sites. They felt that the brown cells were involved in detoxifying.

Scallops

- (Bower, Meyer, 1995) in a study on 4 causes of mortalities in cultured Japanese scallops - one of the agents involved was an unidentified intracellular bacterium that infected the haemocytes. Their observations suggest that the disease is chronic and can be induced by stress. It can also be widespread.
- (Lafrance, *et al.*, 2002) - temperature shocks stressed *Placopecten magellanicus* juveniles. Cold shocks increased the recovery time for swimming making the animals more vulnerable to starfish predation for 6 days. This would have obvious impacts on reseeded operations.
- (Brun, *et al.*, 2003) - *Placopecten magellanicus* did not respond to heat stress by producing HSP70. Bay scallops (*Argopecten irradians*) did show an enhancement of HSP70 and maintained higher levels for 8 days. They also showed a cold shock response with HSP70.
- (Fleury, *et al.*, 1996) - the adenylate energy charge (AEC) and glucides reflected the stress in juveniles that had been reseeded on the bottom. The post seeding behaviour had a high energy cost to the animals and, 3 days after seeding in the summer, they had still not recovered to their original state.
- (Kenchington, 1994) - RNA/DNA ratios could be used as an indicator of nutritional stress in populations of *Placopecten magellanicus*. It could be used to show patchiness in populations on a spatial basis.
- (Lodeiros, *et al.*, 1996) found the RNA/DNA ratios were good for predicting muscle growth in juvenile and mature *Euvola ziczac*. However, maturing scallops were more problematic during reproductive processes occurring.

Current methodologies used to assess stress in shellfish

(continued)

- (Lodeiros, *et al.*, 2001) - *Lyropecten nodosus* would mobilise proteins from the digestive gland and carbohydrates from the muscle during periods of maximal gonad growth.
- (Maguire, *et al.*, 1999) tested several stress indicators: standard salinity stress test, condition index, recessing speed of the scallop, adenylic energetic charge (AEC), and percentage carbohydrate content of the striated muscle. AEC was good for short-term stress while carbohydrate content and condition index was better for long-term stresses. Behavioural traits such as recessing ability looked positive because of the non-destructive nature of the test.
- (Maguire, *et al.*, 2002b) - the adenylate energy charge (AEC) was lower in scallops that had been dredged than those that were not. Scallops just outside the dredge track were also lower than the controls. [This suggests that mechanical stress can cause a decrease in the AEC].
- (Miyazono, 2000) - RNA/DNA ratios and acid protease activity were good indicators to show difference in the stress caused by differing water flows for scallops. The highest flow rates caused poorer growth, but this was not reflected in the protein or glycogen content of the muscle.
- (Roddick, *et al.*, 1999) - RNA/DNA ratios are a useful index of the health of scallop (*Placopecten magellanicus*) populations, but because of the number of factors that affect growth, it should not be the only index used.

Mussels

- (Clark, *et al.*, 1009) found mussels were very sensitive to diluted effluent from a 2-cycle outboard motor and showed physiological stress (?) and degeneration of gill tissue. There was a significant delayed mortality effect. Oysters were not as bad.
- (Pickwell, Steinert, 1988) - TBT did not produce heat shock proteins or metallothioneins in mussels or oysters. Serum protein increased over the time of exposure.
- (Bacchiocchi, *et al.*, 2001) - mussels responded to heavy metal pollution with a decrease in their TOSC (Total Oxyradical Scavenging Capacity). They also showed that Neutral Red retention time in haemocytes was decreased in the treatment mussels (*galloprovincialis*).
- (Brown, *et al.*, 1995) found populations of mussels (*edulis*) from the North Sea and Baltic differed genetically. When they were exposed to similar levels of cadmium, the NS mussels developed more HSP70 than the BS mussels and less cadmium in their tissues. The quantity of HSP70 correlated well with survival.
- (Cajaraville, *et al.*, 1991) - the health of mussels (*galloprovincialis*) could be determined histologically after starvation episodes. After a 10 day starvation period, there was an increase in Type I and Type IV digestive tubules and a reduced Mean Epithelial Thickness" (MET). Quantitative histological diagnoses are recommended over subjective.
- (Dijkema, *et al.*, 1995) - phycotoxins caused stress in mussels reducing the filtering rate.
- (Emmett, *et al.*, 1987) - reproduction in some areas caused a delay in the replenishment of the glycogen stores in some populations that correlated with summer mortality.
- (Fuentes, *et al.*, 2002) - hybrids from *edulis* and *galloprovincialis* had a lower viability than pure *galloprovincialis*. The viability was associated with parasitism from *Marteilia refringens*, and lower levels of the stress proteins calreticulin and HSP70.
- (Gainey, 1987) - during hyperosmotic stress events the addition of dopamine decreased the magnitude of water loss and helped with the recovery of the animal [might be a feature for hatcheries]
- (Hatcher, *et al.*, 1997) - the difference between the respiration rate (based on ammonia excretion) and the maintenance respiration rate may be related to the degree of nutritional stress of the mussel. Reproduction influenced these relationships.
- (Hummel, *et al.*, 1989) - ratios of free amino acids (taurine/glycine) were not particularly useful as the differences between treatments were less than spatial and seasonal differences.
- (Kluytmans, *et al.*, 1988) - exposure of *O. edulis* to cadmium resulted in the inhibition of gamete production, but increased spawning frequency so that the total number of gametes produced was not changed much.
- (Lopez, *et al.*, 2001) - HSP70 were found more predominantly in intertidal mussels than those in suspended culture.
- (McKinney, *et al.*, 1996) used LC50 survival data to evaluate the effect of copper concentrations at two pH levels to determine the stress effect on freshwater mussels (*Elliptio angustata*).
- (Myrand, Gaudreault, 1995) - there was a genetic component to the summer mortality phenomenon rather than environmental effects.

Current methodologies used to assess stress in shellfish

(continued)

- (Myrand, *et al.*, 1999) - the summer mortality was related to the level of heterozygosity in the mussels. Increased heterozygosity was correlated with lower metabolic rates. Reproduction, because of its larger energy demand, would tend to put stresses on the animals reserves and therefore, lower metabolic rates would be an asset.
- (Okumus, Stirling, 1994) - transplanted mussels had higher respiration rates and ammonia excretion than native populations, but after 4.5 months the rates were the same indicating acclimation to local conditions.
- (Pekkarinen, Suoranta, 1995) - freshwater mussel (*Anodonta anatina*) increased the calcium concentration in the haemolymph upon being transferred to a bucket for transportation. Those mussels kept in the lab also had higher calcium and glucose levels in the haemolymph. The levels would return to normal when the mussels were returned to the lake. Storage of samples would bias the results.
- (Romeo, *et al.*, 2003) used several biomarkers to test the effects of environmental stressors: of glutathione S-transferase (GST; exposure to organics), of catalase (exposure to oxidative stress) and of acetylcholinesterase (inhibited by some pesticides) and the lipid peroxidation (thiobarbituric acid reactive substances: TBARS). All showed seasonal patterns and differences between sites characterised by different levels of heavy metal contamination.
- (Steinert, Pickwell, 1988) - HSPs are produced to a variety of stressors and appear to be biologically universal. Heavy metals also tend to increase the level of metallothioneins.
- (Tiscar, *et al.*, 1996) used indirect immunofluorescence and monoclonal antibodies to measure HSP70 in mussels (*galloprovincialis*). The HSP70 increased when exposed to pure *E. coli* cultures but not the broth catabolites.
- (Tremblay, *et al.*, 1998) - lower heterozygosity resulted in higher metabolic rates and lower O/N ratios resulting in lower scope for growth.

Clams

- (Laing, 1993) found *Tapes philippinarum* early juveniles could withstand longer periods of nutritive stress if they went into the starvation period with more carbohydrate reserves and could also resume growing more quickly when food was restored.
- (Oubella, *et al.*, 1993) states that haemocytes represent the most important defense mechanism against foreign material in bivalves. Challenges with *Vibrio* increased the number of haemocytes as haemocytes migrate between tissues and plasma.
- (Belanger, *et al.*, 1986) - clams *Corbicula sp.* Responded to exposure to zinc by increasing the water content in tissues either due to tissue degradation or osmoregulatory impairment.
- (Chou, *et al.*, 1994) - the mortality rate of the clam *Meretrix lusoria*, when injected with a virus, would increase when the temperature was increased post-injection.
- (Figueras, *et al.*, 1996) used histology to look at the positive correlation between brown ring disease and the parasitic load in *Ruditapes* spp.
- (Gallager, Mann, 1984) reported that neutral lipids in larvae are metabolised when the animals are under stress. A practical method for assessing the amount of lipid remaining is by using a lipid-specific stain.
- (Marin, *et al.*, 2003) - biochemical composition of *Tapes philippinarum* varied by season, sex and that condition index and total energy content was influenced by both endogenous and environmental factors.

6 ToR C: Review the ecological factors affecting shellfish production and, more specifically, carrying capacity and fouling

Preliminary remarks and WG comments

After a wide discussion at the 2003 meeting, the WG agreed to the establishment of a working plan for the coming meetings. The subjects were ranked according to their priority. Some of them will need to include additional working group members with the necessary expertise to address these issues.

- 1) Carrying capacity (hydrographic factors, primary productivity, food supply)
- 2) Predation
- 3) Fouling
- 4) HAB Blooms
- 5) Disease
- 6) Pollution (water quality)

During the 2003 meeting, it was agreed that the report should focus specifically on the effects of the physical environment and primary production on the carrying capacity of shellfish production and on modelling approaches for predicting carrying capacity. Participants at the 2004 meeting added to the 2003 report while focused on reviewing:

- food supply limitation as related to carrying capacity approaches for documenting food depletion in nature.
- bivalve performance methodologies for documenting trends in production related to environmental variation and density-dependant growth
- predation and fouling controls on shellfish production.

6.1 Introduction

Knowledge of how coastal ecosystems function is fundamental for developing strategies for improving aquaculture sustainability. An understanding of aquaculture-based ecosystems requires consideration of biological, physical, and chemical factors. Physical processes governing water circulation and mixing determine the transport and supply of dissolved and particulate matter, including bivalve food particles and waste products (faeces, pseudofaeces and ammonia). Important biological processes to be considered include feeding and egestion by cultured and wild organisms, the dynamics of the supporting planktonic ecosystem, interactions between pelagic and benthic communities, and exposure to infectious diseases and pathogens. Important chemical considerations include nutrient and contaminant dynamics and chemical transformations mediated by the cultured organisms and various ecosystem components, including bacteria.

The development of tools for the identification of ecosystem carrying capacity is critical to the management of the coastal zone and for ensuring aquaculture sustainability (economic and ecological). Ecological models are showing great potential for identifying suitable aquaculture sites and sustainable rearing densities (Incze *et al.*, 1981, Raillard and Menesguen, 1994, Dowd, 1997; Bacher *et al.*, 1998, Campbell and Newell, 1998, Ferreira *et al.*, 1998, Meeuwig, 1999, Grant and Bacher, 1998, Pouvreau *et al.*, 2000; Pastres *et al.*, 2001, Beadman *et al.*, 2002). A wide array of oceanographic instrumentation is available that may be used to observe environmental changes at aquaculture sites, for monitoring the status of bivalve food, and to test model predictions of the transport of water and food particles. In addition, methods are available for monitoring density dependent changes in bivalve performance that can be used to test productive capacity predictions.

The following review and recommendations are aimed at supporting decision-making needed to improve the economic sustainability of the shellfish industry. The focus of the WGMASC at its first meeting in 2003 was on identifying some of the major physical and biological processes that may limit bivalve carrying capacity and which may affect sustainability over space- and time-scales relevant to the aquaculture industry. In addition, international modelling efforts to assess the carrying (productive) capacity of coastal systems were examined as they quantitatively address the interactions between bivalves and the environment. The 2004 meeting of the working group expanded the section on carrying capacity beyond theories and model-based predictions to include a review of approaches and methodologies for detecting changes in production trends that may be related to excessive stocking (e.g., food supply depletion and bivalve performance). The WGMASC also began to outline additional biological considerations on shellfish productive capacity, including predation and fouling.

6.2 Carrying capacity

6.2.1 Major environmental controls on shellfish production

A basic understanding of bivalve production requires tracking of the total particulate load (bivalve food abundance) and composition (nutritional value), and the water flux (mixing and exchange) (Grant and Bacher, 2001). Coastal waters exhibit a variety of physical oceanographic processes, for example tidal and estuarine circulation. Dissolved and suspended matter in the water column is transported and mixed by water motion and are eventually exchanged with the adjacent open ocean, or deposited (utilized) locally. It is hypothesized that the carrying capacity of an ecosystem (the maximum production achievable in a given ecosystem) is regulated to a large extent by water motion and mixing. Inclusion of oceanographic parameters in studies of culture systems is essential to a quantitative assessment of the validity of this hypothesis (Cranford *et al.*, 2003).

Some relatively simple scaling exercises have been conducted that provide intuitive indicators of the limiting effect of water motion on aquaculture productive capacity (Cloern, 1982; Officer *et al.*, 1982; Nichols, 1985; Hily, 1991; Smaal and Prins, 1993; Dame, 1996; Dame and Prins, 1998; Prins *et al.* 1998; Cranford *et al.*, 2003). This approach compares factors supplying food to bivalves, including the residence time (RT) of water in an embayment (determined by degree of tidal exchange of water) and phytoplankton turnover or primary production time (PPT), with the time required for the bivalves to clear the whole water body (clearance time; CT). Tidal exchange may limit system productive capacity if CT/RT is less than 1, which means that tidal exchange is unable to compensate for particle depletion by bivalves. Studies of this type suggest that intensive shellfish culture may have the capacity to deplete food supplies on a coastal ecosystem scale in some regions, with potentially negative consequences to aquaculture yields (Dame and Prins, 1998; Prins *et al.*, 1998; Cranford *et al.*, 2003). Dame and Prins (1998) examined 11 coastal ecosystems and suggested that many systems produce sufficient phytoplankton internally to prevent overgrazing by resident bivalve populations. However, these authors concluded that some systems under intense bivalve culture require the physical import of food resources from outside the system to prevent particle depletion.

Carrying capacity is theoretically at maximum if $CT/RT < 1$ and $CT/PPT = 1$ (Smaal and Prins, 1993; Dame, 1996; Dame and Prins, 1998). To date, the only system studied that appears to meet these criteria for overexploitation by shellfish aquaculture is the Bay of Marennes-Oléron, the most intensive growing region of the Atlantic coast of France (Dame and Prins, 1998). Research on the whole-basin environmental effects of intense mussel and oyster aquaculture in Marennes-Oléron has focused on the impact of bivalve overstocking on growth and survival (Héral, 1993; Héral *et al.*, 1986). Excessive culture on two occasions led to large-scale growth reduction and high mortalities in the Bay.

Environmental controls on aquaculture capacity are believed to be greatest in estuaries and inlets where water residence time is long and bivalve biomass is high. In such areas, bivalve feeding could dramatically reduce the concentration and alter the nature of suspended particulate matter with the resultant potential to change pre-culture productivity within a defined area. In areas with greater flushing, water depleted of particles by bivalves can be renewed by tidal exchange. Particle depletion is believed to be greatest in estuaries and inlets where water residence time is long and mussel biomass is high (e.g., Dame, 1996). In such areas, water depleted of particles by mussels cannot be completely renewed by tidal exchange. Comparisons of water residence times in Prince Edward Island (PEI; Canada) embayments with estimates of the time required for mussel cultures to clear this water (clearance time) indicate that food supplies in 12 of 15 embayments are being removed faster than they can be replaced by tidal exchange (Cranford *et al.*, 2003). Meeuwig *et al.* (1998) used a different mass-balance approach to model phytoplankton biomass in PEI embayments and estimated that the mussel farms in six of 15 systems reduced phytoplankton biomass by 45 to 88%. These order of magnitude calculations are strongly suggestive that the intensive mussel culture activities in PEI have the capacity to alter matter and energy cycling for long periods, with the resultant potential to decrease mussel productivity through food limitation.

The above scaling and mass-balance approaches are useful and intuitive indices for addressing the issue of system productive capacity. However, they are limited in that they neglect potentially important physical processes, such as water column stratification, mixing, and flow velocity, that could influence the effects of bivalve culture operations on suspended particles. These approaches also use single flushing estimates for estuaries, when flushing is spatially dependent, and do not include additional particle utilization by fouling organisms associated with shellfish culture as well as by other resident suspension-feeding organisms that include zooplankton and benthic invertebrates. The cumulative grazing pressure on the phytoplankton needs to be considered when assessing system productive capacity for aquaculture.

6.2.2 Ecosystem and carrying capacity models

A variety of models have been applied to assessing the environmental interactions of bivalve aquaculture operations (Dowd, 1997; Grant *et al.*, 1993; Grant and Bacher, 1998; Smaal *et al.*, 1998; Meeuwig, 1999; Dowd, 2003; Bacher *et al.*, 2003). While all of the approaches include a comparison of physical water exchange to some sort of biological process like filtration, there are no standard methods for assessing carrying capacity. Bearing in mind the complexity of interacting factors, this is not surprising. Empirical studies such as the calculation of budgets (e.g., carbon, nitrogen, and energy) and simulation modeling have been some of the more focused approaches used to evaluate potential bivalve aquaculture effects at an ecosystem level. As an example of the former, Carver and Mallet (1990) calculated the mussel

carrying capacity of an inlet in eastern Canada by comparing estimated food demand to food supply based on organic seston concentrations delivered by a simple tidal prism model. The latter approach was used by Raillard and Menesguen (1994) who constructed a simulation model for a macrotidal estuary in France to describe relationships between oyster feeding, primary production and seston transport. Both approaches yielded different, but complimentary, information. Meewig *et al.* (1998) used a mass-balance approach to model phytoplankton biomass in 15 Prince Edward Island embayments and estimated that the mussel farms in six of these systems reduced phytoplankton biomass by 45 to 88%. Dowd (2000) examined a simple biophysical model that quantified the relative roles of flushing, internal production and bivalve grazing on seston levels. These order of magnitude calculations strongly suggest that intensive bivalve culture has the capacity to alter particulate food supplies for long periods in some coastal systems as a result of limitations in the tidal exchange of food from outside the system.

More complex numerical models are powerful tools that may help to guide aquaculture management because they integrate important biological and physical processes that represent the complexity of this system (Cloern, 2001). Fully coupled biological-physical models may be envisioned (e.g., Prandle *et al.*, 1996; Dowd, 1997) that predict shellfish production and carrying capacity as a function of culture density and location. To do this, shellfish ecosystem models, including carrying capacity models, must be integrated with information on water circulation, mixing, and exchange to account for transport and spatial re-distribution of particulate and dissolved matter. Box models (Chapelle *et al.*, 2000; Dowd, 1997; Raillard and Menesguen, 1994) offer a practical means to couple coastal ecosystem models with physical oceanographic processes. The bulk parameterizations of mixing required for these box models can be derived directly from complex hydrodynamic models (Dowd *et al.*, 2002).

Simulation models that focus on estimating bivalve carrying capacity and related ecosystem impacts provide effective tools for quantitative descriptions of how food is captured and utilized by bivalves as well as site-specific information on ecosystem variables and processes (Carver and Mallet, 1990; Brylinsky and Sephton, 1991; Grant, 1996). The ability to predict physiological responses of bivalves under culture conditions permits calculation of clearance, biodeposition, and growth rates and this ability presents tremendous opportunities to manage the sustainability of the industry (Carver and Mallet, 1990; Labarta *et al.* 1998). From a mathematical perspective, the nonlinear functional relationships used to describe bivalve bio-energetics have often led to poor model predictions due to their high sensitivity to inadequately known physiological parameters (Dowd, 1997). Robust mathematical relations are being developed with the needs of simulation models in mind, such that bioenergetic models have been successful in predicting growth (Grant and Bacher, 1998; Dowd 1997; Scholten and Smaal, 1998).

Decision support systems have been developed that integrate available knowledge about natural- and human-driven parts of coastal ecosystems into computer-based models (Crooks and Turner, 1999; deJonge, 2000). Simulation models can be directed towards assessment of bivalve growth and carrying capacity from the standpoint of farm management. For instance, bivalve studies based on physical transport of food particles to bivalves and their bioenergetic use by the animals are part of a growth equation. Coupled with estimates of stocking density, these models produce farm yields, which may then be exported to economic models of profitability (e.g., Samonte-Tan and Davis, 1998). An essential feature of the growth models is that they may be fully groundtruthed using bivalve harvest/growth data from the farm sites. Ultimately, these models may be used to actively manage the location and extent of culture in coastal estuaries for multiple users. Such models will need to take into account culture dynamics such as seed-stocking and fouling biomass, depth of activity, and cumulative effects of neighbouring human activities (e.g., agriculture runoff, construction sedimentation, boating, and ballast activities, etc.).

Another new development, which must be taken into consideration for ecological modelling, is increasing interest in bivalve polyculture. Bivalve culture is rarely conducted in isolation from other bivalve culture. Some leases accommodate several bivalve species. All have different habitat, physiologies and production dynamics. Accurate modelling of single species culture interactions with surrounding habitat ecology needs to take this into account in the future.

6.2.3 Limitation of food supply

Food depletion and subsequent food limitation of bivalve production can result if bivalve populations remove food particles faster than tidal exchange and primary production can replace them. Bivalve filter feeders have a large capacity to filter water, directly altering suspended food concentrations (Bayne *et al.*, 1989; Jørgensen, 1996; Dame, 1993; and 1996; Smaal *et al.*, 1997). Estimates of water filtration rates by dense populations of bivalve filter-feeders indicate a capacity to impact and control phytoplankton and seston abundance at the scale of whole estuaries, bays and coastal systems (Cloern, 1982, Officer *et al.*, 1982, Nichols, 1985, Hily, 1991, Smaal and Prins, 1993, Dame, 1996, Dame and Prins, 1998, Prins *et al.* 1998). Population explosions of introduced bivalve species in San Francisco Bay and dramatic reductions in oyster populations in Chesapeake Bay have been implicated as the cause of the large changes in phytoplankton biomass and production experienced in these systems (Nichols, 1985; Newell, 1988; Nichols *et al.*, 1990; Alpine and Cloern, 1992; Ulanowicz and Tuttle, 1992).

The ability of bivalves to deplete microalgal biomass and/or seston concentrations has been confirmed in relatively small-scale laboratory and field experiments with benthic populations (Fréchette *et al.*, 1989, Cole *et al.*, 1992, Muschenheim and Newell, 1992, Kamermans, 1993, Butman *et al.*, 1994, Wildish and Kristmanson, 1997). Research on the whole-basin environmental effects of intense mussel and oyster aquaculture in the Bay of Marennes-Oléron, the most intensive growing region of the Atlantic coast of France, has shown that high rearing densities caused large-scale

growth reduction and high mortalities in the Bay on two occasions (Héral, 1993; Héral *et al.*, 1986). Similar observations were reported for intensive scallop culture in Mutsu Bay, Japan (Aoyama, 1989). In both cases, increased food limitation caused by bivalve-induced changes in particulate food abundance and quality appears to have contributed to the reduced growth and survival of the bivalves.

Several attempts have been made to measure particle depletion at raft and longline aquaculture sites using a variety of sampling approaches including water sampling and moored or profiling *in situ* instruments. In general, particle depletion within the geographic scale of shellfish farms is substantially greater for mussel raft culture (Navarro *et al.*, 1991, Heasman *et al.*, 1998) than for longline culture methods (Rosenberg and Loo, 1983, Fréchette *et al.*, 1991, Ogilvie *et al.*, 2000, Pilditch *et al.*, 2001, Ibarra, 2003). The close spacing of mussel ropes attached to rafts attenuates water flow through the raft system (Boyd and Heasman, 1998), allowing the mussels to clear much of the seston load and thereby depress growth rates downstream (Navarro *et al.*, 1991, Heasman *et al.*, 1998). Studies of food depletion associated with longline culture has provided more variable results, with no food depletion reported inside some farms (Fréchette *et al.*, 1991, Pilditch *et al.*, 2001), and significant depletions observed inside others (Rosenberg and Loo, 1983, Ogilvie *et al.*, 2000, Ibarra, 2003). Such variability is expected given site differences in culture density, circulation patterns, current speed, and mixing processes (see above).

Measuring food depletion on relatively large geographic scales (bay to coastal ecosystem) is problematic as environmental variability has a tendency to mask any depletion (Bacher *et al.*, 2003). This is particularly true with approaches that rely on limited spatial and temporal sampling (discrete water sampling). Moored *in situ* electronic sensors (e.g., fluorometers, transmissometers) have been used to provide more frequent measurements than can be obtained through manual sampling, but these tend to provide insufficient horizontal and vertical coverage to document the scale of particle depletion. Ibarra (2003) overcame the latter problem (vertical variability) by utilizing an array of light attenuation meters to provide an integrated measurement of particle concentrations throughout the water column. This approach was able to detect particle depletion within a suspended mussel culture farm in Nova Scotia. However, a large number of these moorings would be required to assess the spatial extent of particle depletion and to detect interactions between farms.

Remote sensing methods using multi-spectral and hyper-spectral scanners have been applied to detecting the spatial scale of particle depletion. Owing to the resolution required, relatively low altitude (aircraft mounted) systems appear to have the greatest potential. A problem with using this approach in coastal regions stems from difficulties in distinguishing signals representative of suspended particles (chlorophyll and total suspended sediments) from those of the seabed, including macrophytes. Cost is also an important issue with this approach and this tends to limit observations to very brief periods. To overcome tradeoffs between high spatial resolution (remote sensing) and high temporal resolution (moored instruments), an approach utilizing a combination of moored and towed instruments is being tested in Canada. A towed undulating vehicle has been adapted to include environmental and food supply sensors and is able to provide the intensive horizontal and vertical sampling needed to characterize food supply variations over spatial scales relevant to whole bays.

6.2.4 Bivalve performance

The estimation of the effects of the food supply on shellfish growth is intrinsically connected to shellfish aquaculture effects on their food supply. Bivalve growth and condition measurements are theoretically useful indicators for monitoring the status of food supplies. In addition, information on growth trends and changes in culture density may provide an indication of whether or not the carrying capacity of the ecosystem has been exceeded (i.e., reduced crop yield). However, growth and condition are also controlled by general environmental conditions and are influenced by husbandry practices, contaminant inputs, and other factors that reduce the utility of shellfish-based measures as bio-indicators of environmental quality. Research is needed before such measures are proven to be effective for use in monitoring the impacts of environmental conditions on shellfish production. This research should address unanswered questions regarding;

- the separation of the many factors controlling mussel growth (cause-effect relationships),
- the utility of reference sites when water passing through the culture is rapidly transported throughout bays,
- density-dependent effects on mussel condition, and
- the establishment of farm operational objectives (growth thresholds) that can be used for farm management purposes.

Notwithstanding the need to more fully understand all the factors controlling shellfish growth at aquaculture sites, standardized monitoring of bivalve performance indices provides valuable information that is related to the cumulative effect of all physical, chemical, biological and husbandry factors acting on the culture. These data are therefore of importance for identifying long-term trends at the culture site and can be used by farmers for managing husbandry practices. Given the potential for interactions between all farms, particularly in intensively leased embayments, an open process is needed for the exchange of information and for the establishment of bay-wide management approaches.

The next step in the WGMASC work plan with regard to standardized shellfish monitoring will be to review available and proposed approaches and methodologies (e.g., growth rate, condition indices, meat yields, animals/kg, farm production, weight at harvest, time to harvest, etc). The most promising methodologies will be identified after

assessing the strengths and weaknesses of the different approaches. From this analysis, recommendations will be made on the most useful approaches.

6.3 Predators and fouling

6.3.1 Introduction

Predation and fouling were considered as factors that may have considerable influence on the culture of shellfish species with attendant economic consequences. These factors are inextricably linked and were considered jointly because each may have the potential to generate problems associated with the other. For example, in an attempt to remove a predatory threat an artificial structure may be used which may increase the potential for fouling and negatively impact the culture species in that manner. The goal of this section is to identify some of the problems created by fouling and predation in relation to the culture of marine shellfish and present an initial framework (in the form of questions) to identify information that may be required to better manage the problem.

The primary outcome of predation is to remove the culture individual from the system, which has an immediate economic impact. There are a number of mechanisms by which a predatory species may increase in a system:

- The density of culture organisms can influence abundance of predatory species in a system;
- Culture species introduced into a system may also introduce a predatory species;
- Predatory species may also prey upon less abundant wild organisms in the vicinity of the culture system;
- Migratory patterns/behaviour of predatory species (e.g., birds).

Fouling organisms can cover the species cultured or the containment system and impacts the culture process by restricting flow and hence oxygen and food availability or by actively filtering food (phytoplankton) from the system. Fouling impacts the economics of the culture process by reducing growth rates of the culture species (hence their time to market size) and increases the level of intervention (labour) to replace the fouled material or clean the fouled organism. Fouled organisms may also have reduced market value or marketability. The intensity of fouling may be influenced by:

- The location of the culture system (subtidal vs. intertidal) or the level of husbandry intervention;
- The degree of containment or predator protection required;
- Light regime, turbidity and salinity;
- Culture species introduced into a system may also introduce a fouling species.

The intensity of impact of these factors may be dependant upon a number of factors relating to the location, stage and method of culturing the aquaculture species. Tables 1 and 2 both summarize the primary predatory and fouling organisms, respectively, influencing a variety of shellfish culture methods. The tables do not attempt to provide an exhaustive list of problem species associated with each factor but do highlight the extent of the problems associated with the various culture mechanisms. Each culture area and mechanism used may have specific issues related to it. Intertidal shellfish culture, for example, may reduce the exposure time to exclusively aquatic predatory species (fish, crabs) and those that require considerable time to prey upon the culture species (starfish and whelks, on the other hand some drill species may function even if exposed to air). However, intertidal culture may also increase the risk of avian and mammalian predation. Fouling may be exacerbated by the amount of material used to protect or contain the culture organisms. Increased material may be a function of the stage of development (size) of the culture species where smaller individuals would require smaller mesh size and hence the potential for greater surface area to colonize for fouling organisms.

Table 1. List of Potential Predatory Species on Mussels, Oysters, Clams and Scallops according to the Life Stage and Method of Culture.

n/a – culture method does not apply for species at this culture stage

n/p – predation not a major factor

Phase	Method	Shellfish Species Group			
		Mussels	Oysters	Clams	Scallops
Nursery	Subtidal Floating Cages	n/a	Crabs, Snails	n/a	n/p
	Bottom Cages	n/a	Crabs, Snails	Crabs, Snails, Nemertean	Starfish Crabs
	Bottom Plots/Collectors	Starfish, Sea Ducks, Crabs, Fish,	Starfish, Crabs, Snails, Fish, Birds	Crabs, Snails, Nemertean, Birds	Crabs, Snails, Birds, Fish
	Suspended Collectors	Starfish, Sea Ducks, Fish,	n/a	n/a	Starfish, Crabs
Grow Out	Subtidal Rafts/Tables	N/A	n/p	n/a	n/p Crabs, Starfish
	Subtidal Longlines	Starfish, Birds, Fish	n/p	n/a	Crabs, Starfish, Nematodes
	Subtidal Nets/Cages	n/a	n/p	Fish, Crabs	n/p Crabs, Starfish
	Subtidal Plots	Starfish, Birds, Fish, Crabs, Snails, Copepods	Starfish, Birds, Fish, Crabs, Snails	Fish, Crabs, Snails	Fish, Crabs, Starfish
	Intertidal Plots	Starfish, Birds, Fish, Crabs, Snails	Starfish, Birds, Fish, Crabs, Snails, Nematodes	Fish, Crabs, Snails, Birds	n/a
	Intertidal Tables	n/a	Snails, Birds, Nematodes	n/a	n/a
	Intertidal Poles	Starfish, Birds, Fish, Crabs, Snails	n/a	n/a	n/a

Table 2. List of Potential Fouling Organisms on Mussels, Oysters, Clams and Scallops according to the Life Stage and Method of Culture.

Phase	Method	Shellfish species group			
		Mussels	Oysters	Clams	Scallops
Nursery	Subtidal floating cages	n/a	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans polychaeta	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters polychaeta
	Bottom cages	n/a	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans	macroalgae, barnacles, mussels,	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters
	Bottom plots	barnacles, mussels,	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans	macroalgae, mussels	n/a
	Spat collectors	mussels, hydroids, tunicates, macroalgae, bivalves	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters	n/a	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters
Grow out	Subtidal rafts/tables	tunicates, hydroids, ascidians, holothurians, mussels, macroalgae, barnacles, worms	tunicates, hydroids, ascidians, holothurians, mussels, macroalgae, barnacles, worms, oysters	n/a	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters

Phase	Method	Shellfish species group			
		Mussels	Oysters	Clams	Scallops
	Subtidal longlines	tunicates, hydroids, ascidians, holothurians, mussels, macroalgae, barnacles, worms	??	n/a	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters
	Subtidal nets/cages	n/a	tunicates, hydroids, ascidians, holothurians, mussels, macroalgae, barnacles, worms	macroalgae, barnacles, mussels,	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters
	Subtidal plots	tunicates, hydroids, ascidians, holothurians, mussels, macroalgae, barnacles, worms	tunicates, hydroids, ascidians, holothurians, mussels, macroalgae, barnacles, worms, oysters	mussels	mussels, tunicates, sponges, macroalgae, worms, barnacles, snails, hydroids, bryozoans, oysters
	Intertidal plots	hydroids, mussels, macroalgae, barnacles, worms	hydroids, mussels, macroalgae, barnacles, worms, oysters	macroalgae, barnacles, mussels,	n/a
	Intertidal tables	n/a	hydroids, mussels, macroalgae, barnacles, worms	n/a	n/a
	Intertidal poles	hydroids, mussels, macroalgae, barnacles, worms	hydroids, mussels, macroalgae, barnacles, worms	n/a	n/a

n/a – culture method does not apply for species at this culture stage

6.3.2 Managing the issues

It is clear that there are many issues to consider when managing the problems associated with fouling and predation. Solutions to minimise the problems may seem intuitive but for the most part require an in-depth knowledge of the system and the factors influencing the respective predatory and fouling organisms, as highlighted above. Allied with this knowledge it is important to have a good understanding of the physicochemical requirements and tolerances of the culture species. Information pertaining to the culture species is more likely available as it would have been generated when the species was being assessed for culture. Similar information pertaining to the predatory or fouling organisms is generally less accessible but no less important. Predictive tools to determine settlement times of cultured species in relation to fouling organisms can maximise settlement of the target culture species, while minimising cohabiting culture or fouling organisms, e.g., target *Pecten* to avoid *Mytilus* and tunicate fouling.

For example, a good understanding of factors (e.g., temperature, salinity, hydrodynamic characteristics of a system) that influence the settlement/recruitment patterns and growth rates of the fouling (and predatory) organisms may result in certain predictive capabilities relating to the timing and location (horizontal or vertical) of the fouling event. With this knowledge in hand the aquaculturist is in a position to take a decision to adjust the husbandry practices. Economic considerations will ultimately dictate the degree of adjustment that is required. Table 3 highlights some of the potential information gaps pertaining to fouling and predatory organisms as well as the culture species and systems and identifies some of the applications of this information such that the impacts may be minimised.

Table 3. Information requirements and potential applications to manage problems associated with fouling and predation on cultured shellfish.

Information Required	Potential Benefit – Comment
Temperature and salinity tolerance – predatory and fouling organisms	Can the culture species be successfully cultured under a temperature and salinity regime such that the predatory or fouling species may not function? May be more applicable to controlling fouling as many predatory species are highly mobile and can move from an area when conditions become unsuitable.
Temporal and Spatial Refuge – culture process	Can the culture species be deployed at a specific location and time to avoid the predatory or fouling activity? This may be impractical, as operators may not have the luxury of multiple sites within which to base their operations and relocate product as the need arises. Optimising the timing of deployment may be a more feasible adjustment assuming that the availability of the culture species can be modified accordingly.
Habitat requirements – fouling organisms	Do the fouling organisms have a preference for particular substrates or more importantly reject others? If so, can a non-attractive culture containment material be utilised while maintaining optimum conditions for the culture species.
Habitat requirements - predators	Do the predatory species have specific habitat requirements during non active periods? Do they require sediments within which to bury or rock or boulder within which to shelter. If so the culture location may be selected with these requirements in mind. However, highly mobile predators may be less constrained by such requirements.
Size Refuge – culture organism	Is there a specific size above which a culture organism becomes less susceptible to predation? If so, the need to protect this organism using meshes or cages may become less significant with increasing size over time.

It is clear from the table above that it is very difficult to minimise the impacts of predation and fouling in the absence of basic ecological and environmental tolerances of the organisms in question. Even a trawl of anecdotal accounts (local knowledge) may prove enlightening and may direct management solutions.

What is also clear is that the management of the issues of predation and fouling of cultured shellfish may require a number of trade-offs. To ensure reduced fouling or predation pressure on the culture activity a concomitant decrease in performance (reduced growth rates) of the culture organism may result. A full analysis of the economic costs and benefits associated with the management of these problems will ultimately dictate the course of action taken.

6.4 References

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7 ToR D: Evaluate the current sustainability of shellfish culture and develop a workplan to improve sustainability

Preliminary remark and WG comments

During its 2003 meeting, the WG established the following workplan:

- 1) Identify potential environmental effects of shellfish culture (2003).
- 2) Prioritize level of environmental concern/benefits related to aquaculture activities (2003/04).
- 3) Identify geographic areas, regions, and culture methodologies and species having the greatest potential for exceeding sustainability based on available information on aquaculture development trends (2005).
- 4) Recommend a general management framework for maintaining sustainable aquaculture (2004/05).

The 2004 report of the WG focuses on the items 1 and 2 of this workplan. It is worth noticing that a considerable amount of time has been given to the definition of shellfish culture sustainability, due to the need to share a common view of the tasks corresponding to this ToR.

7.1 Introduction

There is a need to review all aspects of shellfish production related to the environment and assess how they comply with sustainability requirements (Goals 3 and 4 of ICES Strategic Plan). Based on this evaluation, options to improve sustainability can be identified. In our review, sustainable relates to ecological sustainability only and not economic sustainability. Since the subject is interactions with the environment, only those phases of the culture that are being carried out in the marine environment (spat collection, grow-out and harvesting) are being reviewed here.

There is a large difference between the culture of fish and shellfish. Most farmed fish are carnivorous and food is added to the environment, while shellfish are herbivorous and eat algae and bacteria that are available in the water. Unlike finfish aquaculture, bivalve culture requires minimal additions to the environment, except for the animals themselves and the infrastructures used to grow them. Their food is supplied by the environment and their wastes return nutrients and minerals to the ecosystem. Many cultivated species are indigenous to environment and release reproductive products to the surrounding area. Mollusc culture is therefore much more intricately and inextricably linked to its environment than most finfish culture, including mariculture cages. At present, many shellfish operations worldwide do not show a detectable impact at the current level of production (e.g., Crawford *et al.*, 2003). Nevertheless, there are concerns about potential environmental impacts as culture areas expand and biomass increases. The impacts can be both negative (e.g., removal of food for other organisms and organic enrichment of seabed) and positive (e.g., potential reduction in coastal eutrophication). The degree of environmental impact is expected to differ greatly between culture sites depending on the type and extent of culture activities and local environmental conditions (Chamberlain, *et al.*, 2001). The workplan of the WGMASC includes an initial review of the *potential* interactions between shellfish culture and the marine environment. Following the review, these potential impacts will be prioritized according to the degree of environmental concern in order to identify ways to improve sustainability. Further discussion on priorities and options to maintain sustainability is planned for the next meeting.

Different species of shellfish are cultured in various ways. For example, mussels are cultured on rafts, longlines, Bouchot poles and bottom plots, or oysters are cultured on trestle tables, longlines and bottom plots (Table 1). For these different techniques, different structures are introduced in the marine environment. Harvesting from bottom plots involves diving and dredging, while harvesting from lines does not. Not all species and not all culture types will have the same effect on the environment. The effect of these different culture techniques are outlined below.

7.2 Synthesis of scientific knowledge on the potential interactions of shellfish aquaculture production on the environment

Dense populations of bivalve filter-feeders, including both wild and cultured populations, can potentially modify, maintain and create entire habitats through their effects on suspended particles and the formation of structurally complex shell habitat. Suspended and bottom culture of bivalves increase the surface area available for attachment and grazing by other species, and spaces between shells provide refugia from physical stress (currents and waves) predation, pests and disease. Potential mechanisms for ecosystem level effects include the utilization of particulate food resources (primarily phytoplankton and detritus, but including some auto- and heterotrophic picoplankton and micro zooplankton) by the bivalves and associated epifauna, the subsequent release of unutilized materials in dissolved (ammonia) and particulate (faeces and pseudofaeces) form, and the removal of minerals from the system in the bivalve harvest.

The following is a synthesis of the scientific knowledge of potential environmental controls on aquaculture production. A comprehensive literature review of the current state-of-knowledge on the ecosystem-level impacts of marine bivalve culture was recently published as part of a Fisheries and Oceans Canada technical report (Cranford *et al.*, 2003). This report was attached as Annex 5 of the 2003 WGMASC report and served as a basis for discussion by WGMASC. The report focused on the consequences of aquaculture practices in Canada, and particularly on suspended mussel culture. Several additional potential environmental concerns were discussed by the WGMASC to include aquaculture activities conducted in other ICES nations (e.g., bottom, table, and raft culture).

The culture of bivalve molluscs may involve a number of effects on the current state of coastal marine ecosystems. Important biological processes to be considered include feeding and egestion, the dynamics of the supporting planktonic ecosystem, interactions between pelagic and benthic communities, and exposure to infectious diseases and pathogens. Important chemical considerations include nutrient and contaminant dynamics and chemical transformations mediated by the cultured organisms and various ecosystem components, including bacteria.

The rapid and extensive transformations of water bodies into mussel production could potentially change the ecological function of some bays. Potential impacts (positive and negative) related to intensive bivalve aquaculture may occur at different stages of cultivation. The following lists the environmental concerns identified by the WGMASC and should not be considered comprehensive at the present stage. Considerations of the environmental consequences of diseases associated with shellfish aquaculture were not considered as this is the topic of another working group. These will be expanded at future meetings.

7.2.1 Spat collection stage

- Juvenile collection methodologies may result in environmental effects. Bottom dredging for spat removal can cause positive and negative benthic community impacts. The removal of spat also reduces their availability to predators.²
- Heavy spatfall in regions of bivalve culture causes competition with wild populations while also reducing predation pressure on wild species through the addition of new prey. A positive impact can be recruitment to over fished wild beds.
- Introduced Japanese oysters in SW Netherlands reproduced successfully resulting in the creation of extensive reefs that possibly compete for food and space with wild populations (Kater and Baars, 2003).
- The introduction of shell substrate for settlement and protection of cultured bivalves can have beneficial or negative impacts on benthic communities. The substrate can negatively affect existing species, but it can also increase diversity or recruitment of already present wild species.
- There is a potential that suspended spat collectors in coastal regions may alter local sedimentation patterns.
- Pest species can be introduced to systems during transport of spat between culture sites.
- Generally, the area that is needed for spat collection is much smaller than the grow-out areas. Consequently, the interaction with the environment is smaller as well.

7.2.2 Grow-out stage

7.2.2.1 Potential effects of bivalve filter feeding

- Bivalve filter feeders have a high filtration capacity and high density cultivation may deplete the resident phytoplankton and seston so that they depend on the tidal input of offshore phytoplankton to sustain high density culture.
- The spatial scale of particle depletion is an important issue. Depletion has been documented within culture sites (mussel rafts and long-lines) and there is the potential for interaction between leases in extensively cultured bays. Large scale particle depletion would impact food webs within the system.
- Bivalve aquaculture may help to reduce excess coastal phytoplankton caused by eutrophication through their feeding activities.
- Competition for food between cultured bivalves and zooplankton, and direct ingestion of zooplankton by the bivalves may alter pelagic food webs.
- Bivalve selective feeding behaviour may cause a shift in phytoplankton size spectra.
- Feeding activity could potentially alter marine particle dynamics (i.e., aggregation and sedimentation) by reducing particle concentration or by disrupting flocs.
- Extra grazing on phytoplankton can reduce competition with macroalgae, which may result in the formation of macroalgae blooms (Sfriso & Pavoni, 1994).
- Bivalve filter feeders may be forced, through depletion of important nutritional requirements, to browse on algal species toxic to human health, delaying harvest and increasing pressure within a system

² This subject is also covered by the Benthic Ecology Working Group.

7.2.2.2 Potential effects of biodeposition

- Bivalve faeces and pseudofaeces contain organic matter (15 to 50% organic content) that can cause benthic enrichment effects. The recycling of these organic biodeposits increases the oxygen demand in sediments, potentially generating an anaerobic environment that promotes ammonification and sulfate reduction, leading to alterations in benthic species abundance and community composition.
- By diverting suspended organic matter to the seabed, suspended culture may impact food webs and nutrient dynamics. The rate of nitrogen cycling in coastal regions is increased through the rapid biodeposition of suspended organic matter and the subsequent nutrient regeneration in sediments. A shortened cycle of nutrients between the benthos and phytoplankton can increase local nutrient availability as less material is exported. The increased sedimentation of organic matter through biodeposition therefore can act to retain nutrients in the coastal region. The greater availability of nutrients may lead to enhanced primary production, potentially contributing to more frequent algal blooms, including toxic species.
- The degree of organic enrichment is closely related to hydrodynamics such that the potential for impact is highly site specific. Estuaries identified as having the greatest risk of biodeposition effects are generally shallow, have a relatively small tidal exchange and have a high percentage of the total estuarine volume under culture.
- Oxygen depletion in the water column resulting from the high BOD of biodeposits is generally limited and localized near the seabed.
- Mussel fall-off from suspended culture creates additional organic loading and alterations to benthic communities.

7.2.2.3 Potential effects of excretion

- Shellfish excrete ammonia. The dominant form in which nitrogen is occurring in the marine environment is nitrate. Thus, excretion of ammonia may alter coastal nutrient dynamics. For example, the change in abundance of ammonia may alter phytoplankton species (Philippart *et al.*, 2000). This in turn can have an effect on grazer species composition and abundance. In addition, a change in the ratio of nitrogen to phosphorus may have similar effects. For example, the formation of *Phaeocystis* blooms may depend on this ratio (Riegman *et al.*, 1992). Also, there is much speculation on the contribution of bivalve culture to phytoplankton blooms, including HABs (Parsons *et al.*, 2002; Cloern, 2001).
- Harvesting of nutrients via removal of bivalves may remove nutrients from the coastal system. This method can be used to alleviate the local effects of nutrient addition by fish farms. A number of salmon farms in Scotland and Canada are combining the culture of mussels and salmon on an experimental basis (Robinson *et al.*, 2003; Cook *et al.*, 2003).

7.2.2.4 Other potential effects

- Shellfish culture uses various materials such as ropes, buoys, rafts, poles or shells. In addition, the shellfish themselves are introduced into the marine environment. This material can act as a substrate or refuge for epibionts. Increased shell substrate/refuge for epibionts (fouling organisms and parasites) caused by the introduction of cultured species and associated holding structures will compound any impacts from the bivalves due to the additional food utilization, biodeposition, and excretion. Epibionts or fouling organisms can make up a large part of the biomass of suspended cultures of bivalves. They comprise of a variety of organisms such as macro algae, sponges and ascidians. When filter feeding the epibionts may have effects that are comparable to those of bivalves such as removal of algae, biodeposition, or excretion. In the Thau lagoon (France), ascidians are 5% of the total stock of cultured bivalves (Gangnery, 2003). These ascidians can maximally filter $205 \text{ l d}^{-1}\text{ind}^{-1}$, while oysters can filter $211 \text{ l d}^{-1}\text{ind}^{-1}$ and mussels $162 \text{ l d}^{-1}\text{ind}^{-1}$. Tunicates can retain particles smaller than $2 \mu\text{m}$, which is smaller than what most bivalves can take up, but they also ingest larger particles. Thus, competition for food with the cultured bivalves is possible. The introduction of parasites, such as *Polydora* species, can impact on both food availability, and inadvertent mortality of the cultured stock.
- Diseases are introduced with the movement of aquaculture species, introducing and transmitting pathogens, often resulting in significant mortalities which add to the organic load within a system
- The reef effect caused by the aquaculture structures and animals increases habitat and food availability to some species (positive effect). For example, it is known that Eider ducks feed on ropes of mussel longline culture (Ross, 2000). A higher abundance of crabs and flatfish was observed under rope cultures of mussels. Sea bream feeds on mussels that are cultured in the Mediterranean (Lagardère *et al.*, 2001).
- The potential interactions of shellfish installations with the hydrodynamic conditions may lead to reduced waters currents within the area occupied by shellfish culture, and an increase in the sedimentation beneath the installations. It has been observed that the tables used for the intertidal culture of oyster in France are provoking an increased localised sedimentation underneath the tables (Sornin *et al.*, 1983).

- Predation of cultured bivalves by birds, sea bream, and other predators may also cause increased predation on wild species while having a positive effect on the predators.
- The dedication of certain areas to aquaculture can exclude other activities that may negatively influence the environment (e.g., protection from trawling impacts).
- Hydrocarbons and wastes introduced to the environment from increased boating activity in culture areas can have deleterious effects.
- The structures utilized for bivalve culture are frequently visited by the farmers and this may create some disturbance for birds and marine fauna. These disturbances can even become intentional, to frighten those birds which predate on cultured shellfish.
- Any attempt to assess environmental effects on bivalve aquaculture must consider the complexity of natural and human actions in estuarine and coastal systems. Shellfish responses to multiple stressors (nutritive, contaminant, fishing activities, invasive species, habitat loss, climate change, coastal construction, etc.) are intimately connected. For example, infectious diseases associated with shellfish overstocking, combined with enhanced food limitation and exposure of cultured organisms to "exotic" pathogens introduced with seed or broodstock, can have a significant and frequently permanent impact on shellfish physiological and nutritional status.

7.3 Prioritizing potential environmental interactions

Table 2 summarizes the potential interactions between shellfish culture and the environment identified in Section 7.2 (above). The WGMASC, at the 2003 meeting, began to assign different priority ratings (low, medium, high and unknown) to each interaction to reflect the degree of environmental concern associated with each interaction. At present, the discussion was limited to identifying interactions that presently appear to be of greatest environmental concern. This exercise will be completed at the next meeting of the WGMASC. Reasons for a 'high' rating include considerations of the relative stocking density per unit area (related to the type of culture), the potential for the environment to assimilate the impact, and considerations of the recovery rate. *It should be noted that any actual impact will be site specific and will only occur under specific environmental and culture conditions.*

7.4 Relative impact of different husbandry practices

It is believed that certain culture methodologies and practices potentially have a greater degree of environmental impact than others. Suspended culture of mussels on longlines, rables and rafts permits the holding of large numbers of bivalves per unit area, and this culture method is therefore believed to have the greatest potential for impacts. Even with these types of culture, impacts may not be significant if local hydrographic conditions permit the dispersion of wastes. Table 2 also includes an initial comparison of the degree of environmental interaction associated with different culture activities. This activity is incomplete and the WGMASC work plan for 2004 includes assessing the degree of environmental interactions associated with different types of shellfish culture activities in greater depth. The relative environmental impacts of the culture of traditional and exotic species will be assessed, as will the impact of different culture structures.

7.5 Discussion on sustainability of shellfish culture

After considerable discussion, sustainability was defined by the working group as: "the husbandry and future development of cultured shellfish stocks without compromising the structure and function of the ecosystem". This definition was derived from a scientific perspective (FAO, 1995) for use by the WGMASC and would otherwise have to be refined to include social and economic factors. The WG found this ToR somewhat ambiguous as our definition of sustainability does not allow for multiple levels of sustainability. Therefore, it is difficult to produce a workplan to "improve the sustainability" of shellfish culture. Our interpretation of this ToR is:

- 1) to assess whether or not shellfish aquaculture is currently conducted in a sustainable manner, and
- 2) to provide recommendations on how future developments can be conducted without compromising sustainability.

The level of concern regarding aquaculture sustainability is related to the rate of development. Aquaculture production trends vary by geographic region and by species. Evaluation of the current sustainability of shellfish culture therefore requires an assessment of cultured shellfish production through time for each of the ICES countries and regions within each country. Specific data on production trends by country, region, and species are available. Production data need to be acquired for the next WG meeting, with the plan of identifying specific species and areas of concern.

The WGMASC cannot state what impacts are acceptable. There are valid socioeconomic aspects to the sustainability question that cannot be addressed by scientists alone. Major roles of science are to advise on the potential consequences associated with aquaculture interactions with the environment, and to make recommendations towards the development of approaches for managing cultured shellfish stocks in a sustainable manner. Given the direct relation known to exist between the financial sustainability of the shellfish aquaculture industry and the ecological sustainability

of coastal systems, environmental considerations need to be incorporated within management and development plans for shellfish aquaculture.

Environmental effects monitoring (EEM) is an integral component of environmental management and protection frameworks developed for other industries and are designed to minimise the consequences of our actions. The evaluation of EEM results advances our understanding of the level of impact of the aquaculture industry, and this contributes to improvements in husbandry practices, mitigation measures, and the revision of monitoring and management plans. Environmental management frameworks consist of a linked series of processes that are developed to address predictions of significant potential environmental effects. Predictions are inherently subject to some degree of uncertainty as a result of gaps in knowledge and the influences of unforeseen factors. Monitoring programs address this uncertainty by ensuring that actual effects do not exceed the predictions and are within predetermined acceptable limits. Management frameworks consist of a feedback-loop from prediction to monitoring to management response and also include additional feedbacks between regulations, scientific research and public perceptions. It is generally accepted that management responses to monitoring should be timely and follow a predetermined course of action in the event that measured environmental changes exceed predefined acceptable limits.

The WGMASC workplan includes an examination of available environmental management and protection frameworks and methodologies available for addressing potential impacts identified as having an enhanced level of concern (Sections 3 and 4 above). The workplan includes: identification and evaluation of impact mitigation measures, incentives that improve culture and environmental performance, the identification of environmental performance indicators and reference points, and the development of decision-support systems.

A preliminary recommendation is that monitoring programs could ideally follow a tiered approach that recognizes that increased risk requires an increase in monitoring effort. Various levels of monitoring could be triggered based on the perceived environmental sensitivity (e.g., presence of valued ecosystem components) and risk (e.g., dispersive vs. depositional environment), the nature of the operation (size and type), and the measurement and verification of environmental impacts.

7.6 References

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Table 1. Overview of different methods used for the cultivation of different shellfish species groups in different culture phases.

Phase	Method	Shellfish species group			
		Mussels	Oysters	Clams	Scallops
Spat collection	Bottom dredging	X			
	Use of shell substrate		X		X
	Use of artificial collectors	X	X	X	X
	Hatchery produced	X	X	X	X
Nursery	Subtidal floating cages		X	X	X
	Bottom cages		X	X	X
	Bottom plots			X	
Grow out	Subtidal rafts/tables	X	X		
	Subtidal longlines	X	X		X
	Subtidal nets/cages		X	X	X
	Subtidal plots	X	X	X	X
	Intertidal plots	X	X	X	X
	Intertidal tables		X		
	Intertidal poles	X			
Harvesting	Stripping ropes, poles	X	X		
	Subtidal and intertidal dredging	X	X	X	
	Intertidal digging			X	
	Removing bags, nets/cages, diving		X	X	X

Table 2. Classification of the interactions between shellfish culture and the marine environment. Effects will be classified as low, medium, high, or not applicable (n.a.). In situations where appropriate the activity was considered according to high (h) and low (l) energy levels.

Type of culture →	Suspended			Bottom			
	Subtidal rafts/tables	Subtidal longlines	Subtidal nets/cages	Subtidal plots/beds	Intertidal plots/beds	Intertidal tables with bags, cages	Intertidal poles
Interaction ↓							
Dredging for spat	n.a.	n.a.	n.a.	Low-h Med-l	Medium	n.a.	n.a.
Seeding of shells for spat collection	n.a.	n.a.	n.a.	Low	n.a.	n.a.	n.a.
Placement of spat collectors	Low	Low	Low	n.a.	Low	n.a.	Low
Removal of collectors	n.a.	n.a.	n.a.	n.a.	Low	n.a.	n.a.
Filter feeding: algal consumption and zooplankton predation	Medium	Low-h High-l	Medium	Low	Low	Medium	Medium
Filter feeding: nutrient removal	Low	Low	Low	Low	Low	Low	Low
Excretion of nutrients	Medium	Medium	Low	Low	Low	Low	Low
Deposition of (pseudo)faecal pellets	Med-h High-l	Med-h High-l	Low-h Med-l	Low	Low	Low	Low
Changed current, sedimentation	Medium	Low-h Med-l	Low-h Med-l	Low	Low-h Med-l	Low-h Med-l	Medium
Increased substrate for epibionts	High	High	High	Low	Low	Medium	Medium
Increased refugia	High	High	High	Low	Medium	Medium	Medium
Increased predation	Low	Medium	Low	Medium	Medium	Low	Medium
Competition for space	Low	Low	Low	High	High	Low	Low
Pollution	Low	Low	Low	Low	Low	Low	Low
Stripping ropes and poles	Low	Low	n.a.	n.a.	n.a.	n.a.	low
Dredging	n.a.	n.a.	n.a.	High	High	n.a.	n.a.
Intertidal digging	n.a.	n.a.	n.a.	n.a.	Medium	n.a.	n.a.
Diving	n.a.	n.a.	n.a.	Low	n.a.	n.a.	n.a.
Removing bags, nets, cages	n.a.	n.a.	Low	n.a.	n.a.	Low	n.a.

Table 3. Identifying positive effects associated with shellfish aquaculture activities such that ecosystem structure and function is enhanced.

Activity	Description of Benefit
Dredging for spat	Reducing competition for space and food in existing seed beds; Improved cohorts and overall population in growout areas
Seeding of shells for spat collection	Increase habitat complexity; increased population of target species and other species (near and far field impacts)
Placement of spat collectors	Enhance larval survival and recruitment success of target and other species
Removal of collectors	Removal of potential competing individuals (for food resource)
Filter feeding: algal consumption and zooplankton consumption	Additional research required
Filter feeding: nutrient removal	Additional research required
Excretion of nutrients	Additional research required
Deposition of (pseudo-)faecal pellets	Low/moderate deposition provides energy to the system resulting higher production and the potential for increased biodiversity
Changed current, sedimentation	Increased food supply to benthos and biodiversity
Increased substrate for epibionts	Higher community production and the potential for increased biodiversity
Increased shelter	Habitat complexity and extent increased resulting in higher biodiversity and biomass
Increased food availability	Increase in predatory organisms
Increased predation	Increase food-web complexity
Competition for space	None
Pollution	None No aquaculture activity, allowing biodiversity within the ecosystem.
Stripping ropes and poles	Create habitat for fouling organisms
Dredging	Reducing competition for space and food in existing beds and potentially enhancing spat settlement. Increases food supply for predators. Increases benthic-pelagic exchange.
Intertidal digging	Reducing competition for space and food in existing beds and potentially enhancing spat settlement. Increases water porosity in sediments that encourages burrowing.
Diving	None
Removing bags, nets, cages	Fallowing allows natural recovery of an area (breaking disease cycle)

8 MCC Sessions at the Annual Science Conference in Vigo (spain)

Three sessions are convened under the Mariculture Committee (MCC): Towards Sustainable Aquaculture (co-conveners H. Ackefors, P Kamermans and J.Doyle), Shellfish Culture in the ICES Area: Perspectives and Limitations (co-conveners A. Smaal and A. Bodoy), Mariculture in Integrated Coastal Zone Management (co-conveners E. Black and J. Stotrup). Another session is of interest to the WGMASC: Life history, Dynamics and Exploitation of Marine Living Resources (co-conveners: O. Kjesbu, P. Kamermans, and I. Boyd). Information was distributed previously at the previous WGMASC meeting. Members of the WG are encouraged to participate in the Annual Science Conference and attend these sessions.

9 Recommendations to MCC

Recommendations on ToR A (hatcheries for shellfish production)

The survey conducted on shellfish hatcheries has been restricted to the ICES waters. These are all waters connected to the North Atlantic Ocean. However, we believe it is important to expand the survey to other parts of the world where species of interest for ICES members are cultured in hatcheries, such as the Pacific coast of North America and the Mediterranean. In this way, a complete overview of existing expertise and technology is achieved.

Most of the information not available is associated with countries that have yet to designate members to the WGMASC. We encourage those countries to participate in these activities.

Recommendations on ToR B (the literature on stress indices)

Research is needed to identify and test stress indicators of shellfish to evaluate: 1) the commonality of some metrics among phyla; 2) the cause-effect relationship between stressor and the indicator; 3) the scale of temporal and spatial variability of the indices.

Closer relationships should be created with other working groups (see above) on this issue as it spans several TORs. Discussions should be initiated to provide a more comprehensive review on the subject as well as the scope of application for the technique.

Recommendations on ToR C (ecological factors affecting shellfish production)

Research is needed on indicators of carrying capacity and standardization of methodologies, including identification of bivalve performance measures.

To enhance the chapter on predation and fouling, a comprehensive literature review of regional problems regarding predation and fouling should be conducted.

10 Draft resolutions (Terms of Reference for 2005)

The **Working Group on Marine Shellfish Culture** [WGMASC] (Chair: A. Bodoy, France) will meet in La Rochelle (France) from 13–15 May 2005 to:

- a) Update the synthesis and prepare a publication on the development of shellfish hatcheries within ICES countries. This will examine the technical infrastructure and methods (water treatment, broodstock conditioning, feeding schedules, etc.) of the different hatcheries, the proportion of cultured animals to wild conspecifics being used as broodstock and the application of genetic tools (e.g., triploids) to develop hatchery strains.
- b) Prepare a state of knowledge report comparing and contrasting the standard methods used to measure stress indicators in shellfish and provide a discussion of how they would be used to diagnose incidents of cultured shellfish mortality.
- c) Access and critique the standard methodologies used to estimate shellfish performance indices as related to examining the carrying capacity of the growing area. This will include a review of the effects of HABS, disease and pollution in relation to the performance and carrying capacity of shellfish culture.

WGMASC will report by 1 June 2005 for the attention of the Mariculture and Living Resources Committees and ACME.

Supporting Information

Priority :	WGMASC is of fundamental importance to ICES environmental science and advisory process and addresses specific issues of the ICES Strategic Plan.
Scientific justification and relation to Action Plan:	<p>Action Plan references:</p> <ol style="list-style-type: none"> a)- 1.3, 2.4, 2.5, 2.6, 3.9, 6.3 b)- 1.10, 3.10, 3.14, 4.6, 6.3 c)- 2.12, 3.11, 4.7 <p>a) Shellfish hatcheries are essential for the genetic improvement of broodstock and for securing adequate quantities of high quality spat (juvenile shellfish) for industry grow-out. There is no centralized information base documenting the use of different species by different countries. Current research identifying broodstock sources, broodstock conditioning (for reproduction) techniques, larval rearing and spat settlement for hatchery-reared species needs to be reviewed, synthesized and documented (in collaboration with WGAGFM). Information will be gathered with a 'survey questionnaire' of commercial shellfish hatcheries. Participants will also be asked to identify their R&D priorities.</p> <p>b) Shellfish production is based on the use of the natural productivity of coastal waters as a source of food. Shellfish require a clean environment and is, in essence, integrating the environmental conditions of the growing area. Unexplained mortalities have an immediate negative effect on both producers and market share. These can be caused by predation, HAB, diseases, pollution, and water quality or any combination thereof. Stress in animals can be caused by either chronic or acute extrinsic factors that may act to decrease the fitness of the animal or to cause its premature death. Use will be made of the biological effects of contaminants literature that is available within ICES. Chronic factors will often be manifested by changes in the overall health of the animal (i.e., in a stepwise cellular-tissue-organ-physiological levels). Acute factors produce immediate responses that may only show up at the molecular/cellular levels. Therefore, detecting acute effects is usually done with biochemical techniques. Different bio-indices will be reviewed and their usefulness determined: adenylate energy charge (AEC), heat shock proteins, total oxy-radical scavenging capacity (TOSC), glycogen content, metallothioneins (MT), cytochrome P4501A1 activity, DNA damage, total lipids and histology.</p> <p>c) Despite the extensive progress made in salmon culture in ICES Member Countries, shellfish production still accounts for half of the mariculture production. As such, issues related to shellfish production, in relation to the environment and technological development of the industry need to be addressed within ICES. The effects of the</p>

	environmental perturbations on shellfish production are dramatic and sudden. There is a need to identify the causes of potential impacts and their effects on the production of shellfish, in order to establish the requirements for the industry in terms of water quality, ecological perturbations and biological competitors. This should be prepared with the view of establishing Ecological Quality Objectives (EcoQOs) regarding the shellfish activity. The information will also be useful for determining the ecological requirements for shellfish production in coastal areas. The Working Group has given priorities to the different impacts : 1- Carrying capacity (Hydrographic factors, Primary productivity, food supply), 2- Predation, 3- fouling, 4- HAB Blooms,5- Disease, 6- Pollution (water quality).
Resources requirements :	None required other than those provided by the host institute.
Participants :	Identification of membership from ICES countries and invited participants from shellfish hatcheries
Secretariat facilities :	None required
Financial :	N/A
Linkage to advisory Committee :	ACME
Linkage to other Committees or groups :	MARC, MHC, WGHAB, WGITMO, WGPDMO, WGAGFM
Linkage to other organisations :	European Aquaculture Society, World Aquaculture Society

11 Closing of the meeting

The 2004 meeting of WGMASC in Portland, Maine (USA) was formally closed at 5 pm, 15 May 2004.

12 Annexes

Annex 1 List of Participants

Names	Addressee	Phone	Fax	E-mail
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Annex 2 Meeting agenda

Thursday, 13 May 2004

- 9:30 Welcome of the participants by Chair
- Plenary session:
- General subjects
- Comments on the Terms of reference
- 11:00 Health Break
- 11:30 Plenary session
- discussion on the terms of reference
- Adoption of agenda and subgroups organisation
- 12:15 Lunch break
- 13:00 Subgroup ToR A, ToR B and ToR C
- 16:30 Health Break
- 17:00 Subgroup ToR A, ToR B and ToR C
- 18:30 End of session

Friday, 14 May 2004

- 9:00 Subgroup ToR A, ToR B and ToR C
- 10:30 Health Break
- 11:00 Subgroup ToR A, ToR B and ToR C
- 13:00 Lunch Break
- 14:00 Plenary session
- exchanges on the subgroups reports
- 16:00 Health Break
- 16:30 Subgroup ToR A, ToR B and ToR C
- 18:30 End of session

Saturday, 15 May 2004

- 9:00 Subgroup ToR D
- 11:00 Health Break
- 11:30 Subgroup ToR D
- 13:00 Lunch break
- 14:00 Subgroup ToR D
- 15:30 Health Break
- 16:00 Plenary session

- Approval of the recommendations
- Proposal of ToRs for 2005
- Meeting location in 2005

16:30 Any other business, Adoption of the report

18:00 Meeting Adjournment

Sunday, 16 May 2004

9:30 Departure for a field trip (oyster farm)

14:00 Return downtown

Annex 3 New model of form proposed for use with hatcheries and representatives within ICES Countries



The Fish Health Inspectorate
Fisheries Research Services
Marine Laboratory
PO Box 101
375 Victoria Road
Aberdeen
AB11 9DB

Dear Sir/Madam

ANNUAL RETURN OF SHELLFISH FARM PRODUCTION-2004

As part of an ICES Working Group on Marine Shellfish Culture, I seek data for a report on the Production and Development of Shellfish Hatcheries in the UK.

Attached are forms requesting information on your shellfish hatchery enterprise(s), please return completed forms by email to fraserdi@marlab.ac.uk. Guidance notes are included.

A national and international summary of returns will be produced, and presented in such a way to maintain confidentiality of individual businesses.

Our aim is to produce a report on the activities and outputs of hatcheries within ICES countries, which is both useful and relevant to the aquaculture industry, highlighting environmental issues and areas of priority for research and development.

If you have any concerns regarding the completion of the survey form, please do not hesitate to contact me on 01224 295698.

Thank you for your co-operation.

Yours Sincerely,

David Fraser

PROJECT

**ICES- QUESTIONNAIRE ON THE PRODUCTION AND DEVELOPMENT
OF SHELLFISH HATCHERIES**

GUIDANCE NOTES

Q1. Please enter the site name and a short description of the site location.

Q2. a) Please enter the number of full time (FT) and part time/seasonal (PT) staff.
b) Do you have a quarantine facility, if so indicate purpose, e.g., holding broodstock, quarantine of imports
c) Indicate i. the type of water supply, i.e., estuarine, pump ashore, artificial sea water.
ii. if the water supply is treated prior to use, e.g., filtered, ozone, disinfected.
d) Do you treat effluent water (Y/N), what is used, e.g., filtered, ozone, disinfected.

Q3. Please indicate a) the number of individuals produced,
b) the stage (spat, larvae, etc),
c) whether the site is a hatchery or a nursery (H/N).

Please also indicate d) any mortality (estimated annual %),
e) the causes of mortality,
f) any treatments.

Q4. Please enter a) the country of origin of each broodstock species and indicate whether it is wild stock, hatchery stock, or hybrid,
b) the criteria used for species/strain/origin selection, i.e., disease resistance, fast growth rate, high survival, morphological characteristics.

Please also indicate c) conditioning method, e.g., temperature, chemical,
d) if triploidy is employed (Y/N).

Q5. Please enter larval rearing systems employed (e.g., static, flow-through, conical vs. flat-bottom tanks, high aeration vs. low aeration, etc.), and problems encountered during larval rearing.

Q5. Please enter techniques and problems encountered during spat settlement.

Q7. Which microalgal species are used to first feed and ongrow larvae and spat and how you produce your cultured algae (batch, continuous, other).

Q8. Please indicate the destination of spat, please name country of destination

Q9. Please highlight any Research & Development concern, issues or requirements on the reverse on the form.

IN CONFIDENCE

**ICES- WORKING GROUP ON MARINE SHELLFISH CULTURE
FISHERIES RESEARCH SERVICES**

**QUESTIONNAIRE ON THE PRODUCTION AND DEVELOPMENT
OF SHELLFISH HATCHERIES**

1. IDENTITY OF HATCHERY/NURSERY

Site Name	Site Location

2. HATCHERY INFRASTRUCTURE

No. Employees	Quarantine Fac.	Water Supply	Water Treatment	Effluent treatment
FT- PT-				

3. SPECIES PRODUCED - Estimated production for 2004

Species	Number	Stage	Hatchery or Nursery	% Mortality	Cause of mortality	Treatment
Mussels- <i>M. edulis</i>						
Pacific oysters- <i>C. gigas</i>						
Native oysters- <i>O. edulis</i>						
Scallops- <i>P. maximus</i>						
Queens- <i>C. opercularis</i>						
Venus clams- <i>M. mercenaria</i>						
Short-necked clam- <i>P. philippinarum</i>						
Calico clam- <i>R. decussatus</i>						
Other- Specify						

4. BROODSTOCK

Species	Origin	Selection Criteria	Conditioning Method	Triploid
Mussels- <i>M. edulis</i>				
Pacific oysters- <i>C. gigas</i>				
Native oysters- <i>O. edulis</i>				
Scallops- <i>P. maximus</i>				
Queens- <i>C. opercularis</i>				
Venus clams- <i>M. mercenaria</i>				
Short-necked clam- <i>P. philippinarum</i>				
Calico clam- <i>R. decussatus</i>				
Other- Specify				

5. LARVAL REARING

a. Please outline all larval rearing systems employed

b. Please outline any problems encountered during larval rearing

6. SPAT SETTLEMENT

a. Please outline all techniques used for spat settlement

b. Please outline any problems encountered during spat

7. MICROALGAE SPECIES AND REARING TECHNIQUES

Which microalgal species are used to first feed and ongrow larvae? How are microalgae reared?

8. DESTINATION OF SPAT

Species	Destination

9. RESEARCH & DEVELOPMENT CONCERNS/ISSUES/ REQUIREMENTS