

COOPERATIVE RESEARCH REPORT

NO. 159

*CODES OF PRACTICE AND MANUAL OF PROCEDURES FOR CONSIDERATION OF
INTRODUCTIONS AND TRANSFERS OF MARINE AND FRESHWATER ORGANISMS*

Prepared by the "Working Group on Introductions and
Transfers of Marine and Freshwater Organisms" of the
International Council for the Exploration of the Sea
and by the "Working Party on Introductions of the
European Inland Fisheries Advisory Commission"

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PREFACE

Introductions or transfers of marine and freshwater organisms in support of aquaculture or various fishing initiatives have been increasing in numbers quite rapidly in recent years. This document, prepared as a more detailed follow-up to the "Codes of Practice" related to these movements which were prepared by the International Council for the Exploration of the Sea (ICES) and the European Inland Fisheries Advisory Commission (EIFAC), addresses some of the concerns and provides advice related to proposed introductions or transfers. Areas covered are inspection and certification, quarantine, pathology, genetics and ecology. Universal concerns in the above mentioned areas which are common to any introduction or transfer are outlined, as are those related to importations or other movements which are part of established commercial practice or those related to scientific study at research facilities. Specific examples of protocols, mainly related to controlling disease organism spread, are included as are items related to the methods of handling requests for introductions either at the national or international level.

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1. INTRODUCTION

There are long-standing concerns in countries throughout the world regarding the effects of the introduction of non-indigenous aquatic species into oceans, lakes, rivers and estuaries. Many country's concerns have compounded within their jurisdiction with the rapid growth of aquaculture in recent years.

Some countries, through national and international agencies, are involved in reviewing policies and procedures related to proposed introductions of exotic (non-indigenous) species, as well as transfers within and between countries. Examples of groups concerned about exotic species introductions include: the International Council for the Exploration of the Sea (ICES), the European Inland Fisheries Advisory Commission (EIFAC), and the American Fisheries Society (AFS). Countries in both Europe and North America have also individually spent considerable time addressing this issue.

At its Statutory Meeting in 1973, ICES adopted a "Code of Practice to Reduce the Risks of Adverse Effects Arising from Introduction of Non-indigenous Marine Species." Regulatory agencies of all member countries have been encouraged to use the strongest possible measures to prevent unauthorized or unapproved introductions. At its Statutory Meeting in 1979 the Council adopted a revised Code as follows (ICES Cooperative Research Report No. 130, 1984):

1. Recommended procedure for all species prior to reaching a decision regarding new introductions (A recommended procedure for introduced or transferred species which are part of current commercial practice is given in Section 4).
 - (a) Member countries contemplating any new introduction should be requested to present to the Council, at an early stage, information on the species, stage in the life cycle, area of origin, proposed place of introduction and objectives, with such information on its habitat, epifauna, associated organisms, potential competition to species in the new environment etc., as is available. The Council should then consider the possible outcome of the introduction and offer advice on the acceptability of the choice.
 - (b) Appropriate authorities of the importing country (including fishery management authorities) should examine each "candidate for admission" in its natural environment to assess; the justification for the introduction, its relationship with other members of the ecosystem, and the role played by parasites and diseases.
 - (c) The probable effects of an introduced species in the new area should be assessed carefully, including examination of the effects of any previous introductions of this or similar species in other areas.
 - (d) Results of (b) and (c) should be communicated to the Council for evaluation and comment.

2. If the decision is taken to proceed with the introduction, the following action is recommended:

- (a) A broodstock should be established in an approved quarantine situation. The first generation progeny of the introduced species can be transplanted to the natural environment if no diseases or parasites become evident, but not the original import. The quarantine period will be used to provide opportunity for observation for disease and parasites. In the case of fish, broodstock should be developed from stocks imported as eggs preferably or juveniles to allow sufficient time for observation in quarantine.
 - (b) All effluents from hatcheries or quarantine establishments should be sterilized in an approved manner which should include the killing of all living organisms present in the effluents.
 - (c) A continuing study should be made of the introduced species in its new environment, and progress reports should be submitted to the International Council for the Exploration of the Sea.
3. Regulatory agencies of all member countries are encouraged to use the strongest possible measures to prevent unauthorized or unapproved introductions.
4. Recommended procedure for introduced or transferred species which are part of current commercial practice.
- (a) Periodic inspection (including examination by microscopic techniques) by the receiving country of material prior to mass transplantation to confirm freedom from introducible pests and diseases. If inspection reveals any undesirable development, importation must be immediately discontinued. Findings and remedial actions should be reported to the International Council for the Exploration of the Sea.
 - (b) Inspection and control of each consignment on arrival.
 - (c) Quarantining and disinfection whenever possible and where appropriate.
 - (d) Establishment of broodstock certified free of specific pathogens.

It is appreciated that countries will have different attitudes toward the selection of the place of inspection and control of the consignment, either in the country of origin or in the country of receipt.

At its fourteenth session held in Bordeaux, France, from 27 May to 3 June 1987, EIFAC endorsed the efforts of the first session of its Working Party on Introductions and of the joint meeting of the EIFAC Working Party on Introductions with the ICES Working Group on Introductions and Transfers of Marine Organisms (see paragraphs 58 to 60 of the report of the fourteenth session published as FAO Fisheries Report No. 364). The report of the EIFAC Working Party on Introductions includes, as its Annex E, the following revised Code of Practice, based upon the ICES Code of Practice, to reduce the risk of adverse effects arising from the introduction or transfer of inland aquatic organisms:

(a) Recommended procedure prior to reaching a decision regarding proposed introductions (This procedure does not apply to introductions or transfers which are part of current commercial practice).

- (i) Member countries contemplating any introduction should be requested to present to EIFAC at an early stage information on the species, area of origin, proposed place of introduction and objectives, with such information on its habitat, associated organisms etc., as is available. EIFAC should then consider the possible outcome of the introduction and offer advice whether to proceed with further evaluation.
- (ii) Appropriate authorities of the importing country should examine each "candidate for admission" to assess the justification for the introduction, its relationship with other members of the ecosystem, details of its biology and ecology and the possibility of introducing associated pathogenic organisms and parasites.
- (iii) The probable effects of introduction into the new area should be assessed carefully, including an examination of the effects of any previous introductions of this or similar species in other areas, and a prediction of the final range of the species assuming it could form breeding populations in natural waters.
- (iv) The above procedures (i, ii, iii) should be carried out by following the Review and Decision Model as set out in a subsequent section.

(b) Recommended action If the decision is taken to proceed with the introduction, the following action is recommended:

- (i) A brood stock should be established in an approved quarantine situation. Brood stocks should be developed from stocks imported as eggs, in order to minimize the possibility of contamination by pathogenic organisms, parasites or by other species of fish. All effluents from establishments used for quarantine purposes should be sterilized in an approved manner.
- (ii) If no communicable pathogenic organisms including parasites become evident, the first generation progeny, but not the original import, of the introduced species can be transplanted to culture sites or to the natural environment, preferably to small, isolated and restricted river basins or lakes.

(c) Recommended actions after introduction

- (i) A continuing study should be made of the introduced species in its new environment and progress reports submitted to EIFAC.
- (ii) Every effort should be made to contain the species within the water bodies or water courses into which introduction was made.

(d) Regulatory agencies of all member countries are encouraged to use the strongest possible measures to prevent unauthorized or unapproved introductions and transfers.

(e) Recommended procedure for introductions or transfers which are part of current commercial practice (The procedures laid down by the "Draft Convention to prevent the spread of major communicable fish diseases" should be adhered to, especially:)

- (i) Periodic inspection (including adequate microscopic and microbiological examinations) by the receiving country of material for prior mass transplantation to confirm freedom from introducable pests and diseases. If inspection reveals any undesirable development, importation must be immediately discontinued. Findings and remedial actions should be reported to EIFAC.
- (ii) Inspection and control of each consignment on arrival.
- (iii) Quarantining or disinfection where appropriate.
- (iv) Establishment of brood stocks certified free of specified pathogens.

While these codes give a broad direction towards control of introductions and transfers, there is still a need for more specific instructions on their implementation, i.e., a checklist of procedures to be followed for consideration of an introduction and follow-up instructions should one be approved. This document has been prepared to provide initial direction in areas such as: ecology, genetics, inspection and certification, quarantine and pathology.

Although there will continue to be problems related to importations, particularly in the areas of genetics and ecology, this document introduces methods of handling matters related to consideration, approval and monitoring of introductions or transfers.

The protocols put forward herein address first the broad administrative procedures required within countries to handle requests for introductions or transfers. Then, common or "universal" protocols which should be carried out when contemplating these movements for commercial purposes are dealt with, followed by those for importations to support established commercial practice and those solely for scientific study at research establishments. In Appendix I, examples of procedures in various stages of development now being utilized to handle selected species group movements are presented. These examples demonstrate how varied the control procedures are at this time.

Additionally, a "Review and Decision Model for Evaluating Proposed Introductions of Aquatic Organisms into and within Europe" is reprinted in Appendix II from EIFAC Technical Paper 44, to provide discussion on and to evaluate introduction or transfer requests.

2. CONSIDERATION OF REQUESTS FOR INTRODUCTIONS OR TRANSFERS AT THE NATIONAL LEVEL

Any country dealing with or contemplating introductions or transfers of aquatic organisms (marine or freshwater) between countries or within national boundaries should have or enact legislation for regulating such activity. A national coordination/consultation mechanism to review, recommend on and monitor (when approval is granted) any such activity should be established to advise administrators on the use of the regulating legislation.

This mechanism in the form of a national committee or working group, would ensure that all prospective applicants, private or government, wanting to introduce or transfer species would submit properly prepared requests to be vetted for acceptability. Such a committee or working group could actually be duplicated on a regional basis within a country to reflect different environmental conditions. Continuity on the national scene could be addressed by regions periodically meeting to discuss common goals and regional concerns and through adoption of ICES/EIFAC "codes of practice". Appropriate regulations would be required to ensure compliance with approved protocols should permission be granted. Potential applicants would be made aware of the regulations through a national education campaign.

Where an introduction into one country may adversely affect another, the national committee or working group on introductions and transfers should forward the application to ICES for a marine species (including anadromous species) or to EIFAC for a freshwater aquatic species, for a risk analysis and recommendations prior to making a decision on an application (Appendix III, A and B).

3. UNIVERSAL PROTOCOL FOR NEW* INTRODUCTIONS OR TRANSFERS FOR COMMERCIAL PURPOSES

3.1 Ecology

For the purpose of this document, "ecology" is defined as the study of the interrelations of aquatic plants or animals with their environment. The "environment" is everything that may influence an organism's chance to survive and multiply (i.e., physical/chemical characteristics of water, food, other organisms, habitat etc.)

Throughout the section, no distinction is made between an aquatic organism proposed for introduction to a natural ecosystem (release to the wild) or one proposed for other purposes, such as enclosures. It is assumed that escape is an inevitable consequence of most applications, thus presenting the same potential problems as a release to the wild.

An aquatic introduction or transfer brings with it the possibility of effecting a variety of ecological changes (good or bad), directly or indirectly, on indigenous species in the target area. No group of biologists, ecologists or geneticists is able to predict with certainty the results of an introduction of a foreign organism. It may be that the behaviour of an organism, although well known in its native habitat, will be considerably different in a new habitat. Once introduced, a new species may be difficult if not impossible to eradicate, if it is later found in the short or long term to be undesirable.

Because of the ecological complexity related to introducing or transferring a marine or freshwater organism and because it is unlikely that adequate information will be available from the literature on which to base an assessment of likely

* Protocols for handling introductions which are part of established commercial practices are described in Section 4. Countries may choose to classify movements of transfers which are not part of current commercial practice and which involve transfers of identifiably separate stocks or races of a species to areas outside their natural geographic range, as new introductions.

interrelations with indigenous species, it is important that a thorough examination of any proposal be conducted. This would include:

3.1.1 Examination of the physical and chemical characteristics of the native environment of the introduced species, as well as the new environment to which the species will be introduced.

3.1.2 A full biological analysis of the aquatic organism proposed for introduction, both within its natural range and where previous transplants have taken place (if applicable). This review should cover, but is not restricted to, the following aspects:

- (a) feeding habits and food organisms utilized
- (b) reproductive strategy (when, where, how)
- (c) competition with other species
- (d) predation by or on the species
- (e) migration routes and timing (if applicable)
- (f) disease history

3.1.3 Assessment of the new location for the species with respect to indigenous species likely to interact with introduced species, and with respect to the likelihood of the species establishing wild reproducing stocks.

3.1.4 Examination of potential implications of any new fishery which may develop for the introduced species in relation to harvesting of indigenous species (fishing pressure).

3.1.5 Examination of control methods to prevent overpopulation by the introduced species or even for its total eradication if necessary.

3.1.6 Discussion of possibilities for phasing in introduction through an initial controlled planting to study trophic interaction with indigenous fauna, followed by a full-scale introduction should no problems be encountered.

3.1.7 Long-term monitoring of the dynamics of a species in its new environment to ensure indigenous fauna is not affected detrimentally. This monitoring should utilize baseline data collected in the pre-release period as a reference point on which comparisons can be made.

3.2 Genetics

The genetic implications of introducing or transferring aquatic organisms to a new environment are complex and poorly understood, both with respect to their effects on the organisms imported and on the resident species. It has been shown from animal and crop breeding work that manipulation to adapt species to new environments and to produce certain desirable characteristics results in the "narrowing in the genetic base of the species" (FAO Technical Paper #217, 1982). In the process of this manipulation, genetic determinants controlling disease resistance and fitness in marginal environments may be lost at an early stage.

Warnings from biologists, geneticists, and ecologists state that genetic diversity is essential for the preservation of a species and that importation of new species or strains of aquatic organisms could alter this diversity. The natural gene pool of a stock or species may be directly altered by the new genes,

if interbreeding occurs, or indirectly through modification of the physical or biological environment of resident species by the import.

Utter 1981, in discussing distinct salmonid populations states, "In addition, transplantations interfere with heritable patterns of homing precision (Ricker 1972) and further complicate innate differences among species in the formation of discrete population groupings".

A conservative attitude towards introductions and transfers has been advocated by the ICES Working Group on Genetics at their 1985 meeting (C.M. 1985/F:59/Sess. T) including the suggestion that only non-breeding or sterile individuals be deliberately released. This approach would avoid potential reduced fitness and introgression of genes from such stocks on indigenous species.

The following recommendations are aimed at decreasing the risks of genetic disruption resulting from the introduction or transfer of exotic species or strains:

3.2.1 Carry out a thorough risk analysis (genetic, ecological etc.) prior to any introduction. This should include the accumulation of background data related to the history of the species to be introduced in its "home" waters and in any areas where it may already have been introduced. Biological life-history data and, where possible, genetic data in the form of protein electrophoretic analysis, DNA fingerprinting etc., should be collected.

3.2.2 When the time comes to stock out in the wild or in cages, after quarantine, utilize only non-breeding or sterile individuals if possible.

3.2.3 Protect and preserve, at the earliest possible stage, the broad genetic diversity present within the indigenous aquatic organisms most threatened by an importation, by establishing "reserves" (areas where introductions or transfers are forbidden), by artificially maintaining unselected populations or by cryopreservation of gametes or embryos.

3.2.4 Ensure that stock to be introduced for stock rehabilitation purposes or sea ranching is selected from an environmentally similar area (ideally geographically adjacent) and that it is selected to occupy a niche not already filled by an indigenous species or to fill a niche no longer filled because of the extinction of a stock.

3.2.5 Introduce small numbers, under closely controlled conditions, in stages, in order to monitor genetic effects on indigenous species. In this manner, an introduction could be curtailed immediately if adverse effects appeared.

3.2.6 Evaluate thoroughly, before introduction to enclosures or to areas for sea ranching, any genetically engineered aquatic organisms, to determine their possible effects on indigenous species. New genetic methods are being exploited in aquaculture to produce fish or other aquatic organisms with modified genomes (e.g., by selection, polyploidy, gynogenesis, gene transfers etc.). Although this can confer considerable advantage in aquaculture, it could have a detrimental effect on natural populations through competition or because of introgression of the novel genes into wild genomes.

3.2.7 Ensure that national legislation to control movements (introductions or transfers) are constantly updated to cover new technical breakthroughs. For example, recent developments in methods for the storage and transport of chilled and frozen gametes and embryos can facilitate introductions and transfers and many national legislative controls do not cover movements of unfertilized gametes.

3.3 Inspection and Certification

3.3.1 Each country should prepare a list of species eligible for introduction or transfer (possibly done by the national committee or working group set up to coordinate introductions). Experience obtained from previous introductions should be used to update these lists annually, and they should be made available to inspection staff involved in actual examination and certification of import.

3.3.2 A list of known parasites and diseases of eligible species should also be compiled and periodically updated. The list would be used by inspection staff when examining certification papers and actual shipments as they arrive in the country.

3.3.3 Once approval for an importation (for quarantine and testing) has been given, the agency or group making the request, must obtain from the government of the country from which the species originates, a certificate (permit) confirming the origin of the stock, stage to be exported, disease history (as far as known), parasite/predator history, and other specifics as may be required. If the exporting country cannot satisfy the health requirements of the importing country, quarantine is the minimum requirement.

3.3.4 Inspections aimed at satisfying disease or other certificate requirements of 3.3.3 must be carried out by qualified personnel duly authorized by the exporting and importing countries.

3.3.5 Inspection procedures upon arrival of the introduced species at a quarantine site or other release sites should include the destruction or sterilization of all water, packing materials, containers, or other associated shipping materials.

3.3.6 Coordination between the proponent of an introduction or transfer (could be private or government) and the national agency monitoring importation is very important throughout an initial probationary period. A contact from the agency approving the proposal should be named to liaise with the proponent.

3.3.7 Upon completion of initial examination by inspectors of the importing country, the shipment, depending on the conditions imposed, should be released to quarantine, containment or directly for culture.

3.3.8 Quarantine facilities, where required, should be approved and regularly inspected by competent government specialists to ensure effectiveness.

3.3.9 The microscopic and macroscopic examination required while specimens are in quarantine makes it essential that proponents provide sufficient numbers of individuals for introduction and testing. The numbers required and the schedule for testing would be specified by the agency which approves import and would be dependent on species and stage at introduction.

3.4 Quarantine

Introduced or transferred aquatic organisms which are placed in quarantine are, by definition, a potential health risk. The aim of quarantine is to establish that they are either free of prescribed pathogens and pests or if not, that their progeny may be acceptable if they are proven pathogen- and pest-free. Because aquatic organisms may covertly carry pathogens without showing overt signs of clinical disease, they must in most cases be held in quarantine for life and be subject to repeated tests to establish their pathogen-free status. If they are established as pathogen-free, the F1 generation may be released. If the F0 generation is not pathogen- and pest-free and they are not destroyed but kept for breeding, then it may be necessary to quarantine the F1 generation for life, demonstrate the F1 generation is free from pathogens and pests and then use the F2 generation for release.

3.4.1 Introductions, whether as gametes or fertilized eggs for fish (preferred) or as some other stage for molluscs or marine plants, should be disinfected upon arrival at the quarantine unit (even though an approval certificate is supplied). If young fish are being imported, they should be treated by prophylactic bath. As stated under inspection and certification procedures, all materials in contact with the import during shipment should be destroyed or sterilized and not allowed to enter the holding system area of the quarantine unit.

Acclimation of eggs, larvae, adult organisms etc., to environmental conditions, such as temperature at the quarantine station, should be done in a manner which prevents, as far as possible, any contact between transport and final holding media.

3.4.2 It is recommended that intake waters be sterilized or disinfected. Sterilization means killing all life forms in the water supply. Disinfection means using techniques which will kill all the prescribed pathogens. Spring, ground, artesian, and well waters which have no flora or fauna in them prior to entry to the quarantine unit are best and require no treatment. If surface waters are used, there is a risk that native pathogens and pests may cause disease outbreaks in the quarantine unit causing consequent difficulties in deciding whether the pathogen is native to the water supply or was imported with the introduction.

3.4.3 The quality of water used in the quarantine unit should be monitored at regular intervals to ensure that any mortality in the quarantine population is not due to environmental conditions but rather to disease agents.

3.4.4 The cause of mortality in all animals in quarantine should be investigated and a written report should be prepared. All reports must be submitted to the regulatory authority who may undertake further investigations.

3.4.5 Disposal of solid wastes (faeces, surplus food, settled solids) and dead organisms must be conducted by an approved method, e.g., sterilized such that potential pathogens and pests cannot escape the quarantine unit by this route.

3.4.6 When recirculation of water is practised, both assessment and control of water quality must be carried out.

3.4.7 Records of operating conditions and procedures must be kept and made available for inspection by the regulatory authority on request.

3.4.8 If more than one stock (or species) is kept in the quarantine unit each must be kept in a self-contained compartment and precautionary measures instituted to ensure that staff cannot cause transmission of pathogens or pests between different stocks.

3.4.9 No equipment should enter or leave the quarantine unit without disinfection. If several species or stocks are kept in quarantine in separated modules, separate equipment must be available for each group.

3.4.10 Personnel operating the quarantine unit must be supervised by staff qualified to ensure all biological and operating concerns are appropriately addressed.

3.4.11 Personnel should enter and leave a quarantine unit through a disinfection station (footbath, showers) which should be regularly serviced to guarantee continued effectiveness.

3.4.12 Personnel operating a quarantine unit should not visit other aquaculture establishments on the same day.

3.4.13 The quarantine station should have adjacent, but physically isolated, laboratory facilities for inspection and preparation of material for pathology tests. Physical separation from the quarantine unit should help prevent accidental contact with quarantined species.

3.4.14 Should outbreaks of disease or pests occur while a species is in quarantine, a range of common treatment procedures should be immediately available. However, while these procedures may be successful in killing specific pathogens or removing specific parasites, they should not be viewed as an effective means of destroying all organisms carried by introduced species.

3.4.15 Should the quarantine unit suffer a disease outbreak that cannot be controlled, the diseased stocks must be destroyed and disposed of after sterilization in an approved manner, but not before notification of the appropriate government authority. The quarantine unit or particular module (including the biological filters if recycling system is used) must be disinfected prior to its reuse. It is advisable to operate dual systems to facilitate shutdown and sterilization procedures.

3.4.16 The design of the quarantine unit should minimize any risk that:
(a) operator error causes escape of aquatic organisms.
(b) unauthorized persons gain access and cause the release of the aquatic organisms.

3.5 Pathology

For purposes of this document, pathology is defined as, "the study of disease by scientific methods" (ICES Working Group on Pathology and Diseases of Marine Organisms). The objective of identifying diseases and parasites is to minimize or eliminate the introduction and distribution of organisms pathogenic to both native

aquatic species and those being introduced. Steps required to prevent or minimize introduction of pathogenic organisms or parasites include:

3.5.1 An import permit should list prescribed diseases as outlined under inspection and certification (paragraph 3.3.2). The testing regime and results must be supplied with any shipment of aquatic animal or plant into a country. The permit should also certify that the shipment was examined and found free of all parasites.

3.5.2 Where feasible, the desired species should be imported as fertilized ova, as the range of disease agents that can be carried is much less than for later life history stages. Complete surface disinfection is more likely when treatment is administered at the egg stage.

3.5.3 Wherever possible the imported species, regardless of import stage, should come from a production facility or area which has been certified free of prescribed diseases over a two-year period.

3.5.4 Where an exporting country cannot prove it has the required capability for testing of stocks, the import must be considered a risk and be placed in quarantine upon arrival in the importing country. Appropriate testing must be carried out in quarantine.

3.5.5 Sampling for disease caused by viruses and bacteria or parasites while the species is in quarantine should be carried out under the supervision of a fish health officer or inspector employed by the government.

3.5.6 Sample size should be determined by reference to published techniques (examples, Ossiander and Wedemeyer 1973; Worlund and Taylor 1983), based on achieving a 95% probability of detecting a disease agent carrier in a lot with an assumed incidence of carriers.

3.5.7 Specific examinations should also be carried out on native species maintained in quarantine in same containers with imported species.

3.5.8 In the event of a positive identification of a disease, shipment of animals must be destroyed and disposed of in an appropriate manner to avoid spreading disease (see paragraph 3.4.15)

4. PROTOCOL FOR SPECIES USED IN CURRENT COMMERCIAL PRACTICE

This group includes, but is not restricted to, species which are introduced or transferred in large quantities without permanent occupation of the ecosystem (maintained in tanks or in outside systems with access to open waters). Standard procedures with respect to these species should include:

4.1 Inspection and Certification

If continued movements from one country to another or one area to another are necessary to maintain the commercial enterprise, each shipment should include certification attesting to their pathogen-and pest-free status and should be inspected upon arrival for overt signs of pathogens by a qualified inspector.

4.2 Transport

The transport of the aquatic species should be done in such a manner as to avoid loss of water enroute to site of use. If water loss is inevitable enroute, consideration of use of sterile water or water sterilization should be considered.

4.3 Handling

All packing material and water must be appropriately sterilized upon arrival at stocking or holding site.

4.4 Pathology

Periodic pathogenic sampling should be carried out at holding sites to ensure no disease or parasite has escaped detection when certification was carried out.

4.5 Control

Holding sites must be secure against escape and species stocked out in wild as an ongoing practice must be closely monitored to ensure they do not expand their range beyond what was originally intended.

5. PROTOCOLS FOR SPECIES IMPORTED SOLELY FOR SCIENTIFIC STUDIES IN RESEARCH INSTITUTIONS

5.1 Procedures if Stocked in Open Waters

If the imported organism is to be used in open systems, procedures should be the same as those outlined in 3. Quarantine will be required for those non-indigenous species that will be temporarily exposed to open waters but collected again for later analysis.

5.2 Laboratory Handling

If the imported organism is to be held in strict laboratory confinement, with no subsequent plan for releases into the environment, no feasibility analysis would be required. It is important that investigators be aware of existing laws and regulations related to introductions in general. Quarantine conditions are not required when the receiving laboratory has the appropriate conditions for effective confinement. Effluent sterilization is considered to be necessary whenever the water supply and run-off from the laboratory is directly connected to open inland or marine waters. Equipment and water used and organisms which die during experimentation should be destroyed and disinfected. After the experiments are finished, all remaining organisms should be destroyed and disinfected, and tank systems should be disinfected and cleaned.

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APPENDIX I

EXAMPLES OF SPECIFIC PROTOCOLS AND/OR SPECIAL
REQUIREMENTS FOR SPECIES OR SPECIES GROUPS

The examples utilized in this section demonstrate the varying levels of complexity, especially with respect to disease control, that are adhered to in carrying out introductions. Details could well vary from country to country for particular species or species groups.

1. SALMONIDS

The following are the minimum measures considered necessary for preventing the introduction of specified disease agents with introductions and transfers of salmonid ova and fish. Genetic and ecological considerations would also have to be dealt with:

1.1 The importing country must list which diseases or disease agents are proscribed. Fish shall not originate from any farm site in which the disease agents have occurred in the last two years or from waters where there is cause to consider the agent is present, unless ova only are involved. Where the proscribed pathogens may be carried intra-ovum, e.g., all viruses and Renibacterium, the ova are treated as for fish. Other proscribed pathogens may be discounted if disinfection of ova against bacterial pathogens is practised and ova are incubated in parasite-free waters.

The list of proscribed agents will depend on what diseases are present in the importing country and what precautions its experts consider are necessary against importing exotic strains of pathogens already present. An example of a typical list would be:

- (a) Any filterable replicating agent capable of causing cytopathic effects in acceptable cell lines including but not limited to:
 - Viral Haemorrhagic Septicaemia Virus (VHS)
 - Infectious Haematopoietic Necrosis Virus (IHN)
 - Infectious Pancreatic Necrosis Virus (IPN)
- (b) Renibacterium salmoninarum (Bacterial kidney disease, BKD)
- (c) Aeromonas salmonicida (Furunculosis)
- (d) Yersinia ruckeri (Enteric Red Mouth, ERM)
- (e) Myxosoma cerebralis (Whirling Disease, WD)
- (f) Proliferative kidney disease (PKD)

1.2 Site of origin sampling procedures for (a) to (f) must be carried out twice a year, the first test being in the early summer (May/June) and the second in early winter (November/December), including the spawning period for broodfish.

Each lot of fish on site will be tested. A lot is defined according to species, but specimens must be taken from each tank, pond, raceway or cage, to ensure that strain, water supply, origin and age are sampled in each lot. Prior to testing, each fish lot must be examined for overt signs of disease and then divided up to provide fish for the various tests. Apparently healthy fish should be randomly distributed.

- (a) Testing of lots is required during the two years prior to export of live salmonids. The minimum number of samples of fish from each lot should comprise:

Early summer - 90, 0+ yr fish for virus examination, 30, 1+ yr and 30, 2+ yr fish for virus, bacteria and whirling disease examination.

Early winter - 150, 0+ yr fish for virus, bacteria and whirling disease examination.

Note At least five specimens must be taken from each tank, pond, raceway or cage containing fish of a specific lot and this may result in processing more than the recommended minimum of specimens. All fish in the early winter test must be at least five months old.

In addition, serum for testing anti - *R. salmoninarum* agglutinins must be collected from broodstock at spawning time. Serum extraction is non-lethal and must be carried out on 10, 3+ yr fish; 10, 4+ yr fish and 10, 5+ yr fish.

If any fish in any group have a titre of 1/32 or more, the test must be repeated using 30 fish from the failed group(s). If titres of 1/32 or more are again recorded, lethal sampling of 60 fish in failed group(s) must be carried out using gram stains, fluorescent-antibody tests (FAT) and culture methods. Only if these fish are negative will the site be permitted to proceed to testing as listed in (b).

(b) Testing of lots required for a site after the initial 2-year testing period detailed in (a). The samples of fish from each lot should comprise:

Early summer - 90, 0+ yr fish for virus examination, 30, 1+ yr and 30, 2+ yr fish for virus, bacteria and whirling disease examination.

Early winter (November/December) - 150, 0+ yr fish for virus, bacteria and whirling disease examination.

1.3 Laboratory Procedures for the Examination of Fish for Notifiable Diseases:

(a) Virological tests

(i) Sampling: In fish under 8 cm, transverse body sections are sampled to include the main visceral organs. With larger fish; samples of liver, kidney, spleen and pyloric caecae are taken, except in the case of salmon older than one sea winter, when kidney samples alone are taken. The samples should be processed within 48 hours, during which time they must be stored at 4°C. On no account must samples be frozen before testing since this greatly reduces the sensitivity of the test.

(ii) Extraction procedure: The pooled visceral samples should be processed by one of the following methods:
- ground using a mortar and pestle with sufficient sterile sand until a thick paste is formed. Tissue culture maintenance medium (MM) containing antibiotics (see (a)(iii)) should be added in the ratio 1:1 w/v to the original visceral sample. The visceral extracts are then centrifuged at 1,500 g for 15 minutes and the supernatant collected. This supernatant should be diluted a further 1:25 in MM.

- diluted 1:10 in MM and transferred to a Seward stomacher bag and homogenised in a Seward stomacher "80" for 2 min. The homogenised tissue is then centrifuged at 1,500 g for 15 minutes and the supernatant collected. This supernatant should be diluted a further 1:5 in MM.

- subjected to speed rotary blending or ultrasonification using the methods described by Hedrick et al. (1986) Prog. Fish-Cult., 48, 47-51.

The sampled material should be kept as cool as possible during the whole procedure.

(iii) Elimination of bacterial and fungal contamination: Normally, broad-spectrum antibiotics are included with nystatin or fungizone incorporated to combat fungal contamination.

(iv) Inoculation of cell cultures: Culture wells or flasks of BF or CHSE 214 and/or FHM cells or other acceptable cell line(s)* should be inoculated with each extract at a rate of 1/10th the normal volume of MM should be added. The cultures should then be incubated at 15°C (duplicates at 15° and 20°C in cases of suspect SVC) and examined daily for signs of cytopathic effect (CPE). If no CPE develops after 7 days the cultures should be harvested by freezing and thawing and passed using 1:10 dilution into MM, and then onto fresh cell cultures for a second incubation period of 7 days at 15°C (duplicates at 15° and 20°C in cases of suspect SVC).

If CPE develops, the cultures should be harvested by freezing and thawing, diluted 1:100 and inoculated onto fresh tissue cultures. If no CPE develops during the second incubation the test can be declared negative. If viral CPE develops during the second incubation period the virus must be identified.

(v) Cell cultures*: Only young, actively growing cultures, ie., 1-3 days old and 75-95% confluent, should be used for the isolation tests. BF and CHSE 214 cells should be grown at 20-25°C and FHM cells at 25°-30°C. When testing specifically for one disease a single suitable type of cell culture is used, but for general virological examinations CHSE 214 or BF and FHM cell cultures are used in parallel. Currently, tests for the viruses of viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and spring viraemia of carp (SVC) are carried out on FHM cell cultures, but BF or CHSE 214 cell cultures are used for IPN virus tests.

(vi) Positive controls: It has been reported from several laboratories that some fish cell lines appear periodically to lose their receptivity to some fish viruses. Therefore, the susceptibility of the cell cultures to the virus(es) under test

* Alternative cell lines may be used if jointly agreed to by importing and exporting countries or when specified in the health certification import requirements of overseas countries.

should be confirmed. This may be done at the time of testing by inoculating replicate cultures of the cells with known low infective doses of the viruses.

(vii) Identification of viruses: The cause of any viral-type CPE must always be identified. Where specific antisera against a suspected virus is available, serum neutralization of virus infectivity is the test of choice using either the plaque reduction method or the constant serum (1:100) varying virus method.

(b) Bacteriological Tests

(i) Bacterial Kidney Disease, Renibacterium salmoninarum (BKD)
Presumptive Tests

1) Smears: duplicate kidney imprints should be taken for Gram stain and FAT tests.

2) Serum agglutinins. Serum sampling is non-lethal and may be used for valuable brood fish, e.g., 3 years and older. If any fish in any group have a titre of 1/32 or more, the test must be repeated using 30 fish from the failed group(s). If titres of 1/32 or more are again recorded, lethal sampling of 60 fish in failed groups must be carried out using Gram stains, FAT and culture methods. Only if these fish are negative will the site be viewed as free of BKD.

Culture: swabs must be taken from the mid-to-anterior kidney of all fish in the sample group and must be plated without delay onto selective kidney disease medium (SKDM); Austin, Embley & Goodfellow (1963), FEMS Microbiol. Letters, 17, 111-114), incubated at 15°C and examined weekly for 6 weeks for the presence of Renibacterium salmoninarum.

Confirmation of Renibacterium salmoninarum: slowly-developing colonies on SKDM plates should show Gram-positive diplococci bacilli in Gram-stained smears, give positive fluorescent antibody test results using specific antiserum prepared in rabbits and show characteristic biochemical profiles (Austin et al. (1983); Sanders & Fryer (1980), Int. J. Sys. Bact., 30, 496-502).

(ii) Furunculosis (Aeromonas salmonicida)

Culture: swabs from the kidney and any furuncles must be plated onto tryptone soya agar (TSA), incubated at 22°C and examined daily for 7 days for the presence of furunculosis.

Confirmation of Aeromonas salmonicida: colonies grown on TSA plates often produce dark-brown pigment. The gram-negative rods are non-motile and fail to grow at 37°C. Colonies should give a specific agglutination in the A. salmonicida latex test (McCarthy (1977), Fish Health News, 6(3), 146-147), or meet the confirmatory criteria of Popoff (1984), Bergey's Manual of Systematic Bacteriology.

(iii) Enteric redmouth (ERM), (Yersinia ruckeri)

Culture: swabs from the faeces and kidney must be plated onto TSA, incubated at 22°C and examined daily for 7 days for the presence of ERM.

Confirmation of Yersinia ruckeri: colonies grown on TSA plates should show Gram-negative rods on Gram smears, ferment glucose, produce catalase but not cytochrome oxidase, and exhibit a typical biochemical profile (Green & Austin (1983), Aquaculture, 34, 185-192; Ewing et al. (1987), Int. J. Sys. Bact., 28, 37-44).

(c) Parasitological Tests

(i) Whirling disease (WD) (Myxosoma cerebralis)

1) Fish less than 5 months old showing clinical signs of disease: A transverse section is taken through the head cartilage posterior to the eyes and anterior to the opercula and fixed in 10% formal saline. Following fixation, the tissues are processed histologically and subsequently 5 um sections stained with Giemsa, examined microscopically for the trophozoite or spore stages of Myxosoma cerebralis and accompanying pathology in the cartilage.

2) Fish over 5 months old: Fish should be examined by either:
 - the histological method outlined in (i) or
 - whole heads are removed from the fish and processed within 72 hrs. With large salmon older than one sea winter gill arches may be used instead of whole heads. Samples must not be frozen. Samples are soaked in warm water until the skin and muscle can be easily stripped from the cranial skeleton, or filaments from the gill arches. The material is macerated in a blender and filtered through muslin to remove any large fragments. The sample is then concentrated using the plankton centrifuge method of O'Grodnick (1975), J. Wild. Dis., 11, 54-57. A drop of the final suspension is examined using phase contrast microscopy at x 250 magnification. At least 50 microscope fields are examined for each suspension. Confirmatory identification of spores is based on the criteria of Lom & Hoffman (1971), J. Parasit., 57, 1302-1307.

(ii) Proliferative Kidney Disease (PKD) The following are adopted from the Fish Health Blue Book of the American Fisheries Society:

1) Diagnostic Procedures for Disease Situations, Differential Diagnosis: Clinically, the following diseases have similar manifestations: IHN, bacterial kidney disease (BKD), sanguinicollasis (Sanquincola klamathensis), nephrocalcinosis, and low-grade copper toxicity.

Presumptive Diagnosis: The presence of lightly staining extra- and intramacrophage protozoa containing 1-7 "daughter cells" in stained imprints of posterior kidney and spleen. The "parasitized" macrophages are often surrounded by small lymphocytes, the reported "satellite" condition.

Confirmatory Diagnosis: With transmitted electron microscopy (TEM), the primary cell contains multivesicular bodies, lipid bodies, mitochondria, and electro-dense bodies ("haplosporosomes") which contain an electron-lucent bar. With light microscopy, the organism is PAS-positive. In the kidney, particularly the posterior kidney, there is marked lymphocytic hyperplasia to the point that the nephrons are often compressed. Organisms are often seen in the renal tubules and blood vessels.

In the spleen, there is a marked diminution of the erythrocytic elements due to the lymphocytic hyperplasia.

2) Procedures for Detecting Asymptomatic Infections:

Sample the suspect population in accordance with the method to provide a 5% prevalence detection level.

Collect acetone-fixed imprints of posterior kidney and 10% neutral-buffered formalin-fixed samples of posterior and mid-kidney, spleen and gastrointestinal tract. Early in the "PKD season"; i.e., mid-March to mid-May, acetone-fixed smears of pyloric caecal and large intestinal mucosa scrapings should be examined. The acetone-fixed imprints and smears may be stained using either the methylene blue technique or the Leishman-Giemsa method. The formalin-fixed material, after sectioning, may be stained using the PSA and/or the H&E techniques.

3) Procedures for Determining Prior Exposure to the Etiological Agent; No methods are currently available to detect previous infections with the PKD-causing organisms.

4) Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survivability of the Etiological Agent. All samples must be fixed on site in accordance with the procedure described. The organism will deteriorate very rapidly in iced samples - often to the point that it becomes unrecognizable.

1.4 As the range of disease agents that may be carried by eyed ova (especially after disinfection) is very much less than for fish, conditions for imports of ova may be less stringent. However, all ova must be disinfected with iodophors before leaving the exporting country.

Salmonid eggs are safely disinfected as green eggs following fertilization and water hardening, or as early eyed eggs. Iodophors for disinfection are usually providone or polyalcoholic complexes of iodine, in which the soluble iodine confers its broad-spectrum germicidal activity but is not as corrosive or irritating as in its elemental form. A number of typical disinfectants* of this type are available commercially in North America; among these are Ovadin^R, Bridine^R, Betadine^R, Actomar K30^R, Wescodyne^R and Argentyne^R. Most contain a 1%-2% active iodine concentration.

(a) Preparation of the disinfectant

(i) Dilute the stock iodine-based disinfectant to give a solution containing 100 parts per million (ppm) of active iodine. The disinfectant must be prepared in water with a low organic content to minimize loss of the free iodine. Use a plastic, glass, stainless steel or fibreglass tank for preparing and holding the solution.

(ii) Check the pH of the diluted disinfectant and, if necessary, adjust to 6.5-7.5 using 8% aqueous sodium bicarbonate (baking soda).

(b) Disinfection procedure

(i) Use a fresh solution of diluted disinfectant.

* The products specified have proven satisfactory for the purposes indicated; this, however, does not imply that other products may not be equally satisfactory.

(ii) To avoid temperature shock, adjust the disinfectant solution to the same temperature as the subsequent egg incubation temperature.

(iii) In the case of freshly fertilized eggs, allow eggs to water harden one hour before disinfection.

(iv) Immerse water-hardened green eggs or early eyed eggs in the disinfectant for ten minutes.

(v) Treat approximately 2,000 eggs per litre before discarding the disinfectant.

(vi) Rinse eggs thoroughly in uncontaminated water after disinfection.

(vii) Arrange the egg handling programme to ensure that disinfected eggs do not have subsequent contact with contaminated equipment, water or personnel.

Diluted iodophors can also be used to disinfect work surfaces, utensils, nets and other equipment used during the egg-taking process, but they must be rinsed thoroughly in clean, uncontaminated water following the disinfection.

1.5 The exporting country should have significant experience in testing for the proscribed disease agents, i.e., an officially recognized authority. This authority must have records for two years of testing by approved methods for any fish farm source of ova or fish before consideration can be given for direct introductions without quarantine.

Where the exporting country has little or no relevant experience or no history of testing of relevant stocks, the import must be considered a risk and be placed in quarantine on arrival in the importing country. In quarantine, appropriate tests will be conducted. Quarantine should last until the F1 generation is three months into first feeding for imports of ova, and until the F2 generation is three months into first feeding for fish imports.

1.6 Fish and ova for import should be considered from one of four categories of source:

(a) Fish or ova are from specified pathogen-free (SPF) farms, (see 1.2 and 1.3 for sampling and laboratory test requirements) which conform to certain physical requirements, that is:

(i) The site must be entirely supplied by water from spring, bore-hole or well which, from source to inlet, is under the control of the site owner.

(ii) The water supply must be free of feral salmonids and the site should be screened to prevent their ingress.

(iii) The site water supply must be free from risk of pollution from ground water or flooding by other water courses.

(iv) All piscivorous birds or other animals must be excluded from the site.

(v) Food stores should be sited so that transporter vehicles do not traverse the farm but unload at some peripheral point, thus minimizing any risk of introducing infection from another farm.

(vi) All introductions of live fish or ova must be agreed to by the state or local government certifying authority as being from an establishment of similar health status.

(vii) It is essential that vehicles carrying live fish to or from the farm are thoroughly cleaned and disinfected before accepting their cargo. Such farms, called SPF Category I, must have had SPF status for two years. Additionally, no fish or ova other than from a farm with SPF Category I health shall be or have been introduced in the past two years to the site. Fish or ova from SPF Category I farms may be allowed direct entry without quarantine if accompanied by the appropriate certificates (Appendix IV).

(b) Fish or ova are from farms which have been in the SPF category for two years and to which no fish or ova other than from a farm of similar or better health status have been introduced in the past two years to the farm shall be called SPF Category II farms. These farms do not meet the physical site requirements of SPF, Category I farms.

Fish from such SPF Category II sites may be allowed direct entry only in certain situations, i.e., when pathogens on the proscribed list are common to both countries or absent in the exporting country, but assuming the shipment is certified free of all pathogens on the proscribed list. Otherwise, such fish must be placed in quarantine for life and the F1 progeny not released until testing for the proscribed pathogens is completed to the satisfaction of the "official authority" in the importing country.

(c) Ova are from wild sources. The sampling of fertilized eggs or the sex products of fish cannot be relied upon to detect all disease agents. The threat of introducing disease agents with such ova comes from parent fish; however, even here there is cause to doubt if current methods are sufficient to detect low levels of carrier infection of some disease agents in such parent fish. It is for this reason that four tests are conducted before fish farms are accorded SPF status, during which time it has been found that if low levels of infection are present, they almost always express themselves as higher levels of infection in the confines of culture environments. For these reasons, ova of wild fish must be quarantined preferably for a whole life cycle until the next generation (F1) is three months post feeding and certainly for not less than the 2-year period equivalent to the SPF testing period. Ova for quarantine should come from parent wild fish tested individually and found free of all viral and other agents which may be transmitted via ova.

(d) Fish are from wild sources. A high risk is associated with such fish which should be quarantined for life. Fish should come from

groups where samples are tested and found free of proscribed diseases. The safest procedure would be to allow only the F2 generation from quarantine to be introduced but certainly the minimum period must be two years for the F1 generation.

When an importer seeks to introduce fish or ova, whether directly or via quarantine, it is obligatory that a health certificate from a recognized authority be provided for each shipment. Each approved shipment must be accompanied by an appropriate import permit from the authorities in the importing country. Such import permits should clearly indicate if entry is conditional, e.g., is quarantine to be imposed, and if so, the regulatory authority in the importing country must supervise the conditions.

2. MOLLUSCS

Because of the relatively high commercial value of a number of molluscan species, they have been subject to a great deal of movement within or between countries. This movement continues, both as part of ongoing commercial operations or when a new non-indigenous species is brought in. The introduction or transfer of these molluscan species create the same range of inherent genetic, ecological and pathological problems as would the import of other aquatic organisms. However, because the majority of the molluscs introduced or transferred are sessile or capable of only localized movement, it is possible that one could more effectively deal with an analysis of genetic or ecological risks than might be the case with a fish species which exhibited broader distribution characteristics.

A centre for the study of molluscan introductions (usually as adults for broodstock) could be established in a location well removed from contact with related indigenous species which might be impacted on either ecologically or genetically. With proper containment (barriers, removal before spawning, etc.), indigenous and introduced species could be brought together to permit assessment of genetic and ecological interaction. By limiting the size of the initial importations, it would be easier to eradicate the group should something go wrong.

Pathological considerations could be dealt with at the same time as the genetic and ecological considerations.

2.1 Control of On-going Commercial Operations:

2.1.1 General recommendations:

(a) A list of known infectious undesirable pathogenic diseases must be established by the importing country. An example of a list would be:

- Iridovirosis of adults and larvae
- Marteilirosis
- Bonamiosis
- Haplosporidiosis
- Perkinsus type parasite.

(b) The exported molluscs can not be provided from a site where one of these diseases has been identified during the previous four years.

(c) Disease inspection by the exporting country must be carried out four times per year in the different production zones as well as in the hatcheries.

(d) Each exporting country must have accredited laboratories which agree to carry out the disease control regulations.

(e) Each shipment must be accompanied by a health certificate attesting to the absence of listed diseases and which indicates the presence or absence of any abnormal mortalities.

(f) The mollusc for export must originate from production areas in which predator populations (Urosalpinx, Turbellariums, etc) and competitors (crapudula, algae) are not prevalent. In all cases the export molluscs will be sorted and cleaned to remove surface predators and other potential competitors.

2.1.2 Preparation and analysis of samples: For contagious diseases described in the literature all the tests can be made by microscopy, except for Bonamia which now can be identified by simple serological diagnosis. This can be done because of the advanced state of diagnostic techniques now applicable to molluscs.

(a) By utilizing a sample size of 100 for analysis of all the disease organisms results will be sufficiently precise to indicate their presence even at lower levels than (1% at a 95% confidence limit).

(b) After carefully opening the shell, in order not to damage the different organs of the animal, each individual is inspected in vivo. During this inspection the external quality of the flesh will be noted as well as all other abnormalities such as abscess, lesions etc. on different organs.

(c) Other samples are cut sagittally, and fixed in Carson's liquid fixer which offers the advantage of allowing one to use these samples for subsequent treatment and observation under the electron microscope.

(d) One is advised to search for Bonamia with the help of the diagnostic kit ELISA which has proven slightly more sensitive and quicker to use.

(e) Identification of abnormalities or pathogenic agents would lead to the specimen being reexamined using the electron microscope.

2.2 Controls and operations to carry out for other introduction:

In addition to the analyses carried out by the exporting and importing countries as described above, operators concerned only with smaller lots of spawner should carry out the following procedures:

(a) Destroy all shipping materials and carefully brush the shells of each spawner to remove attached living organisms.

(b) Place in a quarantine station where all effluent will be treated by sterilizing techniques such as ultraviolet; ultrafiltration, chlorination, bromination, etc.

(c) Maintain strict health controls on site.

(d) After spawning and successful production of an F₁ generation, the broodstock should be destroyed or utilized to study interactions with indigenous species at special isolated sites.

(e) The F₁ generation can be released if further pathological testing of larvae and juveniles is satisfactory.

Problems encountered as a result of F₁ pathological, genetic or ecological testing could necessitate holding oysters to the F₂ or F₃ generation prior to release from quarantine.

(f) Hatcheries could be established to provide disease-free stock to commercial enterprises, rather than continually importing new animals.

3. EELS

Eel culture is mainly dependent on the supply of elvers, the migrating young stage, which are caught at sea or in rivers and transported to aquaculture installations where they are cultivated. Transportation may also occur for restocking in natural waters. In many cases this implies an import from one country to another. These movements introduce considerable risk of transfer of infectious agents. For example in 1976 Sano & al. reported that a Rhabdovirus had been isolated in Japan from elvers imported from France. Castric and Chastel (1980) presented studies on viruses recovered from elvers on the French Atlantic coast in 1977 and 1978 which stated an IPN virus (type Sp) and two different types of Rhabdovirus (B₁₂ and C₃₀) had been found in eels from the Loire area.

Proscribed diseases and disease agents for eels are:

- (a) All viral disease as defined in salmonids (Appendix I, 1.1)
- (b) Cauliflower disease
- (c) Branchionephritis
- (d) Rhabdovirus sp. (other than in salmonids)
- (e) *Vibrio* sp.
- (f) *Chondrococcus* sp.
- (g) *Anguillicola* sp.
- (h) *Dactylogyrus* sp.

The following procedures for handling the transfers of eels are aimed at minimizing the chance of introducing disease agents into new areas:

3.1 Each country should compile an inventory of current practices in eel transfers, including the following data:

- (a) A list of species of eels introduced and the developmental stage involved (glass eel, elver, sub-adult, silver eel).
- (b) A list of countries or origin, including river systems.
- (c) Routes of overland or overseas transport with details of sites where carrying water has been discharged.
- (d) List of waters in which eels have been released to develop under natural conditions.
- (e) List of aquaculture installations where eels are cultivated.
- (f) List of open waters or installations in which eels are held prior to marketing.
- (g) History of outbreaks of fish disease in introduction areas associated with import of elvers or of other species.

Imports of eels (at any stage) should come from certain areas i.e., areas known to be free from disease and disease agents according to lists previously developed.

3.2 Before export of local elver populations to another country, these populations must be sampled for pathogens or pests (virus, bacteria, etc.) to avoid introduction of any new disease to importing country.

3.3 A six week quarantine upon arrival in the importing country should be mandatory and should follow all the elements of quarantine outlined under universal protocols in section (3.4), including the destruction or sterilization of all material in contact with the eels during import. Transport to importing country should take place without exchange of transport water. A schematic diagram, Figure 1, based on Swedish practice is included for consideration.

Water leaving the quarantine installation must either be filtered through soil of an appropriate particle size or treated with calcium or sodium hydroxide to raise the pH to 10 or higher.

The quarantine station and its operation must be inspected and approved by qualified government inspectors before use. When in use the facility should be supervised by a local veterinarian, authorized by the government to ensure equipment maintenance and hygienic conditions in the installation are maintained.

Sampling of elvers (at least 20-30 animals per sample) for disease agents must be carried out within 1-3 weeks after arrival of the elvers at the quarantine facility. Standard virological examination of pooled 5 and 5 elvers should be carried out using RTG-2 cells. If results of testing are positive for virus or other disease agents, elvers must not be released for farming or other stocking purposes.

3.4 The quarantine must be supplied with a test-fish system consisting of two test tanks (Fig 1).

- The test fish must be juvenile (5 g-15 g) salmonids, e.g. rainbow trout or salmon. Juvenile fish at this stage are considered to be most sensitive to viral diseases.
- The test-fish tanks (100 litres water/tank) must be stocked with at least 100 fish.
- In one of the test tanks the test fish should be exposed to effluent water from the eel tanks, diluted and adjusted to 10°C. In the other test tank the same number fish should be kept in water which is not contaminated by effluent from the eel tanks.
- After exposure to eel tank effluent (minimum 14 days) the test fish (and reference fish) are examined virologically. Until testing is completed no eels will be released from quarantine.
- If the examination results are positive elvers (eels) must not be released for farming or stocking purposes. If the results are negative the eels can be released immediately (about 5-6 weeks from the start of the quarantine).
- Infected fish must be destroyed.
- The quarantine period should be prolonged, if necessary, to thoroughly investigate suspected disease or infectious agent carrier conditions.

A number of factors (species, age, condition of fish, water temperature, virus concentration or infection dose, and the virulence of the pathogen) apparently influence the eventual outcome of a test-fish exposure in situations as

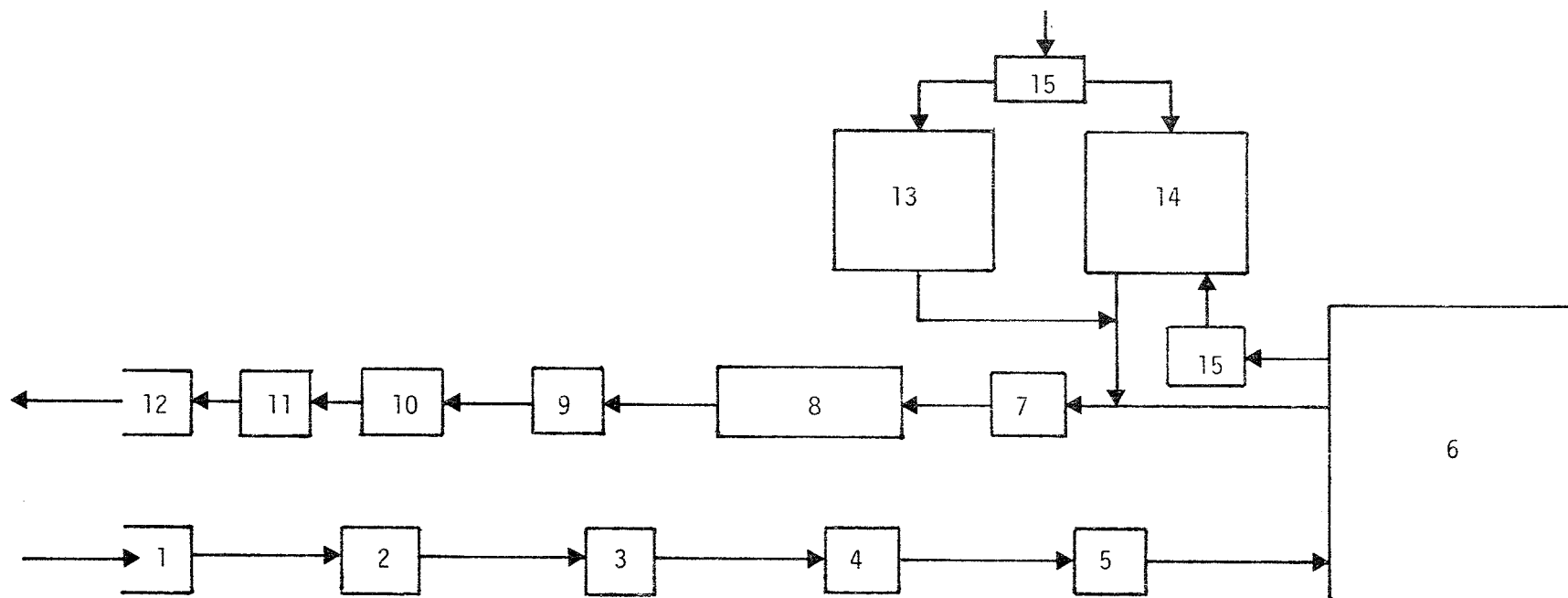


Fig 1. Principle for a quarantine installation for elvers (Modified after Ackefors et al., in press).

Water is pumped through (1) and passes through a screen (2) where particles bigger than 2 mm (e.g., mussels, coarse sand) are removed. The water then passes through a heat exchanger (3), where the temperature is adjusted to the desired level, and through one or two glass-fiber filters (4) or triangle filters, where particles down to 10 μ are removed. After aeration (5) the water then enters the elver tanks (6). After leaving these tanks, faeces and food waste are removed from the water swirl separator (7) or similar arrangement and collected in a reservoir (8). The water is then pumped (9) to an installation (10) where the pH is increased to 10. The pH adjusted water is normally held for one or two hours (11) before releasing it (12).

There are two test-fish tanks (13 and 14). One of them (14) is partly fed with water from the elver tanks and partly with fresh incoming water; the other is fed with only fresh incoming water (13). All the ingoing water is temperature adjusted to 10°C (15). The outgoing water from the fish-test tanks is treated as is the outgoing water from the elver tanks.

Note: UV-light may be inserted between (4) and (5), but because of several technical drawbacks this treatment is generally not recommended.

those described above. Absence of clinical symptoms in virologically IPN-positive salmonids, as well as negative results in experimental Rhabdovirus infection, could be explained in a number of different ways. As we are unable to control some of the important factors for the efficiency of a test system we must admit that negative results can not necessarily be taken as absolute evidence for absence of virus carriers in a tested population.

A French study of viral infections among elvers taken from a certain area of the eastern Atlantic coast in 1984 recovered viruses in 10 of 23 samples examined. Against this background it is realistic to consider the risk of introducing infectious agents as very high in elvers collected and sold for fish culture or stocking purposes from such areas, even if viral examinations of samples from the lot in question are negative.

APPENDIX II

REVIEW AND DECISION MODEL FOR EVALUATING PROPOSED
INTRODUCTIONS OF AQUATIC ORGANISMS

The model (Fig. 1) is composed of five levels of review and five corresponding "Decision Boxes". Components of the model are described below, with decisions being based on scale values obtained from an "opinionnaire" (Table 1).

Note - A simplified model has been enclosed as Figure 2.

(a) Proposal for introduction of aquatic organisms

An entity desiring to realize an introduction would prepare a proposal that includes the answers to the following questions:

- (i) What organism do you propose to introduce (common and scientific name)?
- (ii) What is its native range? What is the present range?
- (iii) What is the purpose of the introduction?
- (iv) Where and into what type of system would this organism be introduced and how many would be introduced?
- (v) What precautions have been or will be taken to ensure that the organisms are not harbouring communicable pathogenic organisms and parasites?
- (vi) If the organisms are to be maintained in a closed system, what measures would be taken to guard against accidental escape to open waters?
- (vii) What is the current state of knowledge concerning the acclimatization potential of the organism?
e.g., (a) Thermal requirements: tropical, temperate, Arctic; (b) Habitat requirements: stream, river, lake, pond, etc., (c) Reproduction: describe the spawning habitat and reproductive strategy of the organisms.

A bibliography of pertinent literature should be appended to the proposal.

(b) Level of Review I

- (i) Purpose of introduction
Does the proposing entity have valid reasons for introducing the aquatic organism? Could no native species serve the same function?
- (ii) Abundance in native range
Knowledge of the population abundance of the organism in its native range is an important aspect of the evaluation. Is it endangered, threatened or rare? Is it exploited from the wild or under culture?
- (iii) Communicable pathogenic organisms and parasites
The evaluation would include assessing the safeguards for avoiding transmission of communicable pathogenic organisms or parasites to the proposed receiving system(s).
- (iv) Site of introduction
It is important to discern from the outset whether the organism would be stocked in an open or closed system. Would it be stocked in or have potential access to a major drainage? If it is to be maintained in a

Figure 1. Review and Decision Model for evaluating proposed introductions of aquatic organisms. Mean "opinionnaire" values (see Table 1) are used at decision-making points (Kohler and Stanley, 1984)

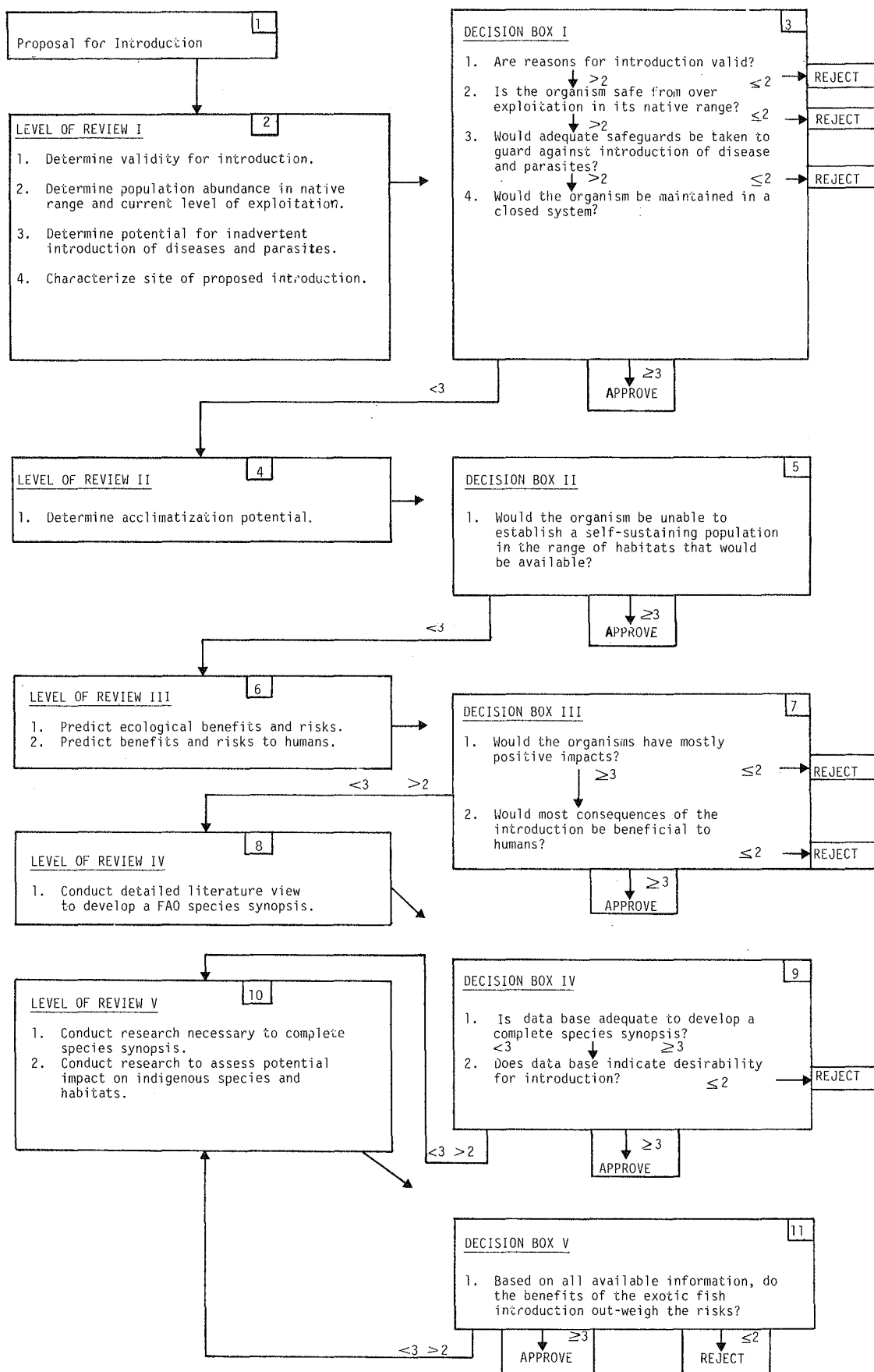


Table 1. Opinionnaire for appraisal of introductions of aquatic organisms.
 Each member of an evaluation board or panel of experts circles the number most nearly matching his/her opinion about the probability for the occurrence of the event.
 If information is unavailable or too uncertain: "don't know" is marked
 (Kohler and Stanley, 1984)

	Response					Don't know
	No	Unlikely	Possibly	Probably	Yes	
1. Is the need valid and are no native species available that could serve the stated need?	1	2	3	4	5	X
2. Is the organism safe from over-exploitation in its native range?	1	2	3	4	5	X
3. Are safeguards adequate to guard against importation of disease/parasites?	1	2	3	4	5	X
4. Would the introduction be limited to closed system?	1	2	3	4	5	X
5. Would the organism be unable to establish a self-sustaining population in the range of habitats that would be available?	1	2	3	4	5	X
6. Would the organism have mostly positive ecological impacts?	1	2	3	4	5	X
7. Would most consequences of the introduction be beneficial to humans?	1	2	3	4	5	X
8. Is data base adequate to develop a complete species synopsis?	1	2	3	4	5	X
9. Does data base indicate desirability for introduction?	1	2	3	4	5	X
10. Based on all available information, do the benefits of the exotic fish introduction outweigh the risks?	1	2	3	4	5	X

Figure 2. Review and Decision Model for evaluating proposed introductions of aquatic organisms (Kohler and Stanley, 1984) (Simplified by B. Steinmetz, unpublished correspondence).

Review level	Opinionnaire value*	Decision
I		
1. Is the need valid and are no native species available that could serve the stated need?	≤ 2 > 2	- <u>reject</u> - <u>to next question</u>
2. Is the organism safe from over-exploitation in its native range?	≤ 2 > 2	- <u>reject</u> - <u>to next question</u>
3. Are safeguards adequate to guard against importation of disease/parasites?	≤ 2 > 2	- <u>reject</u> - <u>to next question</u>
4. Would the introduction be limited to closed system?	≥ 3 < 3	- <u>approve</u> - <u>to review level II</u>
II		
5. Would the organism be unable to establish a selfsustaining population in the range of habitats that would be available?	≥ 3 < 3	- <u>approve</u> - <u>to review level III</u>
III		
6. Would the organism have mostly positive ecological impacts?	≤ 2 $< 3 > 2$ ≥ 3	- <u>reject</u> - <u>to review level IV</u> - <u>to next question</u>
7. Would most consequences of the introduction be beneficial to humans?	≤ 2 $< 3 > 2$ ≥ 3	- <u>reject</u> - <u>to review level IV</u> - <u>approve</u>
IV		
8. Is data base adequate to develop complete species synopsis?	< 3 ≥ 3	- <u>conduct detailed lit. rev. 1)</u> - <u>to next question</u>
9. Does data base indicate desirability for introduction?	≤ 2 $< 3 > 2$ ≥ 3	- <u>reject</u> - <u>conduct research 2)</u> - <u>approve</u>
10. Would benefits exceed risks?	≤ 2 ≥ 3	- <u>reject</u> - <u>approve</u>

1) thereafter next step question 9.
2) research focused on potential impact on indigenous species and habitats. thereafter question 10. Value $< 3 > 2$ restart research.

* see Table 1 of Kohler and Stanley.

closed system, the proposing entity must identify steps it would take to guard against accidental escape.

(c) Decision Box I

A proposal for an introduction would be rejected if:

- (i) reasons for introduction were not deemed valid;
- (ii) the introduction is for reasons other than conservation where the organism is endangered, threatened, or rare in its native range; or
- (iii) the proposing entity has not established that adequate safeguards would be taken to avoid introduction of communicable pathogenic organisms and parasites. The proposal would be approved at this stage when the above criteria are met and provided that the introduction is perceived as being limited to a closed system. When this last condition is not fully met, the evaluation process would proceed to the next level of review.

(d) Level of Review II

This and subsequent levels of review are directed to experts selected by the Working Group. In Level II, the acclimation potential is assessed (Question 5 of the "opinionnaire", Table 1). Should pertinent information be insufficient, as evidenced by more than 50 percent of the experts marking "don't know" on the "opinionnaire", the Working Group might suggest that the proposing entity conduct research with a limited number of specimens under confined conditions for the purpose of obtaining the required data. The Working Group may suggest that all research be conducted within the organism's native range.

(e) Decision Box II

The proposal for the introduction would be approved when there is a strong chance that the organism would not establish a self-sustaining population (average value ≥ 3 for Question 5 in Table 1). Alternatively, further evaluation would be mandated for those organisms that would likely produce self-sustaining populations, or when evidence is insufficient for making a seasonable prediction.

(f) Level of Review III

This level of review is based on predicting the potential impact of the organism on the ecological integrity of the system(s) where it is proposed for introduction. In addition, the analysis of benefit and risk would include assessing the array of potential impacts on man. Review at this level requires detailed knowledge on the ecological relations of the organism in its native habitat, as well as considerable information on the community structure of the proposed receiving system(s).

(g) Decision Box III

The introduction would be rejected if the available information suggests (average "opinionnaire" values ≥ 2) that the organism would exert a major adverse impact on the receiving system(s) or on man. The proposal would be approved when indications are for the opposite outcomes. If the available information is not considered conclusive, the evaluation should proceed to level at Review IV.

(h) Level of Review IV

Level of Review IV requires development of a detailed literature review based on the format for a Food and Agriculture Organization (United Nations) Species Synopsis. However, additional sections concerning impacts of introduction (documented or potential) would also be required. Once the synopsis is prepared, this information will be sent again to the experts so they can attempt to arrive at a recommendation.

(i) Decision Box IV

On the basis of an analysis of the second round of "opinionnaire" data, the Working Group would either approve or reject the proposed introduction. Additional review (Level V) would be necessary whenever the current data base is not considered sufficient, or if it is unclear whether the introduction is desirable.

(j) Level of Review V

This level of review requires that research be conducted to complete the species synopsis or to assess the potential impact of the introduction on the indigenous flora and fauna and habitats. It might be suggested that research be conducted under controlled conditions near the site where the introduction is contemplated or the Working Group may suggest that all studies be carried out within the organism's native range.

(k) Decision Box V

Using all information collected at this stage, the Working Group should be able to make an informed recommendation regarding the proposed introduction. However, the Working Group may find it necessary to suggest additional research if important questions remain to be resolved. In such a situation, the fifth and final evaluation stage would become a loop of the "Review" and "Decision" models until a recommendation could be made.

APPENDIX III

A. METHODOLOGY FOR PRESENTING TO ICES, PROPOSALS
FOR INTRODUCING OR TRANSFERRING MARINE ORGANISMS

At an early stage prior to introducing or transferring a marine species, the country contemplating such a measure and having found it justified should request advice from ICES on the feasibility of the project in accordance with what is said in the Code of Practice, para 1(a).

The request should have the form of a proposal containing detailed information on the objective, the distribution, biology and ecology of the species in question, known risks regarding introductions or transfers of parasites and diseases, area of origin, place of introduction, possible impact on the natural environment at the introduction site, and protective measures to be taken.

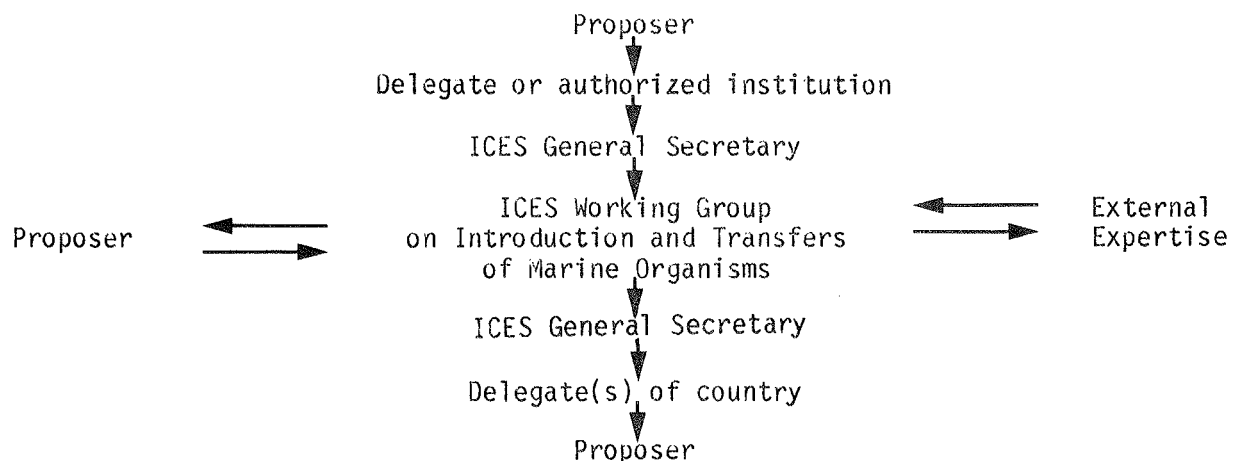
The request should be sent via the ICES delegate(s) of the country (or via an appropriate official body) to the General Secretary of ICES.

The general secretary will forward the proposal to the Chairman of the Working Group on Introductions and Transfers. The group will examine the proposal in detail in accordance with the recommendations and rules in the Code of Practice, the Guidelines and the Manual of Procedure. The work can be carried out by correspondence and, if necessary, in a meeting, preferably the yearly meeting of the group.

When required, the group is free to contact relevant outside expertise. It can also make direct contact with the proposer for further information and consideration. Such discussions may lead to modifications in the original proposal.

After the Working Group has completed its task, its chairman will send back the proposal together with the results of the examination to ICES.

The flow chart below summarizes the handling of a request:



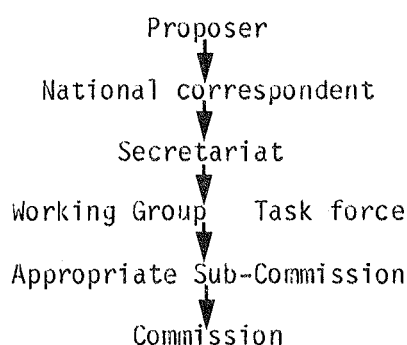
The proposer who gets positive advice to go on with the introduction or transfer should see to it that the implementation of the project is carefully carried out in accordance with the recommendations of the Working Group and the Manual of Procedure in its relevant parts.

The project should further be followed up by at least yearly reports on the activity, sent to ICES and forwarded to the Working Group. Should the group find that there are negative results it should have the opportunity to intervene with new advice or recommendations.

B. PROCEDURE FOR CONSIDERATION BY EIFAC
OF INTRODUCTION OR TRANSFER REQUESTS
(REPRINTED FROM EIFAC TECHNICAL PAPER #44)

- (i) A documented proposal for introduction or transfer of a new species of fish either from a country outside Europe into Europe, or between European countries, should be transmitted via the appropriate Government authority to the National EIFAC correspondent. This assumes that such an introduction would not contravene national laws and that the earlier steps of the Protocol for evaluating introductions would already have been completed by the proposer.
- (ii) The National Correspondent would forward the request to the Secretariat of EIFAC, accompanied by documentation on the scope and purpose of the introduction, the source of the stocking material, the locality into which it is to be stocked and as much detail on the biology and ecology of the species as is available.
- (iii) The Secretariat would transmit this to the Chairman of the Working Group.
- (iv) The Working Group would then consider each request, passing it to a task force if necessary, and would recommend the rejection or acceptance of the proposal or request further information according to the Protocols laid down for evaluating proposed introduction.
- (v) The advice of the Working Group should be transmitted through the appropriate Sub-Commission to the plenary session of EIFAC for endorsement.
- (vi) This decision would then be transmitted by the Secretariat to the National Correspondent who would inform the original proposer.
- (vii) The Commission may consider giving a mandate to the Chairman of the Working Group to directly communicate advice to the proposer via the National Correspondent in exceptional circumstances.

Flow chart for considering request



APPENDIX IV

Certificate of Health of the Origin of Live Fish/Fish Ova

I, _____ as an authorized Fish Health Official of the Federal State Government of _____, certify that the source of live fish/fish ova given below has been inspected by methods approved by the Government of _____ and that no evidence was found of the diseases or disease agents of live fish as required by the Fish Health Regulations of the Government of _____

Source of Live Fish/Fish Ova
and full Postal Address _____

In addition, record any other diseases or disease agents found during the previous two years in farm stock:

Record of the dates of the last four inspections of the site.

Signature in Ink of Certifying Officer _____

APPENDIX IV (continued)
EXPORTER'S DECLARATION

I _____, owner/manager of the site of origin, as recorded below,
(Name in Print)

of all the fish/ova in this consignment which were last inspected on _____
(Date)

declare that no introductions of fish or fish eggs from an uncertified source as
defined by the Fish Health Regulations of the Government of _____
governing the import of live fish/fish ova has been made to this site and that
the shipment described below is derived solely from this site.

The shipment is due to depart _____ on _____ by _____
(City and County) (Date) (Carrier Name)

with anticipated arrival in _____ at _____ on _____ and consists
(Port/Airport) (Date)
of _____

(Species, numbers, age, and size)
Date

Signature of Owner/Manager in Ink

APPENDIX V

GLOSSARY

Applicant - A private or public group or agency, or its representative, which requests permission to introduce or transfer any aquatic organism within or between countries.

Aquaculture - The (commercial) culture or husbandry of aquatic flora or fauna other than:

- (a) the rearing of exotic hobby fish not viable under ambient local conditions
- (b) provincial, state, or federal fisheries enhancement activities
- (c) the use of aquatic organisms for experimental research purposes.

Aquatic organism - any plant or animal growing or living in fresh or salt water.

Bacteria - Extremely small, relatively simple prokaryotic microorganisms traditionally classified with the fungi as schizomycetes.

Biomass - The amount of living matter in the form of one or more kinds of organisms present in a particular habitat.

Broodstock - Specimens of a species, either as eggs, juveniles, or adults, from which a first or subsequent generation may be produced for possible introduction to the environment.

Carrying capacity - The population (as of one species of aquatic organism) that a given area will support without undergoing deterioration.

Competition - More or less active demand by two or more organisms or kinds of organisms at the same time for some environmental resource in excess of the supply available, typically resulting in ultimate elimination of the less effective organism from the particular ecologic niche.

Containment - Sometimes introductions may have adequate health certification but still be viewed as potential ecological risks. To determine the potential of such risks it may ultimately be necessary to establish some animals in an escape-proof situation to carry out tests or for breeding, e.g., to establish monosex or sterile progeny.

The essential features of containment facilities are that:

- a) animals cannot escape and that the regulatory authority agree on the design
- b) the design minimizes any risk of operator error causing animal escape
- c) unauthorized persons cannot gain access and cause the release of contained animals.

Country of origin - (= exporting country)

ICES - the country from which a specific consignment of a species (regardless of its native range) is received.

EIFAC - the country where the species is native.

Country of receipt - (= importing country) The country to which a specific consignment of a species is sent for introduction, transfer, or quarantine.

Current commercial practice - Established and ongoing cultivation, rearing, or placement of an introduced or transferred species in the environment for aquaculture, commercial, or recreational purposes.

Cytopathic - Destruction of cells.

Disease - A deviation from the state of complete physical or social well-being of an organism involving a well defined set of symptoms and aetiology and leading to an impairment of its normal functions. For the purpose of the codes of practice and the protocol document, the word disease is also understood to mean all organisms, including parasites, that cause disease.

Ecology - A branch of science concerned with the interrelationships of organisms and their environments.

Electrophoretic analysis - Analysis of movement of suspended particles through a fluid under the action of an electronegative force applied to electrodes in contact with the suspension.

Epidemiological effect - The effect relating to or involving the incidence, distribution, and control of disease in a population, or: The sum of the factors controlling the presence or absence of a disease or pathogen.

Established species - Species with existing naturally reproductive populations.

Exotic species - (see introduced species)

Fitness - The quality or state of being fit or fitted. Also, a measure of the reproduction success of an individual.

Gamete - Mature germ cell (as a sperm or egg) possessing a haploid chromosome set and capable of initiating formation of a new individual by fusion with another gamete.

Gene - a segment of DNA that occupies a specific position (locus) on a chromosome, is heritable and has one or more specific effects upon the phenotype of an organism.

Gene pool - The elements of the germ plasm of a population serving as specific transmitters of hereditary characters.

Genetics - A branch of biology that deals with the heredity and variation of organisms and with the mechanisms by which these are effected.

Genetic base - The genetic make up and phenomena of an organism, type, group, or condition.

Genetic diversity - All of the genetic variation in an individual, population or species.

Gynogenesis - The production of offspring having all maternal inheritance (all chromosomes and genes obtained from the mother).

Indigenous - Existing and having originated naturally in a particular region or environment.

Introduced species - (= non-indigenous species which includes exotic species) Any species intentionally or accidentally transported and released by man into an environment outside its present range.

Introgression - The entry or introduction of a gene from one gene complex (pool) into another.

In vivo - In that which is alive.

Maintained species - A species of aquatic organism which must be maintained artificially (no natural reproduction) in the environment into which it was introduced or transferred.

Marine species - Any aquatic species that does not spend its entire life cycle in fresh water.

Metabolic by-products - Of, relating to, or worked by metabolism.

Metabolism - The sum of the processes concerned in the building up of protoplasm and its destruction incidental to life.

Niche - The sum of the physical and biotic life-controlling factors (as climate, food sources, water supply, enemies, etc.). A site or habitat supplying these factors characteristically necessary for the successful existence of an organism or species in a given habitat.

Non-indigenous species - (see introduced species) Not originating or developing or not produced naturally in a particular land or region or environment, or; Introduced directly or indirectly from outside into a particular land or region or environment.

Parasite - An organism living in or on another living organism, obtaining from it part or all of its organic nutrient, and commonly exhibiting some degree of adaptive structural modification, usually causes some degree of real damage to its host.

Pathology - Is the study of disease by scientific methods. A pathological condition in an organism is a deviation from normal of known or unknown origin.

Pathogenic - Causing or capable of causing disease.

Polyploidy - State in which a cell or cells of an organism contain three or more haploid sets of chromosomes.

Population - A group of organisms occupying a specific geographic area or biome.

- Predation - The killing and eating of an individual of one species by an individual of another species.
- Prophylactic bath - Medicinal bath that prevents or helps to prevent disease.
- Proponent - One who makes a proposal and who subsequently argues in favour of it. (see applicant)
- Protocol - Detailed outline of methods for adhering to a code of practice.
- Quarantine - A limitation of freedom of movement of individuals of an aquatic species exposed to communicable disease, for a period of time sufficient to test for the presence or absence of a disease.
- Quarantined species - Any species held in a confined or enclosed system that is designed to prevent any possibility of the release of the species, or any of its diseases or any other associated organism into the environment.
- Reproductive strategy - Behaviour patterns in different types of animals by means of which the sperm is brought to the egg and the parental care of the resulting young insured.
- Stock - A population of organisms which, sharing a common gene pool, is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed (Larkin 1972).
- Species - A group of interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1970).
- Transfer - The movement of individuals of a species or population of an aquatic organism from one location to another within its present range.
- Transferred species - (= transplanted species) Any species intentionally or accidentally transported and released within its present range.
- Trophic interaction - Interaction of the feeding level through which the passage of energy through an ecosystem proceeds.
- Virus - A large group of infectious agents ranging from 10 to 250 nanometres in diameter, composed of a protein sheath surrounding a nucleic acid core and capable of infecting all animals, plants, and bacteria; characterized by total dependence on living cells for reproduction and by lack of independent metabolism.

Indication of spine colours

Reports of the Advisory Committee on Fishery Management	Red
Reports of the Advisory Committee on Marine Pollution	Yellow
Fish Assessment Reports	Grey
Pollution Studies	Green
Others	Black

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