

Supplement to Minutes

Agricultural Biotechnology Research Advisory Committee

Performance Standards for Safely Conducting Research With Genetically Modified Fish and Shellfish

Part I. Introduction and Supporting Text for Flowcharts

July 31, 1995



For more information or additional copies, please contact:

Tuskegee University

Founded by Booker T. Washington



School of
Agriculture & Home Economics

August 2, 1995

Dr. Karl N. Stauber
Under Secretary
Research, Education, and Economics
U.S. Department of Agriculture
14th and Independence Avenue S.W.
Washington, D.C. 20250

Dear Dr. Stauber:

Enclosed herewith is a copy of the *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* developed by the Agricultural Biotechnology Research Advisory Committee (ABRAC) which I chair. This was a multi-year effort involving over 200 representatives of the aquatic research community, industry, environmental groups, and natural resource officials.

Domestic aquaculture is a rapidly growing component of U.S. agriculture. Research and development involving genetically modified aquatic organisms present special challenges for assessment of environmental effects and, when necessary, appropriate containment and control. Special credit goes to Dr. Anne Kapuscinski of the University of Minnesota who chaired the ABRAC Working Group of Aquatic Biotechnology and Environmental Safety. Dr. Kapuscinski deftly coordinated divergent views on diverse issues and personally shepherded these performance standards through multiple revisions to their final present form.

The ABRAC, at its meeting of June 26, 1995, approved the following three recommendations concerning implementation of the performance standards.

1. The performance standards should be voluntary and their implementation flexible enough that revised versions can be easily incorporated.
2. Convert the performance standards into a user-friendly computerized expert system for dissemination to the aquatic research community and other interested parties.
3. Sponsor a series of Federal/State workshops to introduce the performance standards to aquatic researchers and State natural resource agency officials.

The ABRAC appreciates the opportunity to develop these science based performance standards for aquatic biotechnology research. ABRAC members sincerely hope that the Department can implement the performance standards so that research and development in this area can move forward and help to maintain the global competitiveness of U.S. aquaculture.

Sincerely,

A handwritten signature in black ink that reads "Walter A. Hill". The signature is written in a cursive style with a large initial "W".

Walter A. Hill, Chair
Agriculture Biotechnology
Research Advisory Committee

Enclosure

Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish

Introduction

and

Supporting Text for Flowcharts

Prepared by

U. S. Department of Agriculture
Agricultural Biotechnology Research Advisory Committee
Working Group on Aquatic Biotechnology
and Environmental Safety

July 31, 1995

TABLE OF CONTENTS

ACKNOWLEDGMENTS	i
INTRODUCTION	1
Purpose	1
Importance of fostering environmentally safe research.....	2
Research and development.....	2
Flexibility of performance standards	2
Encourage research on environmental effects	3
Environmental Safety.....	3
Components of the Performance Standards.....	4
Conversion to an expert system.....	4
OVERVIEW OF PERFORMANCE STANDARDS FLOWCHARTS	4
I. APPLICABILITY OF PERFORMANCE STANDARDS	5
Applicable Organisms	6
Non-applicable Organisms.....	7
Rationale for Applicable and Non-applicable Organisms.....	8
Deliberate gene changes	8
Deliberate chromosomal manipulations.....	9
Deliberate interspecific hybridization	10
Intraspecific selective breeding and captive breeding.....	11
II.A, II.B, II.C. SURVIVAL AND REPRODUCTION ASSESSMENT ..	12
Definitions of Terms.....	12
Early Exit Points.....	13
Assessing survivability of GMO in accessible ecosystems	13
GMOs with ability to disperse.....	14
Isolated accessible ecosystem.....	14
II.A.1. IMPACT OF DELIBERATE GENE CHANGES.....	15
Deliberate Gene Changes Posing No Concern	15
Deliberate Gene Changes Needing Further Assessment	16
Interbreeding with Conspecifics or Closely Related Species.....	17
Potential for interbreeding with closely related species.....	17
Permanent Sterility.....	17
Impact on Threatened, Endangered, or Special Concern	
Populations.....	18
When Genetically Modified Organism is a Non-indigenous	
Species	19

II.B.1. IMPACT OF DELIBERATE CHROMOSOMAL MANIPULATIONS	20
Interbreeding or Mating / Permanent Sterility / Non-indigenous Species	20
Extremely Low Survival of Certain Polyploids.....	21
II.C.1. IMPACT OF INTERSPECIFIC HYBRIDIZATION	21
III. POTENTIAL INTERFERENCE WITH NATURAL REPRODUCTION.....	22
Steroidogenesis and Reproductive Behavior.....	22
Impact on Protected Populations	23
Numbers of GMOs Relative to Potentially Interfered Populations.	23
IV.A. ECOSYSTEM EFFECTS - DELIBERATE GENE CHANGES	24
Familiarity with Overall Performance of the GMO.....	24
IV.A.1. ECOSYSTEM EFFECTS - IMPACTS OF INTROGRESSION OF MODIFIED GENE(S)	25
Estimation of Reproductive Potential.....	26
Estimation of Gene Flow.....	26
Estimation of Fitness of Introgressed Descendants.....	27
Genetic Load of Introgressed Modified Genes	27
IV.B. POTENTIAL BARRIERS ASSOCIATED WITH ACCESSIBLE ECOSYSTEM	28
Familiarity with Reproductive Biology of the GMO.....	29
IV.B.1. ECOSYSTEM EFFECTS - POTENTIAL FOR NON- REPRODUCTIVE INTERACTION	29
IV.C. ECOSYSTEM EFFECTS - IMPACTS OF REPRODUCTIVE INTERFERENCE.....	30
Density-Dependent Factors	30
Assess if density dependence in stock-recruitment can offset population decline.....	30

V. EFFECTS ON ECOSYSTEM STRUCTURE AND PROCESSES	31
Interactions with Threatened, Endangered, or Special Concern	
Populations.....	31
Familiarity with Accessible Ecosystem.....	32
Assess Interactions Between GMOs and Other Organisms	33
Significant interactions.....	33
Consideration of other organisms.....	34
Assess Potential for Interactions to Adversely Alter Ecosystems...	34
Decreased predictability of ecosystem state.....	35
Degraded state of ecosystems	35
VI. RISK MANAGEMENT RECOMMENDATIONS PROJECT SITING, DESIGN, OPERATIONS, AND REVIEW	36
Introduction.....	36
Case-specific approach complemented by review	36
Research projects versus commercial operations	37
Project Siting	37
Different criteria for freshwater and marine sites	37
Project siting to avoid certain risks.....	38
Design of Barriers.....	39
Physical or chemical barriers.....	40
Mechanical barriers	40
Biological barriers.....	40
Scale of experiment as a barrier.....	41
Barriers for all possible escape paths of the water system ...	41
Prevent escape via non-aquatic paths.....	42
Security.....	43
Alarms	44
Stand-by Power.....	45
Operational Plan.....	45
Training	45
Traffic control	46
Record keeping	46
Emergency response plan	46
Peer Review and Site Review.....	48
Peer review prior to start-up of project.....	48
Site reviews after start-up of project.....	49
Documentation to submit to proposal and site reviewers	49
Project approval.....	49
LITERATURE CITED	50
GLOSSARY OF TERMS.....	58

APPENDIX A	
EXCERPTS FROM THE AQUATIC NUISANCE SPECIES PROGRAM (ANS)	A1
APPENDIX B	
ASSESSMENT OF GENETICALLY MODIFIED FINFISH AND SHELLFISH WITH NON-DIOECIOUS MODES OF REPRODUCTION.....	B1
APPENDIX C	
CHEMICAL STERILIZATION OF SEA WATER.....	C1
APPENDIX D	
SCHEMATIC DIAGRAMS OF EXAMPLES OF MECHANICAL BARRIERS.....	D1
APPENDIX E	
ABRAC WORKING GROUP ON AQUATIC BIOTECHNOLOGY AND ENVIRONMETNAL SAFETY	E1
APPENDIX F	
PARTICIPANTS IN THE WORKSHOP ON PERFORMANCE STANDARDS FOR RESEARCH WITH GENETICALLY MODIFIED FISH AND SHELLFISH.....	F1

ACKNOWLEDGMENTS

The Performance Standards and associated documents are the outcome of a broad interdisciplinary collaboration involving numerous individuals. The Working Group on Aquatic Biotechnology and Environmental Safety (Appendix E) prepared the first draft of the Standards, convened an international workshop to seek interdisciplinary input into development of the Standards, and guided revisions of the text, flowcharts, and worksheets. The Agricultural Biotechnology Research Advisory Committee, including members appointed in 1992 and 1994, provided important guidance at key steps in this project. Two students at the University of Minnesota made significant contributions: Craig Acomb initiated development of the Flowcharts; and Carolyn Carr contributed significantly to completion of the Flowcharts and to Appendix B. Approximately 100 participants at the 1993 workshop (Appendix F) contributed the majority of the comments upon which this final draft and accompanying documents are based. A number of other individuals from the United States and other countries submitted additional comments. Wendilea LeMay and Julie Karels (University of Minnesota, Dept. of Fisheries and Wildlife), and Eva Russnack (USDA, Office of Agricultural Biotechnology) did the majority of the crucial clerical tasks.

This project was generously supported by the following organizations: the U. S. Department of Agriculture, Office of Agricultural Biotechnology, Cooperative Agreement No. 92-COOP-2-8023; the Minnesota Legislature, ML 1993, Chapter 172, Sec. 14, Subd. 12(o), as recommended by the Legislative Commission on Minnesota Resources from the Minnesota Environment and Natural Resources Trust Fund; Minnesota Sea Grant College Program supported by the NOAA Office of Sea Grant, Department of Commerce, under Grant No. NA90AA-D-SG-149, Journal Reprint No. 400; the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program supported by the NOAA Office of Sea Grant, Department of Commerce, under federal Grant No. NA90AA-D-SG045; the North Central Regional Aquaculture Center Program under Grant No. MISU/61-4082S from the U.S. Department of Agriculture; and the Department of Fisheries and Wildlife, University of Minnesota. This is article 21,753 of the Minnesota Agricultural Experiment Station Scientific Journal Series.

INTRODUCTION

The growing consumer demand for affordable, high quality seafood and the need to protect marine and freshwater resources from overharvest has led to increased interest in aquaculture research targeted to improve performance traits in economically important species and to address basic issues such as those related to biodiversity and sustainable utilization of aquatic resources. Additionally, aquatic organisms are increasingly used as model species for biomedical and environmental pollution research. Advances in biotechnology provide new opportunities for researchers to address these human needs and interests.

Purpose

These voluntary Performance Standards are intended to aid researchers and institutions in assessing the ecological and evolutionary safety of research activities involving genetically modified fish, crustaceans, or molluscs. Where the need is identified, they are also intended to aid researchers in developing appropriate risk management measures so that the research can be conducted without adverse effects on natural aquatic ecosystems. By helping investigators to systematically address limited knowledge and manage risks, if any are identified, the Performance Standards are *intended to expedite* research and development involving genetically modified aquatic organisms. They are *not* intended to impede investigators from proceeding with such work.

As is the case for planning all research activities, scientists must consider the safety/risks of their research, and appropriately design and manage their research to minimize adverse effects. The scientific community would benefit from technical guidance that addresses the assessment of specific safety concerns for research with genetically modified fish, crustaceans, and molluscs and that provides scientific principles of safe research management. Technical guidance that has broad support throughout the scientific community would help stimulate the research needed for aquaculture to meet growing consumer demands and for addressing biomedical and environmental pollution problems by reducing current uncertainty regarding acceptable standards for conducting that research. Such standards also would assure the public that appropriate guidance is available to the research community to address ecological and evolutionary safety concerns. By focusing on the specific needs of research with genetically modified fish and shellfish, these standards build on the approach of a more general document, "Guidelines for research involving planned introduction into the environment of genetically modified organisms" (ABRAC 1991).

Importance of fostering environmentally safe research

Facilitation of environmentally safe research, through use of these Performance Standards, is particularly important because of three features of fish, molluscs, and crustaceans. First, these research organisms are wild-type or nearly so. These organisms are often hatched from gametes collected in the wild. To date, the domestication of populations or genetic strains of aquacultural species is insufficient to prevent escaped individuals from surviving under natural environmental conditions. Second, the United States is the origin of diversity of numerous fish and shellfish species that are of interest in research and development involving genetic modification. Protection of this natural diversity at genetic, population, and species levels is of paramount importance because aquatic biodiversity in the United States has suffered dramatic declines (Miller et al. 1989, Williams et al. 1989, Williams and Mulvey 1994, Norse 1994). Of the remaining aquatic biodiversity (reviewed by Hughes and Noss 1992), 27% of the fish fauna is endangered, threatened, or of special concern; nearly 50% of all mussel species are currently listed or proposed for listing, as threatened or endangered, under the Endangered Species Act; and two thirds of North America's crayfish species are rare or imperiled. Third, many natural populations of fish, molluscs, and crustaceans are themselves of tremendous economic importance, either because of commercial fishing, sportfishing, or other recreational activity. In other words, economic activity and importance is not restricted to aquacultural stocks of fish and shellfish.

Research and development

These voluntary Standards are designed to apply to research and development conducted in the public and private sectors using the applicable organisms addressed below. Although information in the Standards may provide a useful starting point for evaluating the environmental safety of intentional environmental introductions in commercial aquaculture or in fisheries management programs, these activities will require additional considerations beyond those addressed in these Standards.

Flexibility of performance standards

The term "Performance Standards" appears in the name of this document to convey certain attributes of the intended guidance and to distinguish the guidance from that usually provided by a "Design Standard." Performance standards define endpoints or goals to be achieved, and they provide guidance and criteria for achieving those goals. They differ from a design standard in that they are not rigid and prescriptive. A performance standard provides flexibility to choose the best and most appropriate method of achieving the goals and meeting the criteria. To ensure this flexibility, performance standards are structured to accommodate a dynamic, rapidly changing state-of-the-art.

Encourage research on environmental effects

For a number of research or development projects involving genetically modified fish or shellfish, contemporary knowledge is insufficient to clearly determine if the project is environmentally safe. The Performance Standards are designed to identify such cases and provide recommendations on how to conduct appropriately confined laboratory experiments or outdoor experiments.

Application of the Performance Standards should encourage the conduct of safe research to address important information gaps about environmental effects of particular genetically modified fish and shellfish and facilitate safe development of these modified organisms. Refer to Hallerman and Kapuscinski (1993) for further discussion on conducting confined research on environmental effects.

Environmental Safety

These Performance Standards only address issues related to environmental safety with respect to genetic effects on natural populations of aquatic organisms and ecological effects on aquatic ecosystems. Researchers needing guidance on issues related to food safety should consult the U.S. Food and Drug Administration and published guidelines, such as the recent publication of the Organization of Economic Cooperation and Development, "Safety Evaluations of Food Derived from Modern Biotechnology: Concepts and Principles" (OECD 1993). However, when questions of food safety are outstanding, researchers may find the risk management recommendations useful (see Section VI of this text). If approval or execution of the project is a federal action requiring compliance with the National Environmental Policy Act (NEPA), the completed worksheet (described below) could be the basis for NEPA documentation on environmental effects.

Certain states and many institutions require that experiments involving organisms bearing recombinant DNA molecules comply with the National Institutes of Health (NIH) "Guidelines for Research Involving Recombinant DNA Molecules" (NIH 1994), including the most recent amendments (e.g., NIH 1995), and be approved by an Institutional Biosafety Committee, biosafety officer, or other body. All federally-funded research must comply with these NIH Guidelines. In initiating projects involving organisms bearing recombinant DNA, researchers need to contact their Institutional Biosafety Committee or biosafety officer for guidance on complying with NIH Guidelines. These Performance Standards are intended to further assist all researchers working with fish and shellfish in complying with the NIH guidelines and good safety practices. (See the related discussion in section VI "Risk Management Recommendations: Project Siting, Design, Operations, and Review" under the sub-heading "Review prior to start-up of project.")

Components of the Performance Standards

The Performance Standards consist of three interrelated documents. First, the **Flowcharts** provide the decision making pathway for assessment and management of research projects. Second, the **Supporting Text (this document)** provides scientific background for the questions and alternative decisions in the Flowcharts, presents more detailed risk management recommendations, and provides a glossary of scientific terms and other supporting appendices. In navigating through the **Flowcharts**, researchers need only read the portions of the **Supporting Text** below that correspond to flowcharts and questions applicable to their project. Third, a **Worksheet**, once completed by the researcher, traces a researcher's decision path through the Flowcharts, provides supporting documentation for these decisions and, where appropriate, describes the rationale for the project's risk management measures.

Conversion to an expert system

The ABRAC Working Group on Aquatic Biotechnology and the full ABRAC have recommended conversion of the three components of the Performance Standards into one interactive, computerized decision support tool. The U. S. Department of Agriculture, Office of Agricultural Biotechnology has a prototype computerized version and is exploring full conversion. A computerized version would be less cumbersome because the user responds to prompted questions on a screen instead of leafing through various printed flowcharts. Explanatory text, literature citations, and a glossary would be accessible from any point in the decision making path. The computer program automatically generates a trace of the user's path through the decision questions, thus automating completion of most of the Worksheet. A computerized version of the Standards would be easier to update and disseminate.

OVERVIEW OF PERFORMANCE STANDARDS FLOWCHARTS

The Flowcharts begin with an Overview that schematically summarizes the major pathways. If the Performance Standards are applicable to the genetically modified organism (GMO), as addressed in Flowchart I and supporting text, the researcher is directed to one of three assessment pathways. GMOs produced by deliberate changes to single genes are first assessed (Flowcharts whose title begin with II.A), but the questions are designed to also assess cases where the GMO contains both single gene modifications and other modifications (chromosomal manipulation or interspecific hybridization). If the pathway for deliberate gene changes is bypassed, the researcher is directed to assessment of deliberate chromosomal manipulations (Flowcharts whose title begin with II.B); the questions are designed to also assess cases where the GMO results from

chromosome manipulations and an interspecific hybridization. If this latter pathway is bypassed, the researcher is directed to assessment of interspecific hybrids (Flowcharts whose title begin with II.C).

Each assessment pathway begins with Survival and Reproduction Assessment (Flowcharts whose titles begin with II or III). This portion of the assessment poses questions that are easier to answer, in most cases, than the questions that appear later under Ecosystems Effects Assessment (Flowcharts whose titles start with IV. or V.). Use of Survival and Reproduction Assessment leads the researcher to one of four possible conclusions:

1. a specific risk is identified and the researcher is led to Flowchart VI.A which guides management of that risk;
2. information is insufficient to answer an essential question in the assessment, so the researcher is directed to risk management (Flowchart VI.B);
3. a specific reason for safety of the research is identified and the researcher is directed to EXIT the Standards; or
4. additional information is needed to determine risk or safety and the researcher is directed to proceed to the appropriate section of Ecosystem Effects Assessment.

Questions posed under Ecosystem Effects Assessment require more knowledge about evolutionary and ecological issues than the earlier assessment questions. This section addresses the overarching question: if GMOs did end up in an accessible ecosystem, are adverse effects possible or is there a specific reason to rule out such concern? Use of this section leads to one of the first three conclusions listed above. Thus, certain projects will EXIT the Standards whereas others will proceed to risk management (Flowchart VI.A or VI.B).

I. APPLICABILITY OF PERFORMANCE STANDARDS

Researchers begin by using Flowchart I to quickly determine whether or not the Performance Standards apply to the research organisms in question. If the conclusion is that they do not apply, then the researcher has completed voluntary compliance with the Standards and exits at this point. If the conclusion is that the Standards do apply, researchers should proceed to subsequent flowcharts as directed. However, if preliminary perusal of the relevant flowcharts indicates that researchers lack most of the required information, they may wish to proceed directly to flowchart VI.B., which guides risk management when there is insufficient information.

All subsequent Flowcharts are designed to address organisms with a dioecious mode of reproduction because this is the most common mode among species of finfish, crustaceans, and molluscs. In cases of research involving organisms with non-dioecious modes of reproduction, researchers are directed to Appendix B for specific guidance. Two non-dioecious forms, self-fertilizing hermaphrodites and true parthenogens, can establish an entire population from one accidental escapee. Researchers working with such organisms need to consult Appendix B. Other non-dioecious forms can be assessed with the Flowcharts provided that researchers follow the general guidance given in Appendix B.

Regarding finfish, all subsequent Flowcharts and Appendix B apply both to non-bearers and bearers (see glossary definition for bearers). If the proposed project involves a bearing species, particularly an internal bearer, users should keep in mind the following issue when answering questions on all subsequent Flowcharts. Escape of a single, bearing adult fish could result in eventual release of an entire brood of progeny from the one adult, thus increasing the possibility of establishing an entire population of genetically modified descendants.

Applicable Organisms

Genetic modification may alter attributes of the organism that affect its interaction with its environment or create new attributes that affect its safety as addressed by questions in the Flowcharts. Any proposal to create or use genetically modified aquatic organisms should characterize: the method of genetic modification; the molecular characterization (where possible) and stability of the modification; and the expression, functions, and effects of the genetic modifications. Although the process of modification alone is not a determinant of risk or safety, such information can facilitate a determination of whether the modification decreases, increases, or has no effect on environmental safety.

Except as listed in the next section below, the standards apply to freshwater and marine finfish, crustaceans and molluscs whose genomic structure has been deliberately modified by human intervention. In order to direct researchers to appropriate questions and circumvent unnecessary questions in subsequent flowcharts, Flowchart I refers to three categories of deliberately induced changes in genomic structure:

- (1) Deliberate Gene Changes - including changes in genes, transposable elements, non-coding DNA (including regulatory sequences), synthetic DNA sequences, and mitochondrial DNA;

(2) Deliberate Chromosomal Manipulations - including manipulations of chromosome numbers and chromosome fragments; and

(3) Deliberate Interspecific Hybridization (except for non-applicable cases discussed below) - referring to human-induced hybridization between taxonomically distinct species.

Non-applicable Organisms

The standards **do not** apply to organisms whose genomic structure has been modified by humans **solely** by the following means:

(a) intraspecific selective breeding by natural reproductive processes or intraspecific captive breeding, including use of artificial insemination, embryo splitting or cloning; and

(b) interspecific hybridization provided that (i) the hybrid is known to be widespread because it occurs naturally or has been extensively introduced (e.g., through stocking) in the environments accessible to organisms escaping from the research site, and (ii) there are no indications of adverse ecological effects associated with the specific hybrid in question.

Research projects involving genetically modified organisms which meet the applicability criteria (see section above) will not necessarily require precautions beyond those normally practiced in research. Some projects, depending upon combined characteristics of the organism and accessible ecosystems, may be found early in the assessment to have a safety attribute allowing exit from the standards; i.e., further use of the standards for the proposed research is not necessary (see Flowcharts II.A, II.B, and II.C).

Some organisms not included in the applicability criteria also may pose significant environmental risk (e.g., exotic or nuisance species whose genome has not been deliberately modified, or organisms bearing pathogens). Guidance exists elsewhere to address these problem areas. Specifically, researchers should contact the relevant state and federal natural resource management agencies which have authority over fisheries resources found in the state where the proposed research will occur. The fisheries staff in the state agency should include a staff person who is knowledgeable about both state and federal oversight of research with non-indigenous or nuisance species. It is not the intent of these Standards to address all introductions of fish and shellfish species. Rather, the intent is to provide specific guidance regarding the effect of structural, genetic modification on environmental safety and to promote safe research with such organisms.

Rationale for Applicable and Non-applicable Organisms

In defining genetically modified organisms for which use of the Standards is appropriate, clear objective criteria were sought that can be readily applied *a priori* to conducting a comprehensive risk assessment such as that embodied in the Standards. The objective is to make the Standards applicable to those modified organisms more likely to express novel hereditary traits or otherwise present a new genotype for which there is very little familiarity and experience to predict environmental safety.

A novel trait is one that does not occur in natural populations of the parental species of the genetically modified organism. A novel trait may be (1) expression of a compound not normally found in the species, e.g., antifreeze polypeptide in Atlantic salmon, or the coat protein of infectious hematopoietic necrosis virus (IHNV) in Pacific salmon; or (2) a clearly novel value in a quantitative trait, such as changes in: a metabolic rate; reproductive fertility; tolerance to a physical environmental factor; a behavior; resource or substrate use; or resistance to disease, parasitism, or predation (Kapusinski and Hallerman 1991).

Deliberate gene changes

A novel trait resulting from expression of a compound not normally found in the species is most likely to be produced via addition or substitution of a gene, chromosome, or chromosome segment; the latter two cases are discussed in the section below on chromosomal manipulations. Gene transfer also may give rise to mosaics in the parent generation with associated uncertainty about germline transmission to progeny.

A novel trait might also arise from alteration of copy number of genetic material, such as expression of an introduced copy of a gene already present in the genome of the host species (e.g., a gene for a hormone or other growth factor) if the new gene copy is under novel regulatory control. Therefore, not only the structural gene, but also the *regulatory elements* of the introduced genetic construct are at issue in determining whether or not the modified organism presents a novel trait.

The possibility of novel regulatory control of gene expression is also posed by novel pleiotropic or epistatic effects of the introduced genetic construct. The literature contains many examples of modifications where inserted DNA sequences did not act in the new host as they did in the donor organism or where alterations in one part of the genome caused surprising activity in other parts of the genome. For example, novel pleiotropies of introduced genes have been observed in genetically modified livestock (Marx 1988, Pursel et al. 1989). Novel regulation of gene expression has been linked to altered methylation of

host regulatory elements (MacKenzie 1990), and is posed by trans-activation of an inactive host gene by the action of introduced genetic elements.

Genomic rearrangements such as translocations and inversions occur randomly in nature. They involve no new genetic material, although these rearrangements can be deleterious and reduce the organism's fitness. Humans can deliberately induce genomic rearrangements through the use of recombinant DNA technology, ionizing radiation, radiomimetic chemicals, or other physical treatments. The use of non-ionizing radiation, heat, or chemicals with subsequent targeted selection of progeny is another way of producing modified organisms exhibiting certain desired traits. Deliberately induced targeted changes, depending on the resultant phenotype, may present a higher level of risk than the random events occurring in nature. Deliberately induced genomic rearrangements, on average, are less likely to revert to the state that existed prior to the change and their impacts on fitness are less certain, although the intent is to maintain high fitness of the modified organism in environments of its intended use. Until there is improved familiarity with the characteristics of finfish, molluscs, and crustaceans bearing deliberately induced genomic rearrangements, it is considered prudent to proceed to the next step in the Flowcharts so that potential risk can be assessed on a case-by-case basis.

Deliberate chromosomal manipulations

Generally, the intended utility of producing chromosomally manipulated finfish or shellfish (e.g., triploid and tetraploid organisms) is to improve desirable product characteristics or to reduce environmental risk as a consequence of sterility. The risks such organisms pose to natural ecosystems differ as a function of their degree of: sterility/fertility and viability (see Flowchart II.B.1), involvement in mating behavior (see Flowcharts III and IV.C), and the nature and degree of phenotypic change (see suggestion in Flowchart IV.C to use Flowchart V). Further discussion of these factors appears in Hallerman and Kapuscinski (1993). The process of chromosomal manipulation may yield a mosaic individual in which some but not all cells, possibly even germline cells, contain different, paternal chromosome fragments. This can occur, for example, when irradiated sperm from the same or different species is used for chromosome-mediated gene transfer, and the resulting fertilized eggs are then manipulated experimentally to yield gynogenetic diploids (Thorgaard et al. 1985, Disney et al. 1987). This makes it hard to predict the genotype and phenotype of descendants.

Although the sterility offered by inducing triploidy in some aquatic species reduces environmental concerns about a modified organism, the issue of safety is complicated by three factors. First, the effectiveness of triploidy induction varies among species and the methods used. Second, although triploids are functionally sterile, the males may exhibit spawning behavior with fertile diploid females,

leading to losses of entire broods and lowering of reproductive success. Third, in cases where large numbers of individuals are released, sufficient numbers of sterile triploids may survive and grow for an indeterminate number of years beyond the normal life span to pose heightened competition with diploid conspecifics or predation upon otherwise invulnerable prey (Kitchell and Hewitt 1987). In some cases, such prey may be juvenile conspecifics. The assessment path through Flowcharts II.B.1, III., IV.C, and V. is designed to address these three factors.

Tetraploid individuals in natural systems pose a potential risk through mating with normal diploids, yielding all triploid progeny (see Flowchart II.B.1). Large numbers of such matings, resulting in large numbers of sterile individuals in the ecosystem, pose competition with and reduced reproductive success of normal diploids, increasing the risk of extinction of the affected populations.

In spite of these potential concerns, induced sterilization through chromosomal manipulation can be helpful in research projects (and in commercial aquaculture systems) because it reduces the risk of escapees introgressing into natural gene pools. Because a number of factors have to be considered in assessing environmental effects -- factors addressed by subsequent Flowcharts -- the Standards are applicable to chromosomally manipulated organisms as a general class. Sterility and scale of the proposed research, as addressed in Flowcharts II.B, II.B.1, and III, may allow early exit from further use of the Standards in specific cases.

Deliberate interspecific hybridization

Interspecific hybridization has led to the development of new stocks for commercial aquaculture and for fisheries stocking programs. However, the release of fertile interspecific hybrids into an ecosystem containing either or both of the parental species or other closely related species with which the hybrid can interbreed introduces the possibility of introgressive hybridization (see Flowchart II.C.1). Interspecific hybridization is quite common in fishes (Turner 1984, Collares-Pereira 1987). Interspecific hybrids are known to occur in at least 56 families of fishes (Lagler 1977). Natural occurrences of interspecific hybrids and backcrossed descendants usually are at low frequencies but stocking of hybrids or of either parental species can substantially increase these frequencies. For example, instances of backcrossing to striped bass were observed following the stocking of white x striped bass (*Morone chrysops* x *M. saxatilis*) hybrids into the Savannah River system (Avisé and Van den Avyle 1984). In Lake Palestine, Texas, 29% of the *Morone* individuals screened were not first-generation (F_1) hybrids, but second generation (F_2) backcross hybrids with white bass (Forshage et al. 1988). Evidence of introgressive hybridization in commercially important

Chesapeake Bay stocks of striped bass has been documented (Harrell et al. 1993), presumably due to interbreeding with hybrid striped bass originally stocked in reservoirs on tributary rivers.

Introgressive hybridization compromises the genetic integrity and taxonomic distinctness of native species occurring in natural aquatic ecosystems, and can lead to loss of the genetically distinct species in the ecosystem (Campton 1987, Leary et al. 1995). Concern about such introgressive hybridization is heightened when the affected species are threatened, endangered, or of special concern; the Performance Standards recommend risk management in this situation (see Flowcharts II.C.1 and VI.A.). Among the 86 species, subspecies, and populations of U. S. fish listed as threatened or endangered under the Endangered Species Act (ESA) through 1991, species introductions were a contributing factor to the decline of 28 fish species, and nine of these species were threatened by interspecific hybridization (Wilcove et al. 1992). Another analysis of fish species listings under the ESA reached similar conclusions (Lassuy 1995). If the overall performance of interspecific hybrids is novel compared to that of the parental species, these hybrids and their introgressed descendants also present the potential for adverse effects on ecosystem structure and processes.

A number of fertile and sterile interspecific hybrids of fish, mollusc, and crustacean species are produced in nature, although usually at low frequencies. Sterility in hybrids occurs as a consequence of combining incompatible genomes, although rarely is sterility an absolute quality rather than a quantitative or probabilistic quality. Releases of sterile hybrids can disrupt spawning of parental species' populations (see Flowchart III), and depending on their phenotype, may trigger a decline in affected populations (see Flowchart IV.C) or alter competition and predation in an ecosystem with adverse effects on ecosystem structure and processes (see Flowchart V).

Where a naturally-occurring or a stocked interspecific hybrid is known to be widespread in the ecosystems accessible via the proposed research site, and when there is no indication of adverse effects on the ecosystem associated with that hybrid, there should be little concern about accidental escapes from research and development projects involving that hybrid. Only when the research and development involves either a hybrid with unfamiliar, new genotypes or novel hereditary traits, a new hybrid with which there is little familiarity and experience, or a hybrid recognized as a nuisance species, is it necessary and appropriate that the researcher consider the guidance provided in these Standards.

Intraspecific selective breeding and captive breeding

Offspring of parents subjected to intraspecific selective breeding and captive breeding do not contain new alleles or additional loci and, therefore, they are not

likely to exhibit novel, unfamiliar traits. Changes in frequency distributions of alleles or complete loss of alleles at the population level are the only genetic effects of intraspecific selective breeding and captive breeding, and the extent of the trait effect is limited by the ends of a binomial distribution of allele frequencies (or penetrance of the phenotypes). Changes in allele frequency can be environmentally significant, depending on phenotype, when the change is present in progeny at a high enough frequency and such progeny are introduced into a small population. Such changes are relevant in fisheries stocking programs which contemplate releases of large numbers of organisms into natural aquatic ecosystems. They also should be considered before production in commercial aquaculture systems from which selectively bred organisms might escape.¹ However, there is far less concern in the research and development phase where large, repetitive releases are not intended or likely to occur.

Including this large category of organisms in the Performance Standards would impose an unnecessary burden of assessment on the private and public research community for a class of modified organisms which generally poses little or no risk under conditions normally practiced in research and development. Intraspecific selective breeding has been practiced for centuries, and there is no compelling reason to believe that additional guidance is needed in this area.

II.A, II.B, II.C. SURVIVAL AND REPRODUCTION ASSESSMENT

Flowcharts II.A, II.B, and II.C, and their subordinate flowcharts, are designed to allow assessment of organisms bearing one or more of the genetic modifications covered by the Standards. For instance: a transgenic fish with induced triploidy is assessed by proceeding through Flowcharts II.A, II.A.1, and subsequent paths; an interspecific hybrid with induced triploidy is assessed by proceeding through Flowcharts II.B, II.B.1, and subsequent paths; and a fish modified solely by interspecific hybridization is assessed by proceeding through Flowcharts II.C, II.C.1, and subsequent paths.

Definitions of Terms

In using these and all subsequent Flowcharts, researchers should refer to supporting text for Flowchart I for extensive definitions of the terms (1) deliberate change of genes, (2) deliberate chromosomal manipulations, and (3)

¹ Environmental effects of selective breeding and captive breeding, with respect to fisheries stocking programs and commercial aquaculture, are under active discussion in other aquatic resource fora in the United States (e.g., Aquatic Nuisance Species Task Force 1994, Schramm and Piper 1995).

interspecific hybrid / hybridization. Refer to the glossary for definition of the term, accessible ecosystem.

Early Exit Points

These three flowcharts are designed to identify projects that can exit the Standards at this point before proceeding to more difficult questions about the biology and ecology of the GMO. It is likely that the most commonly used EXIT will be the one due to knowledge that accessible ecosystems preclude survival of any accidentally escaped GMOs. It may be desirable to consider this early possibility for exiting the Standards when making long-range plans for siting of research projects (see further discussion under Section VI., "Project siting to avoid certain risks").

Assessing survivability of GMO in accessible ecosystems

Familiarity with the parental organism, particularly results from past experiences with stocking of the parental organism, can provide partial guidance for answering this question. Such results may indicate the range (broad vs. narrow) of environmental conditions under which the parental organism has survived, thus giving a sense of the potential survival range for the modified organism.

Particular attention should be paid to cases where survival and persistence occurred *contrary to expectations* because these indicate the potential for unexpected results with the modified organism. For instance, releases of pink salmon have shown that genetically modified pink salmon could survive, reproduce, and persist in a broader range of accessible ecosystems than would be expected from studies of their biology in their native range. In spite of assumptions that smolts and immature adults could not survive in fresh water, the Laurentian Great Lakes experienced population explosions of pink salmon two decades after 21,000 juveniles were flushed down the drain of a Lake Superior hatchery (Kwain and Lawrie 1981, Emery 1981 - reviewed by Kapuscinski and Hallerman 1981).

A thorough review of the life history and environmental requirements of the parental organism is needed in order to determine the potential effects of the genetic modification on the modified organism's tolerances for physical/chemical parameters (temperature, salinity, pH, dissolved oxygen, etc.). The tolerance of a species to combinations of physical factors is more difficult to assess than tolerances to individual parameters, but if such information is available, it should be evaluated. The distribution of the parental species also may be controlled under natural conditions by biological factors (e.g., habitat, predators, pathogens, nutrient requirements) which may or may not be able to regulate the abundance of the modified organism, especially if the receiving ecosystem is highly modified

by human activities. Therefore, the *zone of tolerance* of the modified organism to physical and chemical factors should be the primary consideration in evaluating its potential to become established in accessible ecosystems. An important information source for determining the modified organism's zone of tolerance is physiological data on lower and upper lethal limits for environmental factors (e.g., water temperature, pH, dissolved oxygen, other inorganic or organic concentrations). These lethal limits set the lower and upper boundaries of the environmental conditions under which the organism can survive; that is, they define the organism's zone of tolerance. It is imperative, therefore, to assess whether or not: (a) the zone of tolerance of the modified organism has expanded beyond one or both of the lethal limits of the parental organism; and (b) in cases where the zone is expanded, the modified organism will survive in accessible environments which are lethal to the parental organism. For instance, if a transgenic fish exhibits a lower lethal temperature limit than its parental counterpart, it is important to know whether or not the minimum water temperature in accessible ecosystems is within the zone of tolerance of the transgenic organism.

GMOs with ability to disperse

The modified organism's potential for delayed mortality and dispersal to more suitable ecosystems must also be assessed to answer the questions about direct and indirect access to suitable environments. In certain situations, the modified organism might not die after entering the accessible ecosystem but might persist until lethal conditions arise, e.g., tilapia may persist a number of months in temperate zone ecosystems until water temperatures decline at the onset of winter. This delayed mortality could give the organism time to disperse to more distant ecosystems where it can survive and reproduce. In such cases, it is also important to assess environmental effects of the GMOs during the period of their persistence in the directly accessible ecosystem. Therefore, researchers contemplating projects posing such potential should respond "Yes or unknown" to the question about suitable ecosystems being indirectly accessible; they *should not exit* the Standards at this point.

Isolated accessible ecosystem

The question regarding isolated ecosystems is worded to fit situations where the research is conducted in a confined system (indoors or outdoors) but not directly in an isolated natural system. Some researchers using these Performance Standards, however, may wish to conduct experiments directly in an isolated, artificial water body in order to collect information needed to assess the more difficult questions about environmental effects of a certain GMO (e.g., to answer some of the questions in Flowcharts IV. through V.).

To obtain some of this important information, it will be necessary to conduct experiments on potential ecological effects in relatively large, outdoor, artificial aquatic systems which are isolated from natural and semi-natural systems and from which the experimental organisms can be eliminated once the experiments are completed. Examples might include isolated reservoirs or ponds or an abandoned quarry with no outlet. Research projects located in such an isolated site should proceed through the Flowcharts, treating the isolated, artificial waterbody as their project site and rearing unit if they are directed to risk management recommendations. Research in such sites should proceed only if: (1) it is feasible and allowed by the appropriate aquatic resource management agency to destroy all GMOs (and perhaps all aquatic life in the system) upon completion of the experiment; (2) the isolated aquatic system being used is not a live gene bank for any rare, threatened, or endangered species; and (3) it is feasible to implement adequate risk management measures not only for the fairly predictable events, such as annual flooding, but for infrequent major disasters such as the 1993 flooding of the Mississippi River or the recent hurricanes in Florida and Hawaii (see further discussion of disaster preparation under several subheadings of Section VI., "Risk Management Recommendations").

II.A.1. IMPACT OF DELIBERATE GENE CHANGES

This Flowchart is designed to assess organisms bearing a deliberate gene change and possibly bearing one or more additional genetic modifications (see additional explanation above on "Overview of Flowcharts").

Deliberate Gene Changes Posing No Concern

The following information about the GMO is needed to answer "yes" to the first question on the flowchart: molecular characterization and stability of the deliberate gene modification, and the expression, functions, and effects of all the deliberate, induced genetic modifications. With this information in hand, this assessment path can be bypassed if the only change is expression of a marker gene that has no impact on traits identified in Table 1 (below). In order to bypass this assessment path, however, researchers cannot simply assume that the marker gene has no effect on the physiology or fitness of the GMO but rather need to test directly for effects of expression of the marker gene. For instance, the pesticidal property of a baculovirus against the cabbage looper, *Trichoplusia ni*, was reduced when a recombinant form of the virus bearing the bacterial lac Z gene and expressing the marker, β -galactosidase, was tested (Wood et al. 1993).

Deliberate Gene Changes Needing Further Assessment

If the project involves a GMO for which the researcher cannot rule out expression of one of the trait changes listed in Table 1, further assessment is needed in order to reach a defensible decision about safety or risk. These phenotypic changes might pose environmental risk, depending on other factors

Table 1. Classes, examples, and possible ecological effects of phenotypic changes in genetically modified fish, crustaceans, and molluscs. For projects involving GMOs expressing one or more of these phenotypic changes, continue assessment (proceed to the appropriate step in the Flowcharts) in order to reach a defensible decision about safety or risk.

Class	Examples of Phenotypic Change	Ecological Effect
Metabolism	<ul style="list-style-type: none"> - Growth rate - Energy metabolism - Food Utilization 	<ul style="list-style-type: none"> - Shift to different prey size - Alter nutrient and energy flows
Tolerance of Physical Factors	<ul style="list-style-type: none"> - Temperature - Salinity - pH - Pressure 	<ul style="list-style-type: none"> - Shift preferred habitats - Alter geographic range
Behavior	<ul style="list-style-type: none"> - Reproduction - Territoriality - Migration - Chemosensory (including pheromones, allelochemicals) - Swimming/navigation 	<ul style="list-style-type: none"> - Alter life history patterns - Alter population dynamics - Alter species interactions
Resource or Substrate Use	<ul style="list-style-type: none"> - Food utilization 	<ul style="list-style-type: none"> - Release from ecological limits - Alter food webs
Population Regulating Factors	<ul style="list-style-type: none"> - Novel disease resistance - Reduced predation/parasitism - Habitat preference 	<ul style="list-style-type: none"> - Alter population and community dynamics - Release from ecological limits
Reproduction	<ul style="list-style-type: none"> - Mode - Age at maturation and duration - Fecundity - Sterility 	<ul style="list-style-type: none"> - Alter population and community dynamics - Interfere with reproduction of related organisms
Morphology	<ul style="list-style-type: none"> - Shape and size - Color - Fin/appendage form 	<ul style="list-style-type: none"> - Alter species interactions
Life History	<ul style="list-style-type: none"> - Embryonic and larval development - Metamorphosis - Life span 	<ul style="list-style-type: none"> - Alter life history patterns - Alter population and community dynamics

about the GMO and the accessible ecosystems, as is addressed by subsequent questions in the flowcharts. The role of such trait changes in posing adverse environmental effects are discussed in detail in Kapuscinski and Hallerman (1991, p. 101-103) and Kapuscinski and Hallerman (1990, p. 6-7). Refer to these papers for more detailed examples of trait changes and possible adverse effects of introducing such modified organisms provided the scale of introduction is sufficiently large to raise concern.

Interbreeding with Conspecifics or Closely Related Species

Presence of conspecifics in the accessible ecosystem(s) confirms that any escaped GMOs could reproduce in these ecosystems and interbreed with the natural population unless the GMOs have been permanently sterilized. Some aquatic species can also interbreed with closely related species existing in the same environment. Either situation presents the need to assess the potential for introgression of novel genes into natural populations (gene introgression).

Except when introgression might affect threatened, endangered, or special concern populations, questions in this Flowchart do not yet lead to conclusions about the environmental safety or risk of introgression. Researchers need to proceed along the assessment path in order to reach such a decision. (This design feature also applies to Flowchart II.C.1, which assesses the potential for introgressive hybridization).

At this point in the flowcharts, researchers wishing to learn more about predicting gene flow should read the text under "Flowchart IV.A.1. Ecosystem Effects - Impacts of Introgression of Modified Gene(s)."

Potential for interbreeding with closely related species

It is essential to assess whether or not novel genes from escaped GMOs could introgress into populations of closely related species in accessible ecosystems because interspecific hybridization among aquatic species occurs at low frequencies in nature, especially among North American freshwater fishes (Hubbs 1955). Hybridization among these species is relatively common because of external fertilization, weak behavioral reproductive isolation mechanisms, and secondary contact of recently evolved species (Campton 1987). Additionally, interspecific hybrids of many aquatic species are fertile.

Permanent Sterility

Flowcharts II.A., 1 II.B.1, and II.C.1 provide different assessment paths, depending on whether or not the GMO is permanently sterile. In some cases permanent sterility affords an earlier EXIT from the Standards. The criterion

for answering "yes" is that the GMO must be *permanently* sterile in order to discourage assumption of sterility without conducting the appropriate evaluations at the appropriate life stage of the GMO. Before answering "yes" to this question, researchers should have evaluated sterility throughout the lifetime of a statistically valid sample of individuals, focusing especially on ages typically associated with sexual maturity. In a recent study of oysters in which triploidy had been induced to make them sterile, some cells reverted to the diploid state in 20% of the oysters that had been held in trays placed in the York River of Chesapeake Bay (Blankenship 1994). This raised the possibility that fertility could be restored over time in these individuals.

The efficacy of induced sterility in fish and shellfish varies greatly, depending on the species, methodologies (e.g. triploid induction, eyestalk ablation, removal of gonadal tissue), specific protocols for a given methodology (e.g., specific level, timing, and duration of temperature or pressure shock in triploidy induction), and even technical skill of the applicator of the methodology. The literature on efforts to sterilize diploid aquatic organisms by induction of triploidy illustrates this variability. Reported frequencies of triploids in treated groups ranged from 3-100%, with many reports in the 40-60% range; however, survival frequently is depressed by *de novo* triploidy induction (Ihssen et al. 1990). Usually, triploid organisms are sterile because their eggs or sperm contain chromosomes which would remain unpaired at fertilization and thus result in unviable embryos. However, triploids do vary among species in terms of development of reproductive structures, reproductive behaviors, and presence or absence of gamete production (Hallerman and Kapuscinski 1993). The degree of sterility appears to be more complete in triploid, female fish and shellfish than in triploid males (Thorgaard and Allen 1992). See Appendix B, however, for discussion of cases where triploidy may be associated with full fertility; this occurs in some aquatic organisms capable of non-dioecious modes of reproduction (e.g., hermaphroditism, parthenogenesis).

Impact on Threatened, Endangered, or Special Concern Populations

Human-induced species extinctions and declines of populations of fish and shellfish have increased dramatically in this century and do not appear to be subsiding. In North America, many freshwater fish species are endangered or already extinct (Miller et al. 1989, Williams et al 1989, Minckley and Deacon 1991). Shellfish species are also in rapid decline. The most diverse freshwater mussel fauna in the world occurs in North America (Williams and Mulvey 1994) but human impacts have triggered dramatic losses in this globally significant component of biodiversity; 141 species, 42% of the United States fauna are endangered, threatened, or possibly extinct (Williams et al. 1993). Marine species are also threatened with declines and extinctions. Due to lack of

appropriate monitoring, documentation of recent extinctions is scanty (e.g., the first extinction of a marine invertebrate was reported by Carlton et al., 1991) but actual losses are believed to be much greater and on the increase (Norse 1994). Losses of genetically distinct populations and species which reside in freshwater and seawater environments at different life stages, such as anadromous salmon, are also on the increase (e.g., Nehlsen et al 1991). All of these losses of aquatic fauna have been brought about by one or more factors, such as habitat alteration or destruction, introductions of exotic species, and overfishing.

Societal desire to recover depleted aquatic populations and reintroduce locally extirpated species is increasing although financial and other resources needed are rarely available to undertake such efforts. Recovery and reintroduction efforts are technically difficult and demand a long-term commitment to reach success. Given this context, it is clearly prudent and cost-effective to prevent exposure of endangered, threatened, or special concern populations (protected populations) to additional, human-induced alterations via interbreeding with escaped GMOs. Thus, the decision path is designed to encourage such research projects to be conducted so long as they include adequate risk management measures (see Flowchart VI.A and supporting text in Section VI).

When Genetically Modified Organism is a Non-indigenous Species

If the GMO is a non-indigenous species, researchers need to consult the relevant state and federal natural resource management agencies which have oversight authority for uses of non-indigenous fish, crustaceans, or molluscs. These Performance Standards are not applicable to evaluation of the environmental safety or risk issues specific to the experimental organisms being a non-indigenous species. Depending on the non-indigenous species in question, government approval may be needed for the proposed research or development project. The approval process varies for different species and across states, ranging from submission and approval of a letter to the proper authority explaining the research project's facilities and protocols to formal application for a permit to use the non-indigenous species under specified conditions. In the case of no prior knowledge about which agencies to contact, the researcher should start by contacting the state agency with authority over the state's fisheries resources. Common names for such state agencies are the Department of Natural Resources, Department of Fisheries and Wildlife, or some variant of these names. The fisheries division of such a state agency should have a staff member who is knowledgeable about both state and federal oversight of research with non-indigenous species.

To gain background knowledge about the biological principles involved in assessing the environmental safety or risk of a non-indigenous species,

researchers can consult a number of papers and suggested protocols developed by various organizations concerned with aquatic species. Examples include a discussion of conceptual models (Kohler 1992), a discussion of genetic impacts of non-indigenous molluscs (Gaffney and Allen 1992), a suggested protocol for fish introductions in the United States (Kohler and Stanley 1984), an American Fisheries Society position statement on introduction of aquatic species (Kohler and Courtenay 1986), the "Revised Code of Practice to Reduce the Risks for Adverse Effects Arising from Introduction of Marine Species" developed by the International Council for the Exploration of the Sea (Sindermann 1986, 1992), recommendations of European Inland Fisheries Advisory Commission (1988), and the protocol proposed in 1992 by the U.S. Aquatic Nuisance Species Task Force (reproduced in Appendix A).

II.B.1. IMPACT OF DELIBERATE CHROMOSOMAL MANIPULATIONS

This Flowchart is designed to assess organisms modified (1) solely by chromosome manipulations, such as induced tetraploidy and induced triploidy, and (2) by both chromosome manipulations and interspecific hybridization. The impetus to produce the latter type of GMO is that some interspecific hybrids show increased viability when triploidy is induced. For instance, some triploid salmon hybrids exhibit higher viability than the corresponding diploid hybrids (Chevassus et al. 1983, Scheerer and Thorgaard 1983).

Interbreeding or Mating / Permanent Sterility / Non-indigenous Species

Most of the decision path of this Flowchart is aimed at determining the potential for chromosomally manipulated GMOs to interbreed or attempt to mate with natural populations in the accessible ecosystems. The rationale for this focus appears under headings above (marked with *), located under the following headings:

I. Applicability of Performance Standards

Rationale for Applicable and Non-applicable Organisms

- *Deliberate Chromosomal Manipulations
- *Deliberate Interspecific Hybridization

II.A.1. Impact of Deliberate Gene Changes

- *Interbreeding with Conspecifics or Closely Related Species
- *Permanent Sterility
- * When Genetically Modified Organism is a Non-indigenous Species

Extremely Low Survival of Certain Polyploids

To date, most tetraploid fish produced in the laboratory have demonstrated very low survival, so that few individuals reach sexual maturity. This is a mitigating factor against the capability of escaped tetraploids to interbreed with diploids and possibly trigger declines in natural populations through the production of many sterile triploid progeny. Therefore, this flowchart contains an EXIT for research involving polyploids that exhibit extremely low survival with the caveat that the research project is small-scale. Researchers seeking guidance on how to identify an experimental scale appropriate for taking this EXIT should proceed to Flowchart III so that they can compare the factors that would lead to an EXIT versus to a need for risk management.

II.C.1. IMPACT OF INTERSPECIFIC HYBRIDIZATION

This flowchart is designed to assess risk of losing natural populations of genetically distinct species; rationale for this concern was given under subheading, "Deliberate Interspecific Hybridization" under "I. Applicability of Performance Standards." Questions address presence of both parental and other closely related species in the accessible ecosystem because the interspecific hybrid might hybridize with more species than just its parental species (see further explanation under "Potential for Interbreeding with Closely Related Species under section II.A.1.). If there are no parental or closely related species in the accessible ecosystem, risk assessment is greatly simplified and the user is directed to either simply EXIT the standards or EXIT but consult relevant state and federal agencies for guidance on use of non-indigenous species. The rationale for and additional information on seeking guidance about use of non-indigenous species is provided in section I under the subheading "Non-Applicable Organisms" and in section II.A.1 under the subheading "When Genetically Modified Organism is a Non-indigenous Species."

If any parental or closely related species is present in the accessible ecosystem, the user must answer whether or not the interspecific hybrid is permanently sterile (see rationale provided under "Permanent Sterility" under section II.A.1). If the interspecific hybrid is indeed permanently sterile, the flowchart bypasses assessment of risks associated with introgressive hybridization and directs the user to Flowchart III. Otherwise, the user next determines whether or not the accessible ecosystem contains populations of threatened, endangered, or special concern species with which the hybrid could interbreed. The rationale for how this issue is addressed is provided under "Impact on Threatened, Endangered, or Special Concern Populations" under section II.A.1. If this is not an issue, this

flowchart poses a final question in order to assess another possibility for EXIT from the Standards.

Some interspecific hybrids of fish or shellfish produced and reared in the laboratory have exhibited extremely poor survivorship, often at early stages of development. This is a mitigating factor against the capability of escaped hybrids to interbreed with a parental or closely related species and thus against the risk of losing a natural population of a genetically distinct species due to introgressive hybridization. Therefore, this flowchart contains an EXIT for research involving interspecific hybrids that exhibit extremely low survival with the caveat that the research project is small-scale. Researchers seeking guidance on how to identify an experimental scale appropriate for taking this EXIT should jump ahead to Flowchart IV.A.1, which initiates assessment of ecosystem effects of introgression. This will allow researchers to compare the factors that would lead to an EXIT versus leading to a need for risk management.

III. POTENTIAL INTERFERENCE WITH NATURAL REPRODUCTION

This flowchart assesses the risk of lowering the reproductive success of natural populations due to reproductive interference by escaped GMOs. Questions are designed to cover at least two ways that reproductive interference might occur: (1) escaped GMOs are functionally sterile but still enter into mating behavior with fertile individuals in natural populations, yielding infertile broods; and (2) escaped GMOs are fertile tetraploids that breed with natural diploids, yielding sterile triploid progeny. An example of the first concern is evidence that presumably sterile, triploid male masu salmon and ayu exhibited normal courtship behavior toward mature conspecific females (Inada and Taniguchi 1991, Kitamura et al. 1991).

Steroidogenesis and Reproductive Behavior

Triploid males of some fish species exhibit testosterone levels comparable to those of diploid males. Despite abnormal gonad development, triploid rainbow trout exhibit normal sexual differentiation, and at least some triploid males produce sperm. Should courtship and spawning behavior of triploid males sufficiently duplicate that of diploid males, the triploid males could successfully mate with diploid females. No viable progeny would result because the embryos would be aneuploids. However, were many triploids to secure matings, the loss of entire broods could reduce the reproductive success of the naturally existing population, increasing risks of loss of within-population genetic variation or of population extinction due to a demographic catastrophe. In section I, the text

under "Rationale for Applicable and Non-Applicable Organisms" presented additional rationale regarding these risks under the sub-heading, "Deliberate Chromosomal Manipulations."

Flowchart III is designed to focus on testing for evidence of steroidogenesis in individuals of a reproductive age because a negative result from properly controlled assays can clearly rule out the possibility that escaped GMOs will enter into reproductive behavior. In contrast, it is difficult to draw inferences from laboratory behavior experiments about reproductive behavior in natural ecosystems. Absence of a certain behavior in a laboratory environment is an equivocal predictor of that behavior in the field.

Impact on Protected Populations

If reproductive interference is possible, the flowchart leads the user to one of three decisions. When threatened, endangered, or special concern populations are at issue, *exposure* to this risk is minimized by directing the user to risk management (see rationale under "Impact on Threatened, Endangered, or Special Concern Populations" under section II.A.1). When protected populations are not at issue, the response to a final question determines if the user can EXIT from the standards or should proceed to assessment of ecosystem effects of reproductive interference.

Numbers of GMOs Relative to Potentially Interfered Populations

This final question directs the researcher to assess if the numbers of GMOs are so small, relative to the size of potentially interfered populations in accessible ecosystems, that even an accidental escape of *all* GMOs from the project would not cause reproductive interference. A case-specific approach to answering this question is strongly recommended. A useful starting point, however, might be to give an affirmative answer only if the number of GMOs is at least two orders of magnitude less than the number of reproductive age adults in each potentially interfered population. To give an affirmative answer to this question and thus EXIT the Standards, the researcher must base the response on an accurate count of the number of GMOs involved in the project and defensible estimates of critical demographic variables for the potentially interfered populations. Regarding the latter, necessary estimates of demographic variables include: the expected number of individuals of a reproductively mature age; the expected proportion of these individuals which will reproduce successfully (produce at least one viable offspring); and the expected reproductive success (number of viable offspring) per reproducing adult. Expected values involve estimating the mean and variance of a variable. Natural populations show temporal variability in these demographic variables but will be most vulnerable to reproductive

interference when they are at low ends of the range. Thus, an affirmative answer to this question must account for low values in the natural range of these variables.

Useful background information on how to estimate demographic variables in natural populations appears in texts on fisheries population dynamics (e.g., Rothschild 1986) and in extensive literature in peer-reviewed journals. Researchers may find it particularly useful to consult experts on population dynamics of local fish and shellfish populations. These experts can be identified by contacting university departments covering the field of fisheries or aquatic biology, Sea Grant Extension staff in states with Sea Grant College Programs, and government agencies involved with local fish and shellfish resources (e.g., state departments of natural resources or of fisheries and wildlife, U. S. National Biological Service, U. S. Fish and Wildlife Service, U. S. National Marine Fisheries Service).

IV.A. ECOSYSTEM EFFECTS - DELIBERATE GENE CHANGES

Assessment of ecosystem effects of introgression of modified genes from escaped GMOs into natural populations begins by asking if the GMO expresses one or more phenotypic changes listed in Table 1. To answer "no" and thus EXIT the Standards at this point, the researcher must have supporting evidence about the organism's overall performance (see section below on familiarity). Via the phenotypic changes listed in Table 1, organisms affect ecosystem structure and processes. The potential for adverse effects depends on the numbers of the GMO accidentally or deliberately introduced into the accessible ecosystem and other factors addressed in subsequent flowcharts (IV.A.1 and V.). Refer to Kapuscinski and Hallerman (1991, p. 101-103) and Kapuscinski and Hallerman (1990, p. 6-7) for detailed discussions of the role of such trait changes in posing adverse ecosystem effects.

Familiarity with Overall Performance of the GMO

To correctly determine if the genetic modification produces changes in one or more traits listed in Table 1, the researcher must be familiar with the overall performance of the GMO throughout its life cycle. Familiarity is based on a combination of information sources, including: (a) knowledge and past experience with the parental (non-modified) organism grown in the same or similar environments; and (b) results of preliminary indoor or outdoor experiments specifically designed to test for intended and unintended phenotypic changes in the modified organism. Regarding empirical tests for phenotypic changes, two complementary approaches are suggested (Kapuscinski and

Hallerman 1991, Hallerman and Kapuscinski 1993): a battery of laboratory experiments, where a few environmental factors are varied while others are held constant; and studies in more ecologically realistic but securely confined mesocosms (Odum 1984, Voshell 1989).

For some GMOs, information from current research, scientific literature or experts may be insufficient to assess the overall performance of the GMO and thus insufficient to give a clear affirmative or negative answer to the question about phenotypic changes. Following the precautionary principle, research projects involving such unfamiliar GMOs are directed to risk management in order to develop appropriate confinement measures for the project. Lack of familiarity with the overall phenotype of the modified organism makes it particularly difficult to reliably assess ecological effects if: (1) the *intended* phenotypic changes in the modified organism fit under one of the classes in Table 1, (2) the genetic modifications are novel for the species as a whole (e.g., expression of antifreeze protein in tissues of transgenic Atlantic salmon), and (3) effects of the genetic modification on other traits are unfamiliar (e.g., the potential of antifreeze protein to expand the range of salmon into arctic waters and thereby affect aquatic communities not adapted to salmon predation).

When a modified organism is first studied in confined experimental systems, familiarity with its overall phenotype would be expected to be quite low. After substantial phenotypic testing, the degree of familiarity could increase to the point where it becomes possible to give a clear affirmative or negative answer to the question about phenotypic changes. It is imperative that experiments involve proper measurements for these phenotypic changes and that inter-trait correlations and genotype-environment interactions be considered.

IV.A.1. ECOSYSTEM EFFECTS - IMPACTS OF INTROGRESSION OF MODIFIED GENE(S)

As noted in the upper left corner of this Flowchart, projects that reach this point involve GMOs that are not permanently sterile and have the potential for interbreeding with conspecifics or closely related species. The researcher is prompted to estimate four population variables: reproductive potential of escaped GMOs, frequency of introgression of the modified genes, fitness of introgressed individuals, and potential demographic decline due to genetic load of introgressed genes. Three of these estimates -- reproductive potential, frequency of introgression of the modified gene(s), and demographic decline -- require estimation of the number of GMOs that could accidentally escape into the accessible ecosystem and of the abundance of the potentially affected natural population. A range of possible values for the number of escaped individuals,

from a minimum to a maximum number, can be developed by considering a range of scenarios that might trigger escapes from the proposed project. To develop appropriate scenarios, researchers may find it helpful to read text on "Project Siting" and "Design of Barriers" found below under "VI. Risk Management Recommendations: Project Siting, Design, Operations, and Review."

Estimation of Reproductive Potential

Reproductive potential of escaped GMOs will be a function of: (1) survival rate and fertility of the GMO; and (2) environmental conditions affecting reproduction in the accessible ecosystem, such as length of the spawning season (as determined by suitable water temperatures and similar environmental cues) and availability of suitable spawning habitat. One way to estimate the reproductive potential of a group of escaped GMOs would be to construct a life table, a traditional technique in population biology, taking into consideration impacts of environmental conditions in the accessible ecosystem (e.g. Emlen 1984, chapter 3). This necessitates estimation of: different ages at reproduction, survival rates to each reproductive age; and fertility (or else fecundity) at each reproductive age. Estimation of these variables requires substantial familiarity with the overall phenotype of the GMO, as derived from empirical measurements of GMO phenotypes and knowledge about the parental organism (see above discussion of familiarity under "IV.A. Ecosystem Effects - Deliberate Gene Changes"). Clear supporting evidence is needed for any prediction that escaped GMOs are grossly unfit and thus pose negligible reproductive potential (see discussion below on "Estimation of Fitness of Introgressed Descendants").

Estimation of Gene Flow

Estimation of the frequency of the modified gene(s) in the progeny generation will be difficult in most cases. Both the rate of spread of a modified gene and its rate of increase are strongly dependent on the structure of the potentially affected population, which is determined by the connectivity of patches of interbreeding individuals (demes) (Gliddon and Goudet 1994). Connectivity refers to the number of gene-flow connections and magnitude of gene flow among them. Additionally, researchers need to assess whether or not the phenotypic changes exhibited by the GMO would alter directions or amounts of gene flow due to altered dispersal or mating behavior (e.g., caused by expanded tolerance range for a physical factor in the accessible ecosystem).

Qualitative estimation of the degree of connectivity among demes in a natural population is possible via a method described by Goudet (1993) and Goudet et al. (1994). This method involves computer modelling and requires empirical estimation of the fixation index, F_{ST} , a measure of heterozygosity among demes

which is *inversely* proportional to gene flow among these demes (Wright 1943). Using genetic markers generated by molecular genetic methods, it is readily possible to estimate F_{ST} from data derived from natural populations. Gliddon and Goudet (1994) reviewed the application of this method to three actual populations including that of a marine mollusc, the dogwhelk (*Nucella lapillus*), and outlined its potential application to predicting the flow of modified genes into wild populations of Atlantic salmon (*Salmo salar*).

Clear supporting evidence is needed for any prediction that escaped GMOs are grossly unfit and thus pose negligible gene flow (see discussion below on "Estimation of Fitness of Introgressed Descendants").

Estimation of Fitness of Introgressed Descendants

A prediction that introgressed individuals will have lower fitness than non-introgressed individuals must be supported by clear evidence of disruption of survival or reproduction of the GMO under environmental conditions similar to those of the accessible ecosystem. Research done since the 1960's has led to the understanding that natural populations of organisms rarely show 'perfect adaptation.' In different experiments involving different taxa, 0.2 to 10 percent of *random* mutations were adaptive (Grant 1985). These percentages suggest a lower limit for the frequency of adaptive, *deliberate* genetic modifications and do not rule out higher frequencies. Evolutionary and ecological processes are now understood to be much more affected by *ad-hoc* interactions between species, idiosyncrasies of local communities, and stochastic processes (Regal 1994). Therefore, not all new genetic modifications will be maladaptive.

Some genetic modifications could yield a novel adaptive combination of traits so that near wild-type GMOs could survive, reproduce, and persist in natural environments and disrupt the *ad hoc* organization of natural biological communities (Regal 1994). It is reasonable to view genetically modified fish and shellfish as being near wild-type. Although numerous strains or stocks of fish and shellfish have been partially domesticated through consecutive generations of captive breeding (yielding increased fitness in captivity), no such strains have been shown to be so domesticated that their fitness in the wild is negligible (either due to extremely poor survival or reproduction or both).

Genetic Load of Introgressed Modified Genes

If introgressed individuals exhibit lower fitness than non-introgressed conspecifics (but are not grossly unfit), it is necessary to assess potential imposition of a genetic load onto a natural population by interbreeding with genetically modified organisms. Deleterious genetic modifications impair the

well-being of a population not in proportion to the reduction of the viability or fitness of their individual carriers, but in proportion to their frequency of origin (Haldane 1937), i.e., the frequency of modified organisms in the population. The equilibrium frequency q of a deleterious gene arising at a frequency u and opposed by selection of intensity s will be $q = \sqrt{u/s}$ (Dobzhansky 1970, p. 190). With the frequency of homozygotes of the new allele being q^2 , the population suffers impairment $sq^2 = su/s$, or u , the mutation rate (i.e., the frequency of the modified trait in the population). Elevation of the mutation rate via escapes of less fit GMOs, by increasing the genetic load of the population, will increase the rate of so-called genetic deaths. Note that a genetic death need not produce a cadaver. Genetic death occurs if the carrier of a certain genotype produces fewer young than the carrier of another genotype (Dobzhansky 1970). Thus, the effect of the introgression of maladaptive traits through entry of less fit GMOs into a natural population can pose a risk to the long-term viability of the natural population. Although natural selection is expected to remove maladaptive genes from a population, the number of generations required for the process to be completed can be very large (Hartl 1988). Additionally, if the fitness of escaped GMO or early generations of introgressed descendants is reduced, the fitness of future descendants can increase via adaptive evolution (Lenski and Nguyen. 1988).

IV.B. POTENTIAL BARRIERS ASSOCIATED WITH ACCESSIBLE ECOSYSTEM

Researchers are directed to this Flowchart only if the accessible ecosystem clearly lacks conspecifics or closely related species, thus ruling out risks of reproductive interference by the GMO and of introgression of modified genes either by intra-specific introgression or introgressive hybridization. This Flowchart prompts the user to determine if some abiotic factor in the accessible environment clearly prevents reproduction by any escaped GMOs, thus allowing EXIT from the Standards. If use of this Flowchart does not lead to an EXIT, then one cannot rule out establishment of a self-reproducing population of GMO founded by accidental escapees.

Although environmental conditions of the accessible ecosystem permit survival of the GMO at issue (as determined in Flowchart II.A, II.B, or II.C), they might lack conditions *required* for one or more steps in the reproductive process including gonadal development, ovulation, sperm maturation, or spawning. Examples of abiotic factors that might preclude reproduction of the GMO are lack of the required spawning substrate, stream flows, photoperiods, water temperatures, water salinity, or other chemical factor. To invoke one or more of these abiotic factors as a reason to EXIT the Standards, there needs to be

documentation that the factor *clearly* precludes reproduction. For instance, anadromous fish species typically spend their adult phase in salt water and reproduce in fresh water. Depending on the species at issue, lack of freshwater does not necessarily preclude successful reproduction because some populations naturally demonstrate successful reproduction in saline waters of marine estuaries.

Familiarity with Reproductive Biology of the GMO

To correctly determine whether or not a given abiotic factor precludes reproduction of the GMO, familiarity with the reproductive biology of the GMO is necessary. Knowledge of environmental requirements for reproduction of the parental, non-modified organism is a starting point. Lack of knowledge about these environmental requirements requires answering "unknown" to the question on this Flowchart and proceeding to Flowchart IV.B.1. If there is sufficient familiarity with the environmental requirements for reproduction by the parental organism, the next step is to determine whether or not the genetic modification has altered any of these requirements in a way that would change the response to the question on this flowchart. Ideally, this determination should be based on empirical measurements of reproductive processes in the GMO collected in confined indoor or outdoor studies. Scientific knowledge about interactions between the reproductive system and other parts of the parental organism's physiology may also help.

IV.B.1. ECOSYSTEM EFFECTS - POTENTIAL FOR NON-REPRODUCTIVE INTERACTION

This Flowchart addresses situations where escaped GMOs could establish a self-perpetuating population in the accessible ecosystem but reproductive interactions with other species has been ruled out. Thus, the Flowchart initiates assessment of other types of ecosystem effects.

All issues raised in this Flowchart have been explained in prior sections above. Explanation of the first question about phenotypic changes listed in Table 1 appears under "IV.A. Ecosystem Effects - Deliberate Gene Changes." Estimation of reproductive potential of GMOs is discussed in the section, "IV.A.1 Ecosystem Effects - Impacts of Introgression of Modified Genes." The rationale for estimating the fitness of descendants of the escaped GMOs is the same as provided in a discussion of fitness in section IV.A.1. In this Flowchart, however, fitness estimation is for all descendants of the self-reproducing GMO population, not for introgressed progeny generated by matings between GMO and unmodified adults.

IV.C. ECOSYSTEM EFFECTS - IMPACTS OF REPRODUCTIVE INTERFERENCE

This Flowchart assesses the effect of reproductive interference by accidentally escaped GMOs on the abundance of potentially affected population(s) in the accessible ecosystem(s). Researchers directed to this Flowchart previously concluded that reproductive interference is possible, i.e., it cannot be ruled out. The Flowchart provides an EXIT from the Standards if researchers can document the following: (a) abundance of each potentially affected population is regulated by a density -dependent relationship, and (b) such density-dependence would clearly offset (compensate for) potential decline in the population's abundance triggered reproductive interference by the GMO.

Density-Dependent Factors

The literature on fish and shellfish population dynamics contains numerous examples of density-dependent population responses occurring at different life history stages (Rothschild 1986 - see especially chapters 5 and 8). Because this Flowchart is concerned with population *abundance*, researchers should start by assessing whether or not there is density dependence in the relationship of recruitment as a function of numerical population abundance (also called stock abundance). Curvilinearity in this relationship suggests density dependence, as exemplified by the classical Ricker (1954) and Beverton and Holt (1957) recruitment-stock curves. Recruitment at relatively low population abundance is *density-independent* but at intermediate or high population abundance is *density-dependent*, as evidenced by a decline in the rate of recruitment increase (Rothschild 1986, chapter 5). Stated otherwise, the reproductive effectiveness of the population decreases at high population levels because of one or more density-dependent mechanisms.

Assess if density dependence in stock-recruitment can offset population decline

If there is documentation of a curvilinear stock-recruit relationship in the potentially affected populations, then researchers need to determine where the existing population abundance lies along this curve. If the population is at the lower end of the curve where recruitment is *density-independent*, then reproductive interference would reduce recruitment proportionally and could drive the population towards extinction. In this situation, the Flowchart directs the researcher to estimate the magnitude of potential decline in abundance of the interfered population(s), and then to proceed to Flowchart VI.A for specific risk management recommendations.

Estimation of the magnitude of potential population decline allows design of risk management measures for an acceptable number of accidental escapees, as defined in Flowchart VI.A. When such estimation is not possible, researchers should proceed instead to Flowchart VI.B. which guides design of risk management for no/negligible escapes. The concept of "no/ negligible escapes" is further explained below in the introduction section of "VI. Risk Management Recommendations: Project Siting, Design, Operations, and Review (Flowcharts VI.A & VI.B.)"

If there is clear evidence that population abundance is at the high end of the stock-recruit curve where recruitment is *density-dependent* and reproductive effectiveness is decreased, then it may be possible to build a case that a compensatory response in recruitment will offset any decline in population abundance triggered by reproductive interference. Stock abundance would have to be reduced to a level where the rate of recruitment increase is increasing, but not to a level so low as to risk stock extinction via stochastic processes. Building a scientifically defensible case will not be easy. Researchers are strongly advised to seek substantial input of recognized experts on the population dynamics and ecology of the potentially affected natural populations.

V. EFFECTS ON ECOSYSTEM STRUCTURE AND PROCESSES

This Flowchart guides the most difficult assessments, which demand substantial information about complex and variable ecosystem features. If a researcher is directed to this last Flowchart, it means that prior, easier assessments failed to clearly identify a specific reason for the researcher to EXIT the Standards or to proceed to risk management (see related discussion above under "Overview of Performance Standards").

Interactions with Threatened, Endangered, or Special Concern Populations

The question in this Flowchart focuses on *ecological interactions* between escaped GMOs and such protected populations. It differs from questions about protected populations posed in earlier Flowcharts which addressed the potential for interbreeding with or reproductive interference by escaped GMOs.

These populations are especially vulnerable to extinction risk and, therefore, should be protected from novel interactions with GMOs. This protection is justifiable in light of the dramatic declines of aquatic biodiversity in North America, as explained in greater detail in the discussion of threatened, endangered, or special concern populations under "II.A.1. Impact of Deliberate

Gene Changes." Extinction of such populations can damage ecosystem structure or processes and indirectly threaten sustainability of other species in the ecosystem, including those caught in sport and commercial fisheries.

To determine if protected populations occur in the accessible ecosystem, researchers should consult their state fisheries and wildlife agency (including the non-game management or natural heritage programs if these exist in the state) and the U. S.. Fish and Wildlife Service (for freshwater ecosystems) or the National Marine Fisheries Service (for saltwater ecosystems and anadromous fish). Useful information might also be obtained from state offices of the Nature Conservancy.

Familiarity with Accessible Ecosystem

There must be sufficient knowledge of and experience with the accessible ecosystems to ensure that the assessments guided by this Flowchart are scientifically reliable and defensible. Familiarity should include information about each accessible ecosystem's: (1) structure (i.e., biological interactions among species as manifested by segregation in use of food or space), (2) processes (i.e., patterns of nutrient and energy flow, such as is manifested by food webs), and (3) persistence (i.e., ability of an observed structure or species composition to persist within known limits through time). If there is sufficient familiarity with these attributes, development of a simulation model of the accessible ecosystem could provide a useful tool for conducting the assessments requested in this Flowchart. Then, data on phenotypic changes exhibited by the GMO derived from laboratory or mesocosm experiments could be incorporated into the simulation model to assist with these assessments. Refer to the related discussion of experiments under "Familiarity with overall performance of the GMO." located under "IV.A. Ecosystem Effects - Deliberate Gene Changes."

If familiarity about ecosystem structure is lacking, researchers should conclude that assessment of the type and magnitude of species interactions (the first assessment requested on this Flowchart) is not possible and should proceed to Flowchart VI.B. for appropriate guidance on risk management. Assessment of the potential for adverse alteration of ecosystem structure or processes (the second requested assessment) requires overall familiarity with structure, processes, and persistence. Lack of sufficient familiarity in these areas prevents conduct of a scientifically justifiable assessment and, thus, requires proceeding to Flowchart VI.A for appropriate guidance on risk management.

Assess Interactions Between GMOs and Other Organisms

When conducting this assessment, researchers need to consider how interactions will vary as the GMO and other organisms progress through several trophic positions during their life cycle. Such progression is common among aquatic animal species (e.g., Stein et al. 1988). This assessment should also integrate information about the parental organism with an assessment of whether or not phenotypic changes identified in the GMO (i.e., focusing on changes listed in Table 1) may alter interactions between the GMO and other species. In a review of ecological principles and ecological effects of intentionally stocked fishes, Wahl et al. (1995) presented background information that is also relevant for assessing ecological effects of accidentally escaped GMOs. In particular, they recommended that assessment be based on an ecological, community-based framework that integrates the relative importance of predation, competition, abiotic factors, and interactions among these factors across all life stages (see figure 6 in Wahl et al. 1995).

Significant interactions

A number of species interactions are important to assess (Tiedje et al. 1989, Kapuscinski and Hallerman 1990, 1991). Assessment should focus on the following interactions: (a) predator-prey interactions, particularly if the modified organism is a top-level predator, such as a piscivorous fish (Carpenter and Kitchell 1988, Mills and Forney 1988, reviewed in Kapuscinski and Hallerman 1990, p. 6-7) (b) competitive, symbiotic, and parasitic interactions; and (c) indirect interactions, where the activities of the modified organism make the environment less suitable for other species. In extreme cases, the GMO could become a pest to humans or to other species, either because the parental organism is a pest or the phenotypic changes exhibited by the GMO are major enough to yield pest characteristics. This possibility should be considered if the parental organism is an introduced or non-indigenous species. For example, the feeding activities of common carp greatly increase the turbidity of warm, shallow lakes, eliminating aquatic plant beds and reducing populations of visually feeding predators (such as northern pike) and of waterfowl (which depend on the aquatic plants). Thus, any genetic modification that increases the ability of carp to alter their environment (e.g., more rapid growth) has the potential to increase their effectiveness as a pest.

Likewise, it is important to determine if phenotypic changes exhibited by a GMO could increase its ability to adversely affect other organisms in the accessible ecosystem. For instance, if increased growth leads to larger size-at-age or ultimate size, the modified organism could have an advantage in competition for food, habitat resources, spawning sites, or mates. In short, an interaction is of concern if the activities of the GMO can affect the distribution or demography of another species.

Consideration of other organisms

Assessment of species interactions involving the GMO should specifically address populations of conspecifics and closely related species. There is growing evidence that oversized, hatchery-reared salmonids can socially dominate and sometimes displace smaller, wild conspecifics or closely related species through increased aggressive behavior or increased competition for food and space (e.g., Bachman 1984, Nickelson et al. 1986, Vincent 1987). This raises the concern that such displacement might be a more general phenomenon with GMOs exhibiting certain phenotypic changes that adversely influence their interaction with other organisms. Potential displacement of natural populations is a concern even if the GMO cannot interbreed with them because such displacement is the first step towards decline and extirpation of natural populations. Possible adverse ecological consequences include declines in genetic and species diversity, disruption of the ecosystem, and decreased sustainability of fisheries resources important to humans. This latter point is also relevant for the discussion below on adverse ecosystem alterations.

Assessment of species interactions involving the GMO should also address species caught by sport or commercial fisheries. Populations of exploited organisms are often both economically important to humans and ecologically important to long-term health and sustainability of aquatic ecosystems (e.g., Christie et al. 1987). It is, therefore, important to assess whether or not interactions between escaped GMOs and populations of exploited species will adversely affect these populations, for instance through increased population fluctuations, displacement due to heightened competition or behavioral interactions, or declines in abundance and genetic diversity (Wahl et al. 1995). This latter point is also relevant for the discussion below on adverse ecosystem alterations.

Assess Potential for Interactions to Adversely Alter Ecosystems

This last step in ecosystem effects assessment ultimately leads the researcher either to an EXIT from the Standards or to risk management. Proceeding to the EXIT requires that the researcher has clear scientific evidence to support the conclusion that adverse ecosystem alterations are improbable or negligible.

Aquatic communities function through complex interactions along pathways connecting organisms and abiotic resources through transfers of energy, organisms, nutrients, or information. In most instances, changes in community structure (e.g., changes in relative abundance of species) are prevented from triggering large changes in major ecosystem processes (e.g., primary production) by compensatory dynamics of functionally similar species. However, certain changes can lead to substantial changes in central ecosystem processes (Connell

1975, Carpenter and Kitchell 1988, Wahl et al. 1995). Therefore, it is important to assess whether or not species interactions involving escaped GMOs could adversely affect ecosystem processes. For example, increased mouth gape due to increased size of a GMO might enable the organism to prey on organisms until then not subject to predation. Such novel broadening of prey items could perturb the food web of the aquatic community in difficult-to-predict ways.

The concept of *adverse* effect on ecosystem processes can be illustrated by known examples from species introductions. Examples include: (a) common carp muddying up clear lakes through their feeding activities; by increasing turbidity and affecting the balance between photosynthesis by phytoplankton and rooted macrophytes, carp affect habitat availability and food resources for a range of aquatic organisms; (b) predation by piscivorous fishes on planktivorous fishes; by reducing predation upon large zooplankton, a decrease in planktivorous fish may increase grazing pressure upon phytoplankton, affecting the balance of photosynthesis by planktonic algae and rooted macrophytes; (c) an introduced clam in the San Francisco Bay estuary has, through its filtering action, caused the brackish parts of the system to switch from being dominated by planktonic organisms to being dominated by benthic organisms.

Decreased predictability of ecosystem state

Current understanding in ecology is that the only constant is change, and that all ecosystems are in flux (Pickett et al. 1992). At best, systems have multiple, alternating "steady" states, with "steady" defined in relatively short time scales, no more than a few decades. However, as ecological knowledge increases, the alternating states become more predictable, as does the direction of ecosystem change in response to regional or global factors. Addition of any new organism into a system, including GMOs exhibiting changed phenotypes (refer to Table 1), can change the rules under which the system operates and therefore decrease its predictability to humans. At this point in the Flowchart, therefore, researchers should assess whether or not the modified organism will have effects on the accessible ecosystem that will cause a shift to a less desirable state from which it may not be able to return to its previous, more desirable state.

Degraded state of ecosystems

There is a growing literature on the concepts of ecosystem degradation and health. Ecosystem health is influenced by the diversity of ecosystem structure and processes, including some redundancy (Christie et al. 1987, Karr 1991). Assessment of the potential to alter an aquatic ecosystem to a degraded state must address both environmental sustainability and human utilization (e.g., reduce water quality). Accessible ecosystems which have already been greatly perturbed from "healthy" states are particularly vulnerable to further degradation, and thus are more susceptible to adverse effects due to species interactions of escaped

GMOs. A degraded natural ecosystem *should not* be treated as if it is an artificial system undeserving of protection of natural structures and processes.

If the assessment concludes that adverse ecosystem alterations are improbable or negligible, Flowchart V provides an EXIT from the Standards, meaning that no special confinement measures are advised so that fairly large numbers of GMOs might escape from the research project. Before proceeding to this EXIT, therefore, it is important to assess whether or not, through one or more of the assessed interactions, *large-scale* introductions of modified organisms could act as agents of natural selection on other organisms in the community, and what the ecological consequences might be.

VI. RISK MANAGEMENT RECOMMENDATIONS: PROJECT SITING, DESIGN, OPERATIONS, AND REVIEW (Flowcharts VI.A & VI.B.)

Introduction

This section applies only to research projects determined to need risk management based on completion of all prior portions of these Performance Standards. This section presents recommendations for the design and operations of a research project involving genetically modified finfish or shellfish in order to manage specific risks. Planning and implementation of management measures must address all the factors discussed in this section, including project siting, design of barriers, security, alarms, operational requirements (includes written operational plan, emergency response plan, training, and traffic control), and review before and after start-up of project.

Case-specific approach complemented by review

Different research projects needing risk management will exhibit great variety in the biological features of the GMO, the specific risk(s), and features of the overall research project (e.g., project siting). This makes it unfeasible to anticipate the best combination of management measures for every possible case. This section, therefore, presents general recommendations and leaves it up to the user to develop the most appropriate combination of risk management measures that achieve either "no / negligible escapes" or the "acceptable number of accidental escapees", as specified in Flowcharts VI.A or VI.B. Determination of what constitutes "negligible escapes" should be in reference to the specific risk that has been identified for the proposed project, as reiterated on Flowchart VI.A or VI.B; the objective is to have *negligible environmental consequences*. Users of this section must clearly recognize that these performance standards define the minimum requirements; additional measures may be prudent in certain cases. To

assure that this case-specific approach results in adequate risk management and to fully comply with these Performance Standards, users of this section are *expected to seek peer review of their risk management measures prior to project start-up and site review after start-up of operations, making sure that reviewers include aquatic biology and ecology experts* (see detailed discussion under peer review and site review subsection below).

Research projects versus commercial operations

The recommendations laid out in this section are designed to manage specific risks of research projects only. They are not designed to address all the issues posed by commercial-scale operations and they clearly are insufficient to manage specific risks that might be identified for a commercial operation. However, they may provide a useful starting point for future development of recommendations for risk management in commercial operations.

Project Siting

The ease or difficulty of managing a given project's specific risks will depend to a great extent on the geographical location of the research project. *Siting and physical facilities of projects using genetically modified organisms must prevent accidental releases during flooding, storms, earthquakes, and other natural disasters (Table 2)*. Researchers should try to avoid sites where flooding, wave action, or high winds could allow escape of GMOs into a natural water body; although marine and estuarine sites may be more vulnerable, such scenarios are also possible in certain freshwater sites, for instance when outdoor research ponds are located close to a stream, lake, or ditch leading to a natural surface water. Where these conditions cannot be avoided, rearing units must be protected from flood, wave action, and high winds. Project reviewers and inspectors are expected to evaluate the adequacy of protection against accidental escape of GMOs via flooding, water spray or waves during storm events, such as hurricanes and tornadoes, and other episodes of high winds.

Different criteria for freshwater and marine sites

Although most freshwater research projects can meet the criterion of location above the 100 year flood level (designated in table 2), many marine and estuarine research stations cannot meet this same criterion. Consequently, research projects sited at marine and estuarine locations must place greater emphasis on other management options. In many marine cases, the most feasible approach to preparation for floods, hurricanes, or other natural disasters (e.g., wind and wave damage due to a violent storm) is to *keep the scale of the research project small enough so that all animals either can be moved safely to an alternative site or destroyed within a specified time*. Movement or destruction of animals should be completed between the time a disaster warning is received and before

conditions become too dangerous to complete the action. Hurricanes, while extremely destructive, are tracked by the Weather Service and sufficient time is usually available for animals to be moved or destroyed. The protocol for such emergency actions should be spelled out in the emergency response component of the project's written operational plan (see subsection below on operational plan).

Table 2. Minimum criteria for siting of research project when specific risks of working with an aquatic GMO have been identified.

Event	Freshwater	Marine
Flooding	Above the 100 year flood level Storm drains designed for 100 year rainfall event or storage provided Surface runoff diverted around project site	Flood level and storm drain criteria not applicable - place greater emphasis on management of experimental scale and other factors Surface runoff diverted around project site
Wind Loadings ¹	current requirements for laboratory facilities	current requirements for laboratory facilities
Snow Loadings ¹	current requirements for laboratory facilities	current requirements for laboratory facilities
Seismic Loadings ¹	current requirements for laboratory facilities	current requirements for laboratory facilities
Others ¹	current requirements for laboratory facilities	current requirements for laboratory facilities

1. These criteria apply to the research project's rearing units and mechanical barriers located either indoors or outdoors. For indoor situations, the loading criteria will generally apply to buildings housing the rearing units and mechanical barriers. For outdoor situations, loading criteria will generally apply directly to the rearing units (e.g., fiberglass tanks and tank covers located outdoors) and mechanical barriers (e.g., structure of french drain, perimeter fencing located outdoors).

Project siting to avoid certain risks

As summarized on Flowchart VI.A, the specific risks of some projects involve adverse effects on protected populations of species which are threatened, endangered, or of special concern. Instead of implementing measures to manage these risks, another option is to relocate the proposed project to a site where the accessible ecosystems do not contain such protected populations. If researchers are seriously considering such relocation, they should first utilize the assessment flowcharts (I. through V.) to evaluate the suitability of the relocation site. Specifically, it is important to determine whether or not the relocation site poses other specific risks requiring management.

For freshwater situations, siting of the research project in areas with interior drainage and no permanent waterbodies may be prudent until more experience with genetically modified animals is available. In the more arid parts of the western U.S., there are many areas where all runoff either percolates into the ground or evaporates. Any surface water bodies in these areas are temporary. In some cases, relocation of a project may reduce the numbers and types of barriers needed on the project site. The best reason for relocation is if it allows effluents and drawdown water to be discharged to an environment known to be lethal to all life stages of the GMO. For instance, research on GMOs for whom seawater is known to be lethal at all life stages could be conducted at a marine site where it is feasible to discharge project effluents and drawdown water directly to the ocean (i.e., full strength seawater). In some cases, such a strategy might preclude the need for additional barriers in the project's effluent and drawdown water (see related discussions in subsections below).

Design of Barriers

This subsection discusses factors that must be considered in the design of different barriers used to confine GMOs within the site of the research project. *For each possible escape path in the water system, the minimum expectation for each project requiring risk management is to have sufficient numbers of barriers in series to achieve either "no / negligible escapes" or the "acceptable number of accidental escapees," as specified in Flowchart VI.A or VI.B, of all life stages of the GMO occurring during the duration of the project.* Possible aquatic escape paths are discussed in a subsection below. Protection against escape paths beyond the water system is also necessary (see subsection below on this issue).

The entire set of barriers for the water system must prevent escape of the hardest to retain life stage that will occur during the course of the project; usually this is the smallest life stage. Because no barrier type is 100% effective at all times, the overall reliability of confinement measures will depend heavily on the number of independent barriers present in series. Researchers are expected to determine the appropriate combination of types and total number of barriers needed to achieve the accepted number of accidental escapees. The number of independent barriers is site- and project-specific but will generally range from three to five. Where the surrounding environment (accessible ecosystems) is lethal to all life stages of the GMO (e.g., discharge from a freshwater project into seawater or discharge from a marine project into a hypersaline environment), no barriers beyond the standard types of aquaculture rearing units and effluent screening may be required. Project reviewers and inspectors are expected to evaluate the adequacy of the chosen combination and total number of barriers.

At least four types of possible barriers to aquatic escape paths are available to the researcher:

Physical or chemical barriers

These are manipulations of physical (e.g., water) or chemical (e.g., pH) attributes of rearing water to induce 100% mortality in one or more specified life stages of the GMO before such life stage(s) can reach the accessible ecosystem(s). For example, water temperature or pH can be maintained at lethal values for effluents from incubators or for the final effluent coming from all rearing units. Another example is chemical sterilization of project effluent via addition of a chemical (e.g., chlorine, bromine, ozone) at lethal concentrations followed by appropriate removal of the lethal chemical prior to discharge of effluent water from the project site. Exact dose and contact time with the chemical will depend on species and life stage. Treatment with 10-15 mg/L of chlorine for 15-30 minutes is effective for killing fish in freshwater. Appendix C describes a protocol for chemical sterilization of seawater effluent which has been used at marine research stations. For projects involving molluscs, natural spawning of adults held in isolation tanks should be monitored carefully so that dosage and duration of chemical or temperature treatments can be properly adjusted when spawning occurs.

Mechanical barriers

This category includes mechanical structures (either stationary or moving) that physically hold back one or more specified life stages of the GMO from escaping the project site. Mechanical barriers might be placed in series at one or more locations along the water system of the project. For instance, barriers might be located at each point where effluent from a number of rearing units comes together and at the point where effluents of all rearing units form one final effluent stream. Examples of possible mechanical structures include stationary or moving screens (e.g., floor drain screens, standpipe screens), filters made up of one or more types and sizes of media (e.g., gravel traps), grinders with moving parts, and tank covers. Appendix D illustrates several examples of mechanical barriers including a sock filter for effluents from an indoor fish embryo incubator, a stainless steel rod screen for the final effluent from an indoor fish culture facility, and french drains for outdoor fish culture ponds.

Biological barriers

Biological features or alterations of all or a specific portion of the project's GMOs can serve as barriers if they either (1) prevent any possibility of reproduction at the project site, thus avoiding risks of escape of small gametes, embryos, or larval stages or (2) greatly reduce the possibility of reproduction or survival of the project's GMOs if they accidentally escaped into the accessible ecosystem. A project's entire set of barriers in series cannot consist solely of

biological barriers because inter-individual variability in efficacy of the biological barrier is expected. The project, therefore, must have at least one other type of barrier in its total number of barriers. Examples of biological barriers are the following: (1) the project protocol involves killing or removal of GMOs before they reach a reproductive life stage²; (2) only one sex of a solely dioecious GMO is raised in the project site²; or (3) all cultured GMOs are made permanently sterile before they reach reproductive maturity in captivity.

Scale of experiment as a barrier

This involves holding experimental organisms at a number so small that accidental escape of all organisms would not have the adverse effects associated with the project's specific risks (see risks listed in Flowcharts VI.A and VI.B). *It will be difficult to identify and justify a number that meets this criterion.* If the GMO is a self-fertilizing hermaphrodite or a true parthenogen (see Appendix B), experimental scale cannot be counted as one of the project's barriers because accidental escape of *only one* individual could found an entire population of GMOs in accessible ecosystems. Although experiments with such organisms should be kept as small as possible, multiple barriers of other types are required to achieve containment.

Barriers for all possible escape paths of the water system

The accidental escape of GMOs might occur through any of the following components of the water system: influent water and makeup water (applicable in water reuse systems); effluent and drawdown water; waste slurries collected when filters are backwashed, screens scrubbed, or rearing units cleaned by siphoning; and aerosols from larval hatcheries of some shellfish. Therefore, each water system component must have a sufficient combination and number of mechanical or physical/chemical barriers to prevent escape.

Influent/makeup water. Surface waters require an appropriate set of barriers. Well water, other fully enclosed water sources, and municipal sources do not need barriers.

Effluent and drawdown water. All other factors being equal, the risk of accidental escape increases as the frequency of water discharge increases. Static and closed water systems generally have no discharge except when draining the system. Water reuse systems and ponds may have a minor amount of discharge depending on operations and weather conditions. A flow-through system will

² This type of biological barrier is ineffective and thus unacceptable for species of finfish and shellfish which have a non-dioecious mode of reproduction that permits one escaped individual to found an entire population, such as true parthenogenesis or self-fertilizing hermaphroditism (see discussion in Appendix B).

have a continuous discharge. Although a sanitary sewer can serve as one barrier, discharge into sanitary sewers *alone* does not provide an adequate barrier to accidental escape in most cases because (1) many sewers bypass water to storm sewers or surface waters during high-runoff events, or (2) some aquatic animals can survive transit through the sewer and treatment plants. Prior to discharge to a sanitary sewer, effluent and drawdown water should pass through a sufficient set of barriers on the project site to achieve the acceptable number of accidental escapees. For all types of water systems, the effluent drain capacity must be at least two times greater than the normal inflow capacity in order to handle simultaneous draining of a number of rearing units. Researchers are expected to identify the appropriate drain capacity and reviewers/inspectors are expected to evaluate its adequacy.

For water systems which do not have continuous flow-through, an alternative approach to preventing escapes via effluent and drawdown water is to locate the entire project in an indoor facility with no floor drains and the capacity to retain water from a specified number of experimental units. For instance, the facility could be designed to retain all the water if there was breakage of 5-20% of the experimental units. The researcher is expected to seek input from prospective peer reviewers and inspectors in order to select the appropriate water retention capacity. Additionally, any effluent from such an indoor facility must be treated as waste slurry (see below).

Waste slurries. These may hide small or dormant life stages of viable GMOs at in the mixture of uneaten food, feces, possibly shells from hatched eggs, and other particulate matter. Batch chemical or temperature treatment known to be lethal to smaller life stages of the GMO is recommended to kill any viable GMOs that might be present in waste slurries. For some species, on-site drying of waste slurries might be adequate. Final disposal of treated waste slurries must comply with all applicable environmental regulations; researchers are expected to obtain guidelines and regulations from their institution and, when applicable, from appropriate government units. It is generally illegal to discharge such slurries into an aquatic ecosystem. Examples of appropriate disposal of treated waste slurries might be: discharge to a sanitary sewer; discharge into a septic system, delivery to an institutional hazardous waste facility; or deposit in an approved land site.

Prevent escape via non-aquatic paths

Escape of aquatic GMOs might occur through paths other than the project's water system. Researchers must determine if their project poses one or more of the escape paths described below and implement measures to protect against them.

Secure disposal of experimental animals. Certain life stages of some species can survive long periods of time outside of water. For instance, adult bivalves might survive three or more days outside of water as long as temperatures remain relatively cool and surroundings are slightly moist (e.g., a large number of adults packed closely together in a closed container). Therefore, researchers must anticipate and avoid situations where animals might survive after disposal and get into the hands of persons unaware of the need to prevent their introduction into natural water bodies. The best way to avoid such problems is to: initially place animals destined for disposal in secure, labeled disposal containers on-site; and then deliver the containers to a designated, secure disposal facility, such as a hazardous waste facility or land disposal site.

Aerosols. Larvae of bivalves and of some crustaceans are much smaller than those of fish. Consequently, hatcheries for these organisms must be designed to prevent escape of larvae via aerosols into nearby aquatic ecosystems. Hatchery exhaust fans should be situated so that any aerosols that might be transported outdoors will not reach aquatic ecosystems³.

Equipment cleaning and storage. Certain life stages of certain aquatic GMOs could survive for some time if they are accidentally trapped in damp nets, small puddles in fish egg sorting machines, standing water in buckets, gloves or boots of workers attending to the GMOs, or other equipment. Therefore, all equipment that comes in contact with live GMOs should be properly cleaned and drained after each use. To ensure against accidental transport of live GMOs to another insecure site, such equipment should be either: used and stored solely on the project site; or disinfected using treatments lethal to all GMO life stages and thoroughly drained prior to transport off-site. An inventory of project equipment is recommended.

Security

Security measures are needed to: (a) control normal movement of authorized personnel, (b) prevent unauthorized access to the site, and (c) for outdoor projects, eliminate access of predators who could potentially carry animals off-site. Depending on the abundance and behavior of predator species present in the surrounding area, security measures might need to include electric fences, bird netting, and other exclusion measures. Researchers are expected to design an

³This was recommended by a member of the Aquatic Biotechnology Working Group, Dr. Susan Ford (Haskin Shellfish Research Laboratory, Rutgers University) and by Dr. John Kraeuter (Assistant Director, New Jersey Aquaculture Technology Extension Center), Mr. Walter Canzonier (Maurice River Oyster Culture Foundation and President of the New Jersey Aquaculture Association), and Mr. Gregory Debrosse (Hatchery Manager, Haskin Shellfish Research Laboratory).

appropriate suite of security measures and peer reviewers/inspectors are expected to evaluate their adequacy. Table 3 presents a suite of required and optional measures.

Table 3. Required and optional measures for security at research projects needing management of specific risks. Implementation of optional measures depends on features of the project and project siting.

Measure	Required	Optional
Workers		
background check ¹	yes	-
access control	keys	cards
sign in & out	-	recommended
ID badge with photo	-	recommended
Security training	yes	
Visitors		
sign in & out	-	recommended
Escorts required	yes	-
Animals (outdoor facilities)		
electric fences	-	recommended
bird netting	-	recommended
buried liners or suitable barriers against burrowing animals	yes for outdoor ponds	-
Facility		
Full perimeter control	yes	-
Security alarms	-	recommended
Contracted responder or police response to security alarms	-	recommended
signs/warnings	yes	-
written security plan	yes	-
peer and site review	yes	-

¹ Background checks must comply with relevant laws and institutional policies to protect rights of potential and hired employees.

Alarms

At the project planning stage, the value of installing water level, flooding, and perimeter alarms should be carefully considered (Table 4). All projects, however, must have an appropriately placed water level alarm with a battery or emergency power backup. It must alert designated personnel when the water level goes above or below normal levels but well before the water can circumvent the project's entire suite of barriers. Adequacy of the alarm system should be justified in the worksheet and provided to peer reviewers and inspectors. All installed alarms should be

connected to on-site visual or audible signals and a phone dialer. The dialer should contact personnel in a designated order. Also, all installed alarms should have battery or emergency power backups. Automated alarm systems should not be the exclusive form of monitoring, but rather should provide a backup to human monitoring.

Table 4. Types of required and recommended alarms for projects requiring management of specific risks.

Alarm Type	Required or Optional	Comments
Water Level Alarms	required	require phone dialer, local signal, and power backup
Flooding Alarms	required if project is below 100 yr. flood level; otherwise optional	effectiveness depends on phone dialer, local signal, and power backup
Perimeter Alarms	required if rearing units outdoors; otherwise optional	effectiveness depends on phone dialer, local signal, and power backup
Intrusion Alarms	optional	effectiveness depends on phone dialer, local signal, and power backup

Stand-by Power

Stand-by power is needed, not only to prevent damage to research experiments, but also to avoid possible failure of one or more of the project's barriers and to ensure functioning of alarms.

Operational Plan

All research projects needing risk management must have an approved written operational plan. The plan must describe (a) how the project will be operated under normal conditions; (b) anticipated problems that may occur and how they will be addressed; and (c) an emergency response plan for disaster situations. The plan must address the major components of normal and emergency operations presented below. The entire written plan must undergo peer review prior to its implementation (see peer review and site review section below).

Training

Adequate training must be provided for all personnel accessing the project. Such personnel should read the operational plan. It is recommended that they sign a brief statement that they have read and understand how to implement the plan. Required and recommended types of training are presented in Table 5 below.

Researchers are expected to design an appropriate training program and peer reviewers/inspectors are expected to evaluate its adequacy for the specific research project.

Table 5. Required and recommended personnel training for research projects requiring management of specific risks.

Type	Required	Optional
Routine	Principal Investigators and other Project Staff	-
Emergency	All personnel designated as emergency responder	Refreshers plus drill for all personnel designated as emergency responder
Public	-	Recommended as institutional responsibility

Traffic control

Control of traffic in and out of the confinement facility includes personnel, equipment, wastes, and water and tissue samples. When drafting the traffic control portion of the operational plan, refer to the following previous sections for relevant recommendations: "Barriers for all possible escape paths of the water system" (control of waste slurries); "Prevent escape via non-aquatic paths" (control of equipment and final disposal of animals); and "Security" (control of personnel).

Record keeping

Adequate records must be kept to assess compliance with the operational plan (Table 6). This includes personnel and equipment logs as well as daily experimental logs. Accounting for all genetically modified individuals is an effective means of noting losses and discouraging theft. For groups of small organisms, numbers of individuals should be tracked on a frequent basis; one option is to estimate surviving animals based on daily counts of observed mortalities. Once organisms reach a larger size, exact counts of individuals should be maintained. Wherever feasible, individual tagging of sufficiently large individuals is strongly encouraged because it will permit tracking of every modified individual.

Emergency response plan

An emergency response plan is a required component of the operations plan. The purpose of this plan is to define the most common types of emergencies that a project could face and outline what should be done to prevent loss of aquatic GMOs. As first discussed in the section on project siting, the adequacy of the emergency response plan is particularly important for: marine projects located

below the 100 year flood level; and all projects located in the possible path of wave damage, hurricanes and other natural disasters. For such projects, the experimental scale must be small enough to permit movement to a safe site or destruction of animals before disaster conditions become too dangerous to complete the action.

Table 6. Required and recommended logs for projects requiring management of specific risks

Type of Log	Required or Optional
Personnel logs (in and out of facility)	recommended
Equipment movement logs	recommended
Marking of individual animals	recommended, if possible
Experimental logs	required
Animal inventory logs	larger animals - required smaller animals - recommended to extent possible

Responsible party. The project's principal investigator or a designated proxy must be available in person or by phone at all times to respond to emergency problems.

Notification of loss of confinement. In the event of loss of confinement, the responsible party must notify responsible local agencies and the Institutional Biosafety Committee, if one exists. In most cases, the first local agency to contact is the local office of the state fisheries management agency.

Mitigation or recovery plan. The emergency response plan should include a plan for mitigation or recovery of escaped GMOs in cases where the project site and biological features of the GMO allow recovery or mitigation. The state fisheries management agency should be involved in development of such a plan because it will probably have oversight authority over any recovery or mitigation actions that occur in natural waters.

Movement to safe site or destruction of animals. The responsible party must notify responsible local agencies (probably the state fisheries management agency) and the Institutional Biosafety Committee, if one exists, that such an action will be taken. Oversight of the action by a member of the IBC or a staff person of a local agency is strongly encouraged. The emergency response plan should clearly define the event(s) which activate movement or destruction of animals.

Peer Review and Site Review

This section makes a distinction between review of research projects prior to their start-up and periodic site review after start-up. In some cases, flexibility in this distinction is warranted. For instance, researchers may be planning to conduct a new project, involving new types of GMOs, in a site used previously for another research project with aquatic GMOs that already passed peer review and site review. If the new project clearly has the same specific risks as the old project, less extensive peer review may be adequate but site review should continue. If the new project poses a different set of specific risks, peer review prior to start-up is warranted. The review should address whether or not the existing configuration and components of the project site and barriers are adequate for the new project.

Peer review prior to start-up of project

Peer review of the project's siting, design of barriers, security, and operational plan is required. It is imperative that reviewers include scientists with expertise in organismal and population biology of the project's aquatic GMOs and in ecology of the accessible aquatic ecosystems. It may be beneficial to include a representative of the state fisheries management agency. If the researcher's institution has an Institutional Biosafety Committee (IBC), then peer review should be conducted by the IBC making sure that its membership contains adequate aquatic expertise or that external advisors with such expertise are consulted. If the institution only has a biosafety officer, an interdisciplinary review team including the biosafety officer should be convened. One option is to have the head or supervisor of the principal investigator's department assemble a peer review team. Researchers may find it beneficial to seek advice of both the state fisheries management agency and their IBC or other form of review team in early stages of design of physical facilities and drafting of an operations plan.

Certain states and many institutions require that experiments involving organisms bearing recombinant DNA molecules comply with the National Institutes of Health (NIH) "Guidelines for Research Involving Recombinant DNA Molecules" (NIH 1994), including the most recent amendments (e.g., NIH 1995), and be approved by an IBC or other body. All federally-funded research must comply with these NIH Guidelines. These Performance Standards are intended to further assist all researchers working with fish and shellfish in complying with the NIH guidelines and good safety practices. Other responsible local, state, and Federal agencies should be contacted. All permits/approvals needed from these agencies should be obtained prior to the start of the project.

Site reviews after start-up of project

Site reviews are highly recommended and their scheduling should be the responsibility of the researcher's institution. The number of site reviews should be based on (a) the specific features of the research project, such as the complexity of required risk management measures, and (b) findings during earlier site reviews. The purpose of site reviews is to determine whether or not the project is keeping escapes below the acceptable number of accidental escapes. Site reviews should determine whether: (1) appropriate culture practices are indeed being carried out; (2) physical facilities are performing and are maintained as expected; and (3) the operating plan is being followed. Additionally, records might be checked to ascertain, for instance, if frequencies of routine barrier inspections and maintenance by project staff are adequate. Should problems in compliance with the operational plan be identified, additional unannounced site visits might be appropriate.

Documentation to submit to proposal and site reviewers

Researchers are expected to provide the following documents to reviewers of both the project proposal and of the project site: Performance Standards Flowcharts, Performance Standards Supporting Text, Completed Worksheet with attached documentation, and a Written Operational Plan. It is hoped that a computerized, interactive expert system integrating the Flowcharts, Supporting Text, and Worksheet will be developed. Once such a tool is developed, researchers may prefer to submit documentation in software rather than hardcopy format.

Project approval

Once the IBC or other designated review team has decided that the risk management measures are adequate to address the risks identified for a proposed project, it is advisable to obtain written documentation of this approval. The format of this approval is left up to the discretion of the institutions involved. One option is to have the chair of the IBC or review team attach a brief letter of approval to the final version of the Completed Worksheet.

LITERATURE CITED

- ABRAC 1991. Supplement to Minutes. Guidelines recommended to USDA by the Agricultural Biotechnology Research Advisory Committee, December 3-4 1991. Guidelines for research involving planned introduction into the environment of genetically modified organisms. Document No. 91-04, USDA, Office of Agricultural Biotechnology, 14th and Independence Ave., S.W., Room 1001 RPE, Washington, D.C. 20250-2200.
- Allen, Jr., S. K. and S.L. Downing. 1990. Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. Canadian Journal of Fisheries and Aquatic Sciences 47:1213-1222.
- Aquatic Nuisance Species Task Force 1994. Findings, conclusions, and recommendations of the intentional introductions policy review. Report to Congress. ANS Task Force Coordinator, U.S. Fish and Wildlife Service, Department of Interior, Washington, D.C.
- Avise, J.C., and M.J. Van den Avyle. 1984. Genetic analysis of reproduction of hybrid white bass x stripes bass in the Savannah River. Transactions of the American Fisheries Society 113:563-570.
- Bachman, R.A. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Transactions of the American Fisheries Society 113:1-32.
- Blakenship, K. 1994. Experiment with Japanese oysters ends abruptly: oysters thought to be sterile found capable of reproducing. Bay Journal 4(5):1-4. Alliance for Chesapeake Bay, Baltimore, MD.
- Beverton, R.J.H., and S.J. Holt. 1957. On the dynamics of exploited fish populations. Fisheries Investigations of London, ser 2, 19.
- Buxton, C.D. and P.A. Garrett. 1990. Alternative reproductive styles in seabreams (*Pisces: Sparidae*). Pages 113-124 in M.N. Burton, ed. Alternative Life History Styles of Fishes. Volume 28(1-4).
- Campton, D.E. 1987. Natural hybridization and introgression in fishes: Methods of detection and genetic interpretation. Pages 161-192 in N. Ryman and F. Utter, eds. Population genetics and fishery management. University of Washington Press, Seattle.
- Carlton, J.T., G.J. Vermeig, D.R. Lindberg, D.A. Carlton, and E. Dudley. 1991. The first historical extinction of a marine invertebrate in an ocean basin: The demise of the eelgrass limpet *Lottia alveus*. Biol. bull. 180(1):72-80.
- Carpenter, S.R., and J.F. Kitchell. 1988. Introduction. Pages 1-8 in S.R. Carpenter, ed. Complex interactions in lake communities. Springer-Verlag, New York.
- Chaplin, J.A. 1993. The local displacement of a sexually reproducing ostracod by a conspecific parthenogen. Heredity 71(3):259-268.
- Chevassus, B., et al., 1983. Production of viable hybrids in salmonids by triploidization. Genetique, Selection, Evolution 15: 519-532.

- Christie, W.J., G.R. Spangler, K.H. Loftus, W.L. Hartman, P.J. Colby, M.A. Ross, and D.R. Talhelm. 1987. A perspective on Great Lakes fish community rehabilitation. *Canadian Journal of Fisheries and Aquatic Sciences* 44(Suppl. 2):486-499.
- Collares-Pereira, M.J. 1987. The evolutionary role of hybridization: The example of natural Iberian fish populations. Pages 83-92 in K. Tiews, ed. *Selection, hybridization, and genetic engineering in aquaculture*. Verlag, H. Heenemann GmbH, Berlin, Germany.
- Connell, J.H. 1975. Some mechanisms producing structure in natural communities. Pages 460-490 in M.L. Cody and J.M. Diamond, eds. *Ecology and evolution of communities*. Belknap Press, Cambridge, MA.
- Cooperative State Research Service, Department of Agriculture. 1990. Finding of no significant impact; Research on transgenic carp to be conducted at the Alabama Agricultural Experiment Station (AAES), Auburn University, Alabama. Office of Agricultural Biotechnology, U.S. Department of Agriculture, Room 321-A, Administration Building, 14th and Independence Avenue, S.W., Washington, DC 20250.
- Cooperative State Research Service, Department of Agriculture. 1990a. Availability of an environmental assessment and finding of no significant impact relative to USDA funding of research on transgenic carp. *Federal Register* 55 (225):48661-48662.
- Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. Pages 1-13 in J.N. Spuhler, ed. *Natural selection in man*. Wayne University Press, Detroit, MI.
- Dawley, R.M. 1989. An Introduction to Unisexual Vertebrates. Pages 1-18 in R.M. Dawley and J.P. Bogart, eds. *Evolution and Ecology of Unisexual Vertebrates*. Bulletin 466. New York State Museum, Albany, New York.
- Debas, L., A. Fostier, J. Fuchs, M. Weppe, G. Nedelec, A. Benett, C. Cauty, and B. Jalabert. 1990. The sexuality of cultured hermaphroditic fish species: Analysis of morphological and endocrinological features in a protogynous hermaphrodite, *Epinephelus microdon*, as a basis for further research to control reproduction in the grouper. Pages 543-557 in J. Barret, ed. *Advances in Tropical Aquaculture, Workshop held in Tahiti, French Polynesia, Feb. 20-Mar. 4, 1989*.
- Disney, J.E., K.R. Johnson, and G.H. Thorgaard. 1987. Intergeneric gene transfer of six isozyme loci in rainbow trout by sperm chromosome fragmentation and gynogenesis. *Experimental Zoology* 244: 151-158.
- Dobzhansky, T. 1970. *Genetics of the evolutionary process*. Columbia University Press, New York, NY.
- Emery, L. 1981. Range extension of pink salmon into the lower Great Lakes. *Fisheries* 6(2):7-10.
- Emlen, J.M. 1984. *Population biology. The coevolution of population dynamics and behavior*. MacMillan Publishing Co., New York, NY.
- European Inland Fisheries Commission. 1988. *Code of practice and manual of procedures for consideration of introductions and transfers of marine and freshwater organisms*. Food and Agriculture Organization of the United Nations, EIFAC. Occasional paper No. 23.
- Forshage, A.A. et al. 1988. Natural reproduction of white bass x striped bass in a Texas reservoir. *Proceedings of the Annual Conference of Fish and Wildlife Agencies* 40:9-14.

- Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. Pages 1-98 in W. S. Hoar and D. J. Randall, eds. *Fish physiology*. Volume VI. Environmental relations and behavior. Academic Press, New York, NY.
- Gaffney, P. and S. K. Allen. 1992. Genetic aspects of introduction and transfer of molluscs. *Journal of Shellfish Research* 11: 535-538.
- Gliddon, C.J. and J. Goudet. 1994. Gene flow and analysis of structured populations. Pages 30-40 in Organization for Economic Co-operation and Development (OECD), Environment Directorate, Committee for Scientific and Technological Policy. Environmental impacts of aquaculture using aquatic organisms derived through modern biotechnology. The proceedings of the Trondheim workshop. OECD, Environment Directorate, Environmental Health and Safety Division, 2 rue André Pascal, 75775 Paris CEDEX 16 FRANCE. [FAX (33-1) 45 24 16 75. Document expected to be published in the OECD "Document-on-Sale" series.]
- Goudet, J. 1993. The genetics of geographically structured populations. Ph.D. Thesis. University of Wales, Bangor.
- Goudet, J. T. Meels, A. J. Day, and C.J. Gliddon. 1994. The different levels of population structuring of dogwhelks, *Nucella lapillus*, along the south Devon coast. Pages 31-42 in A. Beaumont, ed. *Genetics and evolution of aquatic organisms*. Chapman and Hall, London.
- Grant, V. 1985. *The evolutionary process*. Columbia University Press, New York.
- Haldane, J.B.S. 1937. The effect of variation on fitness. *American Naturalist* 71:337-349.
- Hallerman, E.M., and A.R. Kapuscinski. 1993. Potential impacts of transgenic and genetically manipulated fish on natural populations: Addressing the uncertainties through field testing. Pages 93-112 in J. Cloud, ed. *Conservation genetics of salmonid fishes*. Plenum Press, New York.
- Harrell, R.M. et al. 1993. Evidence of introgressive hybridization in Chesapeake Bay Morone. *Molecular Marine Biology and Biotechnology* 2: 291-299.
- Hartl, D. 1988. *A primer of population genetics*, second edition. Sinauer Associates, Sunderland, MA.
- Hebert, P.D.N., S.S. Schwartz, R.D. Ward and T.L. Finston. 1993. Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group. 1. breeding systems of Canadian populations. *Heredity* 70(2):148-161.
- Heller, J. 1993. Hermaphroditism in molluscs. *Biological Journal of the Linnean Society* 48(1):19-42.
- Hubbs, C.L. 1955. Hybridization between fish species in nature. *Systematic Zoology* 4: 1-20.
- Hughes, R.M. and R.F. Noss. 1992. Biological diversity and biological integrity: current concerns for lakes and streams. *Fisheries (Bethesda)* 17(3):11-17.
- Ihssen, P.E., et al. 1990. Ploidy manipulation in fishes: cytogenetic and fisheries applications. *Transactions of the American Fisheries Society* 119: 698-717.

- Inada, Y. and N. Taniguchi. 1991. Spawning behavior and after-spawning survival in induced triploid ayu *Plecoglossus altivelis*. *Bulletin of the Japanese Society of Science of Fisheries* 57: 2265-2269.
- Kapuscinski, A.R., and E.M. Hallerman. 1990. Transgenic fish and public policy: anticipating environmental impacts of transgenic fish. *Fisheries (Bethesda)* 15(1):2-11.
- Kapuscinski, A.R., and E.M. Hallerman. 1991. Implications of introductions of transgenic fish into natural ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 48(Suppl. 1):99-107.
- Karr, J.R. 1991. Biological integrity: a long neglected aspect of water resources management. *Ecological Applications* 1:66-84.
- Kitamura, S., H. Ogata, and H. Onozato. 1991. Triploid male masu salmon *Oncorhynchus masu* shows normal courtship behavior. *Bulletin of the Japanese Society of Science of Fisheries*. 57: 2157.
- Kitchell, J. F. and S. W. Hewett. 1987. Forecasting forage demand and yields of sterile chinook salmon (*Oncorhynchus tshawytscha*) in Lake Michigan. *Canadian Journal of Fisheries and Aquatic Sciences* 44(Suppl. 5): 284-290.
- Kohler, C.C. 1992. Environmental risk management of introduced aquatic organisms in aquaculture. *ICES Marine Science Symposia* 194: 15-20.
- Kohler, C.C. and J.G. Stanley. 1984. A suggested protocol for evaluating proposed exotic fish introductions in the United States. Pages 387-406 in W.R. Courtenay, Jr. and J. R. Stauffer, Jr., eds. *Distribution, biology, and management of exotic fishes*. Johns Hopkins University Press, Baltimore, MD.
- Kohler, C.C. and W.R. Courtenay, Jr. 1986. American Fisheries Society position statement on introductions of aquatic species. *Fisheries (Bethesda)* 11(2): 39-42.
- Kwain, W., and A.H. Lawrie. 1981. Pink salmon in the Great Lakes. *Fisheries (Bethesda)* 6(2):2-6.
- Lagler, K.F., J.E. Bardach, and R.R. Miller. 1977. *Ichthyology*. John Wiley and Sons, New York, NY.
- Lassuy, D.R. 1995. Introduced species as a factor in extinction and endangerment of native fish species. *American Fisheries Society Symposium* 15:391-396.
- Leary, R.F., F.W. Allendorf, and G.K. Sage. 1995. Hybridization and introgression between introduced and native fish. *American Fisheries Society Symposium* 15:91-101.
- Lenski, R. E. and T. T. Nguyen. 1988. Stability of recombinant DNA and its effects on fitness. Pages 18-20 in J. Hodgson and A. M. Sugden, eds. *Planned release of genetically engineered organisms*. *Trends in Biotechnology/Trends in Ecology and Evolution Special Publication*. Elsevier Publications, Cambridge.
- MacKenzie, D. 1990. Jumping genes confound German scientists. *New Scientist* 15 December 1990:18.

- Marx, J. 1988. Gene watchers' feast served up in Toronto: Putting foreign genes into domestic animals. *Science* 242:32-33.
- Miller, R.R., J.D. Williams, and J.E. Williams. 1989. Extinctions of North American fishes during the past century. *Fisheries* (Bethesda) 14(6):22-38.
- Mills, E. L. and J. L. Forney. 1988. Trophic dynamics and development of freshwater pelagic food webs. Pages 11-30 in S. R. Carpenter, ed. *Complex interactions in lake communities*. Springer-Verlag, New York, NY.
- Minckley, W. L. and J.E. Deacon (eds). 1991. *Battle against extinction: native fish management in the American west*. University of Arizona Press, Tucson, AZ.
- Monaco, P.J., E.M. Rasch, and J.S. Balsano. 1984. Apomictic reproduction in the amazon molly, *Poecilia formosa*, and its triploid hybrids. Pages 311-328 in B.J. Turner, ed. *Evolutionary genetics of fishes*. Plenum Press, New York, NY.
- Moore, W.S. 1984. Evolutionary ecology of unisexual fishes. Pages 329-398 in B.J. Turner, ed. *Evolutionary genetics of fishes*. Plenum Press, New York, NY.
- Moyle, P.B. and J. J. Cech, Jr. 1988. *Fishes. An introduction to ichthyology*. Prentice Hall, Englewood Cliffs, NJ.
- National Institutes of Health (NIH). 1994. Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). *Federal Register* 59: 34496, July 5, 1994.
- National Institutes of Health (NIH). 1995. Amendment Effective April 17, 1995 to Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). *Federal Register* 60: 20726, April 27, 1995.
- Nehlsen, W., J. Williams, and J. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. *Fisheries* (Bethesda) 16(2): 4-21.
- Nickelson, T.E., M.F. Solazzi, and S.L. Johnson. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. *Canadian Journal of Fisheries and Aquatic Sciences* 43:2443-2449.
- Norse, E.A. 1994. Uncharted waters. Conserving marine biological diversity. Pages 85-86 in G. K. Meffe and C.R. Carroll, eds. *Principles of conservation biology*. Sinauer Associates, Inc., Sunderland, MA.
- Odum, E.P. 1984. The mesocosm. *BioScience* 34:558-562.
- Organization for Economic Co-operation and Development (OECD), Environment Directorate in collaboration with Directorate for Science, Technology, and Industry. 1993. *Safety evaluations of foods derived from modern biotechnology: concepts and principles*. 1993 (93 93 04 1). OECD, 2 rue André Pascal, 75775 Paris CEDEX 16 FRANCE.
- Peralta, M.D. 1988. Aspects on the reproduction of bivalve molluscs. *Acuavision*. 3(16) 14-16.
- Perez-Farfante, I. and L. Robertson. 1992. Hermaphroditism in the penaeid shrimp *Penaeus vannamei* (Crustacea: Decapoda: Penaeidae). *Aquaculture* 103(3-4):367-376.

- Pickett, S.T. A., V.T. Parker, and P.L. Fiedler. 1992. The new paradigm in ecology: implications for conservation biology above the species level. Pages 65-88 in P.L. Fiedler and S.K. Jain, eds. Conservation biology: the theory and practice of nature conservation, preservation, and management. Chapman and Hall, N.Y.
- Pinkert, C.A., D.L. Kooyman, and T.J. Dyer. 1991. Enhanced growth performance in transgenic swine. Pages 251-258 in N. First and F.P. Haseltine, eds. Transgenic animals. Butterworth-Heinemann, Boston.
- Pursel, V.G., C.A. Pinkert, K.F. Miller, D.J. Bolt, R.G. Campbell, R.D. Palmiter, R.L. Brinster, and R.E. Hammer. 1989. Genetic engineering of livestock. *Science* 244:1281-1287.
- Regal, P.J. 1994. Scientific principles for ecologically based risk assessment of transgenic organisms. *Molecular Ecology* (1994) 3:5-13.
- Ricker, W.E. 1954. Stock and recruitment. *Journal of the Fisheries Research Board of Canada* 11:559-623.
- Rothschild, B.J. 1986. Dynamics of marine fish populations. Harvard University Press, Cambridge, MA.
- Sadovy, Y. and D. Y. Shapiro. 1987. Criteria for the diagnosis of hermaphroditism in fishes. *Copeia* 1:136-156.
- Scheerer, P.D., and Thorgaard, G.H. 1983. Increased survival in salmonid hybrids by induced triploids. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 2040-2044.
- Schartl, M., I. Nanda, I. Schlupp, B. Wilde, J.T. Epplen, M. Schmid, J. Parzefall. 1995. Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* 373:68-71.
- Schramm, H. L., Jr. and R. G. Piper, eds. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society Symposium 15. American Fisheries Society, Bethesda, MD.
- Sindermann, C.J. 1986. Strategies for reducing risks from introductions of aquatic organisms: a marine perspective. *Fisheries (Bethesda)* 11(2):10-15.
- Sindermann, C.J. 1992. Role of the International Council for the Exploration of the Sea (ICES) concerning introductions of marine organisms. Pages 367-376 in A. Rosenfield and R. Mann, eds. Dispersal of living organisms into aquatic ecosystems. Maryland Sea Grant, College Park, MD.
- Soto, C. G., J.F. Leatherland, and D.L.G. Noakes. 1992. Gonadal histology in the self-fertilizing hermaphroditic fish, *Rivulus marmoratus* (Pisces, Cyprinodontidae). *Canadian Journal of Zoology* 70(12):2338-2347.
- Stein, R.A., S. T. Threlkeld, C.D. Sandgren, W.G. Sprules, L. Persson, E.E. Werner, W.E. Neill, and S.I. Dodson. 1988. Size-structured interactions in lake communities. Pages 161-179 in S.R. Carpenter, ed. Complex interactions in lake communities. Springer-Verlag, New York.

- Sunobe, T. and A. Nakozono. 1993. Sex change in both directions by alteration of social dominance in *Trimma okinawae* (Pisces: Gobiidae). *Ethology* 94(4):339-345.
- Thorgaard, G.H., P.D. Scheerer, and J.E. Parsons. 1985. Residual paternal inheritance in gynogenetic rainbow trout: implications for gene transfer. *Theoretical and Applied Genetics* 71:119-121.
- Thorgaard, G.H., and Allen, S.K., Jr. 1992. Environmental impacts of inbred, hybrid, and polyploid aquatic species. Pages 281-288 in A. Rosenfield and R. Mann, eds. *Dispersal of living organisms into aquatic ecosystems*. Maryland Sea Grant College Program, College Park, Maryland.
- Tiedje, J.M., R.K. Colwell, Y.L. Grossman, R.E. Hodson, R.E. Lenski, R.N. Mack, and P.J. Regal. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. *Ecology* 70:298-315.
- Triantaphyllidis, G.V., T.J. Abatzopoulos, R.M. Sadaltzopoulos, G. Stamou, and G.C. Kastritsis. 1993. Characterization of two new *Artemia* populations from two solar saltworks of Lesbos Island (Greece): Biometry, hatching characteristics and fatty acid profile. *International Journal of Salt Lake Research* 2(1):59-68.
- Turner, B.J., J.F. Elder, Jr., T.F. Laughlin, W.P. Davis, D.S. Taylor. 1992. Extreme clonal diversity and divergence in populations of a selfing hermaphroditic fish. *Proceedings of the National Academy of Sciences* 89(22):10643-10647.
- Turner, B.J., ed. 1984. *Evolutionary genetics of fishes*. Plenum Press, New York.
- Vincent, R.E. 1987. Effects of stocking catchable-size hatchery rainbow trout on two wild trout species in the Madison River and O'Dell Creek, Montana. *North American Journal of Fisheries Management* 7:91-105.
- Voshell, J.R., Jr., ed. 1989. Using mesocosms to assess the aquatic ecological risk of pesticides: Theory and practice. *Entomological Society of America, Miscellaneous Publications* 75:1-88.
- Vrijenhoek, R.C. 1984. The evolution of clonal diversity in *Poeciliopsis*. Pages 399-429 in B.J. Turner, ed. *Evolutionary Genetics of Fishes*. Plenum Press, New York.
- Wilbur, A.E. and P.M. Gaffney. 1991. Self-fertilization in the bay scallop, *Argopecten irradians*. *Journal of Shellfish Research* 10(1):274.
- Wahl, D.H., R.A. Stein, and D. R. DeVries. 1995. An ecological framework for evaluating the success and effects of stocked fishes. *American Fisheries Society Symposium* 15:176-189.
- Wilcove, D., M. Bean, and P.C. Lee. 1992. Fisheries management and biological diversity: problems and opportunities. *Transactions of the 57th North American Wildlife and Natural Resources Conference*: 373-383.
- Williams, J.D. and M. Mulvey. 1994. Recognition of freshwater mussel taxa. A conservation challenge. Pages 57-58 in G. K. Meffe and C.R. Carroll, eds. *Principles of conservation biology*. Sinauer Associates, Inc., Sunderland, MA.

- Williams, J.D., M.L. Warren, K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* (Bethesda) 18(9):6-22.
- Williams, J.E. Johnson, D.A. Hendrickson, S. Contreras-Balderas, J.D. Williams, M. Navarro-Mendoza, D.E. McAllister and J.E. Deacon. 1989. Fishes of North America, endangered, threatened, or of special concern, 1989. *Fisheries* (Bethesda) 14(6):2-38.
- Wood, H.A., K. Munkenbeck Trotter, T.R. Davis, and P.R. Hughes. 1993. Per os infectivity of preoccluded virions from polyhedrin-minus recombinant baculoviruses. *Journal of Invertebrate Pathology* 62:64-67.
- Wright, S. 1943. Isolation by distance. *Genetics* 28:114-138.

GLOSSARY OF TERMS

Abiotic characteristic limiting reproduction (IV.A.1) an environmental factor (e.g., temperature, salinity) of an accessible ecosystem which precludes the possibility of reproduction by a genetically modified aquatic organism.

Accessible ecosystem means the aquatic environment immediately accessible to an organism if it were to escape from the research site, and more distant habitats in the contiguous environment into which the organism or its offspring reasonably may be expected to disperse.

Adverse decline extremely unlikely (IV.A.1) Population decline, combined with demographic or stochastic effects, can result in local extirpations. Therefore, such population decline should be avoided.

Aneuploid refers to an organism in which the number of chromosomes is not an exact multiple of the typical haploid set for the species.

Anadromous fishes spend the adult phase of their life cycles in salt water (or large bodies of fresh water, such as the Laurentian Great Lakes) but move up streams and rivers to spawn (e.g., Pacific salmon, *Oncorhynchus spp.*). This life history type, called anadromy, is the opposite of catadromy (see below).

Bearers are fishes that carry their embryos (and sometimes their young as well) around with them, either internally or externally (Moyle and Cech 1988). External bearers include transfer brooders, forehead brooders, mouth brooders, gill chamber brooders, and skin brooders. Arrangements of internal bearers include: embryo laying following internal fertilization (ovi-ovoviviparity), internal incubation of embryos with no nutrients provided by the mother (ovoviviparity), or internal incubation with nutrients provided by the mother (viviparity).

Captive breeding the controlled husbandry of an aquatic organism under conditions of confinement.

Catadromy refers to the life history pattern of fishes which spend most of their life in fresh water but spawn in salt water (e.g., eels of the family *Anguillidae*). This pattern is opposite that exhibited by anadromous fishes (see above).

Conspecific refers to an individual belonging to the same species.

Dioecious (Dioecy) literally, two houses; for a given species, (the condition of) having male and female reproductive organs in separate, unisexual individuals.

Diploid refers to an individual bearing the usual two haploid sets of chromosomes.

Environmental safety the execution of an experiment without measurable, undesired consequences upon biotic or abiotic components of the environment.

Environmental effects consequences of execution of an experiment, which might include, but are not limited to: (1) changes in the structure, function, or resiliency of an accessible ecosystem, (2) changes in the gene pool of populations resident in the accessible ecosystem or (3) decline in abundance of a population of threatened, endangered, or special concern species.

Epistasis the situation where one gene affects the expression of another.

Extremely low survivorship (II.C.1) survival rates of an interspecific hybrid which are expected to be so low that the hybrid poses virtually no risk of introgressive hybridization with populations of parental or closely related species in accessible ecosystems.

F_{ST} or Fixation Index, measures the reduction in heterozygosity of a subpopulation due to random genetic drift. The fixation index serves as a convenient and widely used measure of genetic differences between populations. In natural populations, observed values of F_{ST} in natural populations include not only random drift, but also migration, natural selection, and mutation. In spite of the resulting complexity in interpretation, F_{ST} is still useful as an index of genetic differentiation (Hartl 1988).

Gene introgression incorporation of a gene into the gene pool of a population.

Genetic load of a population is the proportion by which the population fitness is decreased in comparison with an optimum genotype (Crow 1958).

Hermaphrodite an individual having both male and female reproductive organs. A simultaneous hermaphrodite has both types of gonads throughout its life. A sequential hermaphrodite may be protogynous (having an ovary first, then a testis) or protandrous (having a testis first, then an ovary).

Indirect interactions (V.) Effects of a genetically modified organism on (an)other organism(s) in the accessible ecosystem which are effected through mechanisms involving abiotic factors or additional species. Examples would include, but not be limited to: (1) modification of the physical environment, affecting its suitability as habitat for another species, and (2) cascading effects of altered trophic function in aquatic communities.

Infectious material or agent means any living stage of any organism or any infectious substances that can cause disease in any fish, mollusc, or crustacean or parts thereof, or any processed, manufactured, or other products of fish, molluscs, or crustaceans.

Interspecific hybridization the process of producing a hybrid individual resulting from mating between an adult from one species and an adult from another, different species.

Interspecific reproduction the production of progeny due to mating between individuals of different species. See: interspecific hybridization.

Intraspecific selective breeding the choosing by humans of the genotypes contributing to the gene pool of succeeding generations of a given population of a species; typically, a subset of available individuals breeding individuals are chosen from the population on the basis of fitness or phenotypic value.

Introgression the incorporation of genes of one species into the gene pool of another, the result of backcrossing of fertile hybrids with one or both of the parent species.

Introgressive hybridization introgression (see above) whereby the fertile hybrids tend to backcross with the more abundant species, resulting in a population of individuals most of whom resemble the more abundant species but which also have some of the characteristics of the other parent species. A consequence of this process is loss of genetically distinct populations of one or both parent species.

Marker sequence a DNA sequence introduced into an organism for the purpose of unambiguously identifying the treated individuals or their progeny.

Mosaic, as used in this document, refers to an individual in which component tissues bear different numbers of chromosomes.

Negligible - biological consequences of number of accidental escapees is so insignificant as to be unworthy of consideration.

Non-dioecious the condition of *not* having male and female reproductive organs in separate, unisexual individuals; monecious.

Non-indigenous species means any species or viable biological material that enters the ecosystems beyond its historic range (i.e., territory occupied by the species at the time of European colonization of North America), including any such organism transferred from one country to another (see Appendix A, Excerpts from the Aquatic Nuisance Species Program).

Non-reproductive interference undesired impacts upon a species by a genetically modified organism by other than reproductive mechanisms, e.g., through heightened competition, predation, parasitism, etc.

Novel trait (1) expression of a compound not normally found in the species, e.g., antifreeze polypeptide in Atlantic salmon; (2) expression of a compound normally present in the species if under novel regulatory control, e.g., expression of a species' own growth hormone gene under transcriptional regulation by any but its own growth hormone gene; (3) possession of a chromosomal complement which differs in number or composition from its normal complement, e.g., as in triploids or interspecific hybrids.

Overall phenotype refers to an organism's overall performance at a given life stage. Its performance results from additive and interactive effects of all its qualitative and quantitative traits, such as physiological and behavioral traits, and is affected by genetic and environmental influences on these traits.

Parental organism refers to (1) the organism (parents) to be used in cross-breeding, or (2) the initial organism which is to be the recipient of introduced genetic material or whose genome is to be altered by addition, removal, or rearrangement of genetic material.

Parthenogen (Parthenogenesis) An organism which develops (the process of development) from an egg without fertilization.

Permanently sterile - see sterile.

Persistence is the ability of an observed ecosystem structure or species composition to continue (within known limits) through time.

Pleiotropy the phenomenon where a single gene is responsible for a number of distinct and seemingly unrelated phenotypic effects.

Polyploidy the condition of having a number of chromosome sets is greater than the usual number.

Processes of an ecosystem refers to the biological, chemical, or physical processes occurring in aquatic ecosystems. Also called ecosystem function.

Protected population - population of a species which is listed by federal or state governments as endangered, threatened, or of special concern.

Recruitment is the number of individuals born each year in a population. It is used in the context of population dynamics of fish and shellfish resources.

Reproductive interference disruption of the reproduction of the species by a genetically modified organism, e.g., through its behavior at a spawning site, or by fertilization of eggs by aneuploid sperm.

Reproductively mature age - also called sexual maturity.

Reproductive potential (IV.A.1) - affected by factors such as fecundity, viability of gametes, survivorship of embryos and older progeny.

Resiliency is the ability of an ecosystem to recover to its previous state after a major disturbance.

Self-fertilizing hermaphrodites organisms having both male and female reproductive organs, and which are capable of reproduction by means of fertilizing their own eggs.

Sterile (permanently sterile) Unable to reproduce (unable to gain or regain the ability to reproduce).

Structure of an ecosystem refers to biological interactions among species as manifested in use of food and space.

Tetraploid refers to an individual bearing four, instead of the usual two haploid sets of chromosomes.

Triploid refers to an individual bearing three, instead of the usual two haploid sets of chromosomes.

True parthenogen an organism which reproduces exclusively through parthenogenesis; i.e., its reproduction never involves normal fertilization.

Unintentional trait changes (IV.A) - might occur due to unexpected pleiotropy or epistasis.

Zone of tolerance is the range of values of a given environmental factor over which the lifespan of an organism is not influenced by the direct lethal effect of this factor (Fry 1971). Stated otherwise, the organism is able to acclimate to changing values of the factor within the zone of tolerance.

APPENDIX A: EXCERPTS FROM THE AQUATIC NUISANCE SPECIES PROGRAM (ANS)

The following excerpts are photocopies from, "Proposed Aquatic Nuisance Species Program, September 1992" prepared by the Aquatic Nuisance Species Task Force. Requests for copies of the entire document should be sent to: ANS Task Force Coordinator, U.S. Fish and Wildlife Service (820 ARLSQ), Department of the Interior, 1849 C Street N.W., Washington, D.C. 20240.

Table 2: Examples of Suspected Generic Pathways Involved in the Unintentional Introduction of Nonindigenous Aquatic Species.

Appendix H: Protocol for Evaluating Research Proposals Concerning Nonindigenous Aquatic Species.

Table 2. EXAMPLES OF SUSPECTED GENERIC PATHWAYS INVOLVED IN THE UNINTENTIONAL INTRODUCTION OF NONINDIGENOUS AQUATIC SPECIES

Shipping

- o Ballast water and sediments
- o Anchor chains and chain lockers
- o Sanitary water
- o Hull surfaces
- o Bilge water and sediments
- o Propeller-shaft housing
- o Trash/refuse/garbage

Floating Oil/Gas Drilling Platforms

Recreational Boating

- o Hull surfaces
- o Waste sanitary water
- o Bait wells
- o Bilge water and sediments
- o Motors
- o Associated tools and equipment

Media (e.g., water, seaweed, soil, etc.), Containers and Equipment Used to Transport Live Organisms

- o Aquarium fish, plants, etc.
- o Bait
- o Aquaculture fish, shellfish, plants, etc.
- o Fishery management (e.g., fish stocking)
- o Research specimens
- o Ornamental, other plants
- o Pathogens in target animals

Fresh or Frozen Seafood Transport and Disposal

Human Created Water Connections

- o Navigation canals (e.g., Erie and Welland Canals)
- o Interbasin water transfers (e.g., for irrigation, municipal/industrial water supply, etc.) (Meador 1992)

Natural Pathways

- o Waterfowl and other water birds
- o Hybrid backcrosses

PROTOCOL FOR EVALUATING RESEARCH PROPOSALS CONCERNING NONINDIGENOUS AQUATIC SPECIES

Introduction

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Act, Public Law 101-646, 104 STAT. 4671, 16 U.S.C. 4701-4741 approved November 29, 1991) requires that an intergovernmental Aquatic Nuisance Species Task Force develop and follow a protocol to ensure that research carried out under Subtitle C of the Act does not result in the unintentional introduction or dispersal of nonindigenous aquatic nuisance species to the waters of the United States. This protocol fulfills the requirements of the Act. The Task Force intends to develop the research protocol further based on experience gained through implementation of this protocol. This protocol will supplement other existing Federal protocols established to control activities with specific major classes of organisms, such as those already established for plants and insects under the Plant Quarantine Act of 1912 and the Federal Plant Pest Act of 1952, and for research involving recombinant DNA molecules under the Public Health Service Act of 1944.

This protocol must be used when research is carried out under Subtitle C of the Act. Individuals, states, corporations, and institutions not otherwise covered by this research protocol are encouraged to follow the protocol to prevent introductions of nonindigenous aquatic nuisance species through research activities. Prevention of introductions through other means is being addressed in the Task Force's proposed Aquatic Nuisance Species Program (which addresses prevention, detection and monitoring, and control of nonindigenous aquatic nuisance species). An Intentional Introductions Policy Review Committee is also meeting to review methods by which intentional introductions are and can be controlled. This committee's report and recommendations and the Task Force's Program will be published and receive public comment.

A Research Protocol Committee (Appendix III) composed of representatives from the Task Force members was established to develop the required research protocol. The committee met in Gainesville, Florida, on June 25, 26 and 27, 1991, drafted the protocol, and prepared policy recommendations to the Task Force concerning implementation of the protocol. The draft protocol was circulated to all Task Force agencies for review. A second draft was presented to the Task Force on September 27, 1991. Following a meeting of the Research Protocol Committee on April 1 and 2, 1992, and receipt of additional comments from Federal and non-Federal sources, a final draft was prepared and presented for Task Force approval on April 21, 1992. The research protocol was adopted by the Task Force on April 22, 1992.

Research Protocol

The research protocol consists of two parts: a risk assessment questionnaire (Part I) and a set of guidelines outlining required preventative containment and confinement procedures (Part II). The risk assessment questionnaire requires the Principal Investigator to evaluate the risk that the species, if it escapes or is released, will be a nuisance, and to determine if preventative measures must be taken to prevent the species from escaping or being released. Research may be conducted with no specific preventative measures if: 1) the research site is within the present range of the species; 2) the species is free of nonindigenous diseases, parasites or other extraneous viable material; 3) the species is not likely to be a nuisance if released; and 4) the species cannot survive in the waters adjacent to the research location; or 5) only non-viable forms are used; or 6) the research does not involve actual handling or transfer of the species (e.g. computer modelling and in situ data collection).

The second part of the protocol is a detailed set of preventative containment and confinement guidelines that the Principal Investigator must follow to prevent the escape or release of any research species that fails to meet one or more of the conditions listed above. If any of these conditions exist, the Principal Investigator must take preventative measures that will contain or confine the species to the research facility.

Appendix I is a list of some of the presently existing guidelines and protocols that may be used as resources by investigators to identify the types of precautions that can be taken to prevent unintentional releases of organisms used in research. The specific precautions needed (which include procedural and facility design and use elements) will depend on the species to be studied, its life stage and size (e.g. macroscopic and/or microscopic, and size range within each), the scope of the project, the characteristics of the research site location with regard to the species' critical environmental factors, and the potential of the species to survive in that locale and to be a nuisance. If the species is a disease-causing organism or a parasite, or the species or the source of the species under consideration is not free of nonindigenous diseases or parasites, extra precautions may be necessary. Most of the guidelines listed require that test species be contained or confined by some combination of physical, biological, chemical, and/or environmental barriers, or by limiting the scope of the research. The number and types of barriers needed depends on the species and the potential problems the species could create if it escapes or is released from the research site.

Procedures to Process Research Proposals

1. The Principal Investigator

The Principal Investigator will submit all research proposals concerning nonindigenous aquatic species to their Research Institution for review -- usually the Research Institution will establish a committee similar in membership, roles and responsibilities to the Institutional Biosafety

Committee (IBC) described in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (Federal Register 51, Number 88, page 16959 (51 FR 16959)). In the proposal the Principal Investigator must demonstrate a knowledge of the life history and biology of the species, provide all information necessary for preparation of an environmental assessment, and provide citations for all supporting data. If the species is found to represent any possibility of being a nuisance (risk assessment questionnaire), the proposal must clearly demonstrate that: 1) adequate confinement and containment procedures will be in place during research and throughout the time that the species is held; and 2) the Principal Investigator has incorporated into the study plan procedures, facility design elements, and other preventative measures analogous to those in guidelines developed by NIH for research in recombinant DNA, and the U.S. Department of Agriculture for research in agricultural biotechnology (49 FR 50856, 51 FR 23302, and 56 FR 4134), which are adequate to contain and confine the species and any pathogens or parasites it may contain or be infested with.

2. The Institutional Biosafety Committee

The IBC must determine if the proposal is complete, and that it includes an accurately completed risk assessment questionnaire, all required life history and biological data, and adequate and detailed containment and confinement measures. The IBC should also determine that the proposal complies with all applicable local, state, and national laws and regulations. The IBC should determine if a species-specific containment/confinement protocol has been approved by the Research Protocol Committee for the species and if so, whether the proposal fully meets all requirements of that approved species-specific protocol (ASSP). If an ASSP exists and the Principal Investigator deviates from that ASSP, the IBC should ensure that the differences and the substituted preventative measures are clearly described, since a further review and approval of the proposal by the Research Protocol Committee will be required. If no ASSP exists, the IBC must be assured that the Principal Investigator has conducted a thorough literature review on the species, is knowledgeable of its life history, biology and ecology, and has developed and described preventative measures to adequately contain and confine the species if necessary. Proposals not conforming to an ASSP or for which no ASSP exists will require a full review by the Research Protocol Committee, and should follow guidelines similar to that outlined in Appendix I. The proposal, along with the appropriate findings and certification of compliance from the Principal Investigator, IBC and the Research Institution, will then be transmitted to the Funding Agency. If there is no IBC at a Research Institution, or if the IBC does not have the expertise to evaluate a particular proposal, the proposal will be transmitted to the Funding Agency accompanied by a request for a review by the Research Protocol Committee.

3. The Funding Agency

The Funding Agency provides technical and programmatic review, determines if the proposal is complete and that it complies with the requirements of the National Environmental Policy Act (NEPA) and other applicable laws and

regulations (Appendix IV). The Funding Agency makes all funding decisions; it selects proposals for funding, submits them to the Research Protocol Committee, and after receipt of the Research Protocol Committee's review, determines which proposals will be funded. The Funding Agency may require that the Principal Investigator make changes in the proposal before submittal to the Research Protocol Committee for initial or re-review. All proposals selected for funding will be transmitted to the Research Protocol Committee within 15 days after the proposal has been selected for funding, either for review, if the IBC and Research Institution have not already certified that the proposal is in compliance with a species-specific protocol approved by the Research Protocol Committee (ASSP), or for informational purposes, if the IBC and Research Institution have certified compliance with an ASSP. The Research Protocol Committee will eventually review all proposals, but proposals following an ASSP do not have to be reviewed prior to funding.

4. The Research Protocol Committee

All proposals concerning nonindigenous aquatic species (including the risk assessment questionnaire and preventative measures to be used to prevent escape or inadvertent release) selected for funding by a Funding Agency will be submitted to the Research Protocol Committee within 15 days of selection for funding. For those proposals where the IBC and Research Institution have certified that the research complies with an existing species-specific protocol already approved by the Research Protocol Committee (ASSP), the Research Protocol Committee will use the proposals for informational needs for revising this protocol and approved species-specific protocols as necessary, and for reporting purposes. For all other proposals, the Research Protocol Committee will review in detail the completed risk assessment questionnaire, the research proposal, and the proposed containment and confinement procedures to insure that the proposed procedures are adequate to prevent the species from escaping or being released during the research. The Research Protocol Committee will review and provide comments and recommendations to the Funding agency within 90 days. Proposals requiring major changes must be resubmitted to the Research Protocol Committee for review. The Research Protocol Committee may call on outside expertise when necessary or may establish subcommittees to review multiple proposals for work on the same species. The Research Protocol Committee will advise the Funding Agency and make recommendations: 1) the proposal (including the completed risk assessment questionnaire and preventative measures) appears to be adequate and thus funding is appropriate; 2) the proposal is not adequate in all aspects and needs to be resubmitted to the Research Protocol Committee after deficiencies identified are addressed and appropriate changes made to the proposal; or 3) the proposal has serious inadequacies that require major changes, and should not be funded until these changes are made and the proposal has been resubmitted to the Research Protocol Committee and deemed adequate.

All proposals (both those complying with an approved species-specific protocol and those with individualized containment and confinement plans) will be reviewed by the Research Protocol Committee to determine if there are problems in the use of the risk assessment questionnaire and to improve both this research protocol and approved species-specific protocols. The Research

Protocol Committee will provide an annual report to the Task Force detailing the proposals reviewed, the species involved, the number of proposals needing detailed confinement and containment procedures, the location of the research sites by species, and problems encountered, and will recommend changes to the Task Force as needed.

The Research Protocol Committee will serve as an advisor to the Funding Agencies, providing comments and recommendations on the risk assessment and adequacy of preventative measures being taken by the researcher. The responsibility of selecting and funding the research belongs entirely to the Funding Agency.

Attachment I is a list of guidelines and protocols used to confine or contain nonindigenous species or organisms involved in recombinant DNA research. Consulting one or more of these will help investigators to identify physical, biological, chemical, and/or environmental preventative measures that may be used to confine or contain the nonindigenous aquatic species during research, transportation and storage.

PART I

Risk Assessment Questionnaire

Completed risk assessment questionnaires must be submitted in narrative form to the Funding Agency along with the research protocol. The reasoning behind each answer must be stated.

- I. Does the research concern a nonindigenous aquatic species as defined by the Nonindigenous Aquatic Nuisance Species Prevention and Control Act of 1990? Nonindigenous aquatic species means any species or other viable biological material that enters an ecosystem beyond its historical range, including transfers from both domestic and foreign sources. [Historical range is the territory occupied by a species at the time of European colonization of North America.]

ALL ANSWERS: go to II.

- II. Does the species carry any known nonindigenous diseases, parasites or any other nonindigenous species or viable biological material? Unless there is knowledge or evidence to the contrary (e.g., oysters being transferred from an area where MSX or dermo or imported oyster drills exist, salmonid transfers from areas where IHN and VHS viruses occur, or warmwater species transfers from areas where the Asian tapeworm occurs) species transfers within the continental U.S. can be considered free of nonindigenous diseases or parasites. Any species recently imported directly or indirectly from a foreign country, Hawaii, or a territory of the U.S. should be considered to have nonindigenous diseases or parasites unless proven otherwise; appropriate preventative measures must be taken (see Part II, Guideline of Preventative Measures).

YES or NOT SURE: go directly to Part II (Guideline of Preventative Measures) and to III;

NO: go to III.

- III. Do or could transportation waters, media or sediments carry any nonindigenous diseases, parasites, or other viable material (extraneous organisms)?

YES or NOT SURE: transfer species to clean water and container, treat waste water to kill all organisms, disinfect original container. If this is sufficient to rid the shipment (transfer) of all extraneous organisms, go to IV; if not, go to Part II (Guideline of Preventative Measures).

NO: go to IV.

- IV. If the research does not concern a nonindigenous aquatic species under the Act and the research could not spread nonindigenous diseases,

parasites or other viable material, this protocol does not apply. If the species falls under the Act, continue on to V.

Answers to I., II., and III. are all NO: the protocol does not apply to your research organism;

Any answer to I., II., and/or III. above is YES or NOT SURE: the species falls under the Act; go to V.

V. Will live, viable, or fresh specimens be required?

NO (specimens must be preserved in a manner to kill the organisms immediately to assure no possibility of infestation if the specimens are released): no additional procedures may be necessary;

YES: go to VI.

VI. Will the species be transferred away from the site where collected?

NO: protocol does not apply.

YES: go to VII.

VII. Will the species be transported through areas which are free of the infestation?

YES: adequate preventative measures must be taken to prevent escape or release during transportation; go to VIII.

NO or NOT SURE: go to VIII.

VIII. Is the species present within one mile of any facility which will receive live nonindigenous species or other non-preserved field material which may be contaminated with a nonindigenous species? Studies may be conducted in more than one research laboratory (including field laboratories). List each laboratory in which the research will be conducted, and discuss and document for each laboratory.

YES (The species is found within one mile of a research facility or its effluent discharge point.): the study may not require more than minimal measures at this facility to prevent the species' introduction. It may however require precautionary measures to ensure that nonindigenous species are not spread between collection sites, from one facility to another facility, or from a facility to non-infested sites by means of equipment or supplies used at more than one study site or used for more than one study.

NO (The species is not found within one mile of a research facility which will receive live nonindigenous species or other non-preserved field material which may be contaminated with a nonindigenous species, or within one mile of the facility's

effluent discharge point.): the researcher should report the nearest known population of the species from each facility and go to IX.

IX. Can the species survive in the surrounding waters?

NO: only minimum preventative measures may be needed.

YES or NOT SURE: go to X.

X. Is it absolutely certain that the species will not be a nuisance if it escapes or is released into surrounding waters? [Note: A nuisance species threatens the diversity of abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural, or recreational activities dependent on such waters.]

YES: only minimum preventative measures may be needed.

NO or NOT SURE: go to XI.

XI. Have you previously been approved for research with this species at your present location using the same facilities?

YES: explain the differences between this proposal and previous funded studies and attach a copy of previous approval letter.

If major changes exist from earlier funded study or the answer is NO: go to XII.

XII. Is there a Research Protocol Committee approved species-specific protocol (ASSP) for the nonindigenous species that is (are) the subject(s) of your research proposal, and will this ASSP be used by you for this proposal?

YES (An ASSP exists and will be adhered to in every particular.) Attach the ASSP and list specifics (e.g., options to be used) that are to be used in your research.

NO (No ASSP exists, or an ASSP exists but will not be used.): go to XIII.

NO (An ASSP exists but will not be exactly adhered to, i.e. additional or different methods will be used, or parts of the ASSP will not be used.) Describe in detail any deviation from the ASSP, specify if any part of the ASSP will be used, and describe preventative methods to be used that differ from those in the ASSP. If any part of the ASSP is to be used, attach the ASSP.

XIII. If the proposal has reached this point in the risk assessment, a preventative containment/confinement plan must be developed which will ensure that the species or any diseases or parasites it might carry cannot escape or be released into the surrounding waters. The species

under consideration is a live or viable nonindigenous aquatic species, a nonindigenous pathogen or parasite of aquatic species, or might be carrying nonindigenous diseases or parasites of aquatic species, is not present in the waters surrounding the research site, could survive if released, and could be a nuisance. The researcher must document knowledge of the literature concerning the species and the problems which could result if released. A plan must be developed to ensure that the research does not result in the release, escape, or dispersal of the species. The investigator will be required to develop a preventative plan (PART II) and submit it with the research proposal to the Funding Agency who will forward it to the Research Protocol Committee for review. The investigator and the supporting Research Institution must agree to comply with the preventative plan, and this protocol or an approved species-specific protocol. The Funding Agency and the Research Institution will ensure compliance.

Every investigator conducting research on a live or viable nonindigenous aquatic species which could be a nuisance, and is conducting the research outside the species' present range, is required to develop containment and confinement procedures and have a secure facility. Reference to guidelines already available (Appendix I) can be of assistance in developing a containment and confinement plan. Table I is an outline of the information and containment and confinement procedures required in most existing guidelines. In the future species-specific protocols may be developed for high visibility species (like the zebra mussel) whose life history, biology, and impacts are known and for which there are multiple studies under consideration. When reviewed and approved by the Research Protocol Committee, approved species-specific protocols (ASSP) may be used by investigators, however compliance to all points of the ASSP will be mandatory. Any or all protocols may be changed by the Research Protocol Committee as new knowledge is accumulated. Deviations from approved species-specific protocols will require case by case approval of research proposals and their preventative plans. Research on nonindigenous species which may also have nonindigenous diseases and parasites will require maximum security for the species and for any diseases or parasites the species may carry. Every effort should be made to conduct research on nonindigenous species in facilities located within the existing established range of the species; in this case only one level of preventative measures may be required.

PART II

Guideline of Preventative Measures

The Research Protocol Committee cannot develop a detailed set of guidelines for every nonindigenous species under research. Investigators and Research Institutions must develop containment and confinement plans taking into consideration the species, its characteristics, diseases and parasites, and critical environmental factors, its capabilities to be a nuisance, the design of the research facilities, and the location of the test site in relationship to the species' present range. Appendix I lists guidelines which have already been developed for groups of organisms. Table I is an outline of the informational needs and preventative measures to contain or confine test species found in most guidelines. The appendix and table are included as reference materials for investigators.

If the investigator determines that live specimens must be used, that the research must be conducted in an area where the species is not already present, that the species could survive if released into surrounding waters, and that the species or its diseases or parasites could be a nuisance, major preventative measures would be required to prevent escape or release. The preventative plan should use a combination of physical, biological, environmental, and/or chemical barriers to contain or confine all life stages of the organism. Reducing the scope of the research should also increase the safety of the research.

For containment of diseases, parasites, small species, or the early life stages of larger species, the procedures outlined in the NIH guidelines (FR 51 No. 88, May 7, 1986, pg. 16959) or guidelines developed by the U.S. Department of Health and Human Services (see references) are the most comprehensive.

For containment or confinement of larger forms, the guidelines developed for whole plants or animals by the Office of Agricultural Biotechnology, USDA, are the most appropriate, especially if the research is to be conducted outside the laboratory (see Appendix I).

Preventative measures should address all life stages present or possible during the research phase. Where feasible, use of juvenile specimens, monosex populations, or sterile individuals is recommended.

Species-Specific Confinement and Containment Protocols

The Research Protocol Committee expects to receive many research proposals on a few high profile, high risk species, such as zebra mussels. Either the Research Protocol Committee, a subcommittee of the Research Protocol Committee, or one of the funding agencies may submit a species-specific confinement/containment protocol for review by the Research Protocol Committee. When such a proposed species-specific protocol is submitted, the Research Protocol Committee will review the adequacy of proposed containment

procedures to insure that the species cannot escape or be released during research. The Research Protocol Committee will complete its review and provide a response to the appropriate Funding Agency or subcommittee within 90 days. The form of the Research Protocol Committee's response will be either: 1) The species-specific protocol is adequate as proposed and is approved for general use by the research community (i.e., the protocol has become an approved species-specific protocol (ASSP)); or 2) The species-specific protocol is not adequate as proposed and is not approved. If the proposed species-specific protocol is not approved, the Research Protocol Committee will state reasons and may suggest modifications to correct problems seen. Since these protocols will only be prepared for species which are considered nuisance species, the risk assessment section can be reduced and the preventative plan can be standardized.

Compliance with all provisions of an approved species-specific protocol (ASSP) must be fully accepted in writing by the Investigator and the Research Institution. Specific preventative measures to be used by the Investigator must be documented in the research proposal. If all aspects of the ASSP are accepted, the Research Institution, through its Institutional Biosafety Committee, can approve confinement and containment procedures and monitor the research. All documentation, including the proposal, completed risk assessment questionnaire, and preventative measures to be used, will be forwarded to the Research Protocol Committee by the Funding Agency. Any deviations from the requirements of an ASSP will require that the research proposal and confinement and containment plan be reviewed by the Research Protocol Committee before funding is approved.

The Research Protocol Committee will use the information in all research proposals (using both species-specific and non-standard protocols), to improve future protocols and to monitor the location of research on nonindigenous aquatic species.

The Research Protocol Committee will report annually to the Task Force the number of proposals requiring confinement/containment measures, the species involved, and the location of research sites. Problems will be identified and recommendations for correcting them provided to the Task Force.

Until a research proposal is funded and becomes public property the confidentiality of the contents of the proposal must be maintained at all levels. All levels of review before funding must be made aware of the legal and ethical responsibilities not to discuss, copy, or share proposals with anyone not directly involved or authorized to assist in the review.

Inspections and Compliance

All proposals which are required to follow a confinement and containment protocol must include certification by the Principal Investigator and the Research Institution that they will comply with the requirements of the protocol, and within the proposal must document the specific containment and

confinement measures to be used. The Institutional Biosafety Committee and the Biological Safety Officer, if appointed by the IBC (see NIH guidelines 51 FR 16963 for specific duties), will monitor the conduct of the research and verify compliance with the containment and confinement procedures agreed to by the Principal Investigator and the Research Institution.

The Funding Agency, the Research Protocol Committee, and appropriate state agencies may inspect the facilities and containment and confinement procedures at any time. The Research Institution should inspect its research at least twice yearly.

Failure to comply with the protocol, or the escape or release of a nonindigenous aquatic species must be reported to both the Funding Agency and Research Protocol Committee immediately. Penalties for noncompliance with the protocol will be administered by the Funding Agency and could include suspension of research funding. The major responsibility for compliance with the protocol falls to the Principal Investigator and the Research Institution.

Existing Guidelines and Protocols

Guidelines for Recombinant DNA Research: [These can also be applied to nonindigenous aquatic species proposals.] (FR 51 No. 8, pg. 16958; FR 51 No. 123, pg. 23367; FR 52 No. 154, pg. 29800; FR 56 No. 22, pg. 4134; FR 51 No. 88, pg. 16959)

Microorganisms

National Institutes of Health (NIH). 1968. Guidelines for Research Involving Recombinant DNA Molecules. Published in Federal Register May 7, 1986 (51 FR 16958-16961) with additional major actions August 24, 1987 (52 FR 31838); July 29, 1988 (53 FR 28819); October 26, 1988 (53 FR 43410); March 13, 1989 (54 FR 10508); March 1, 1990 (55 FR 7438); and August 11, 1987 (52 FR 29800) with appendix P for plants and Q for animals.

Whole Plants and Animals

U.S. Department of Agriculture (USDA). 1984. Coordinated Framework for Regulation of Biotechnology. Federal Register December 31, 1984 (49 FR 50856) and June 26, 1986 (51 FR 23302+).

USDA. 1986. Advance Notice of Proposed USDA Guidelines for Biotechnology Research. Federal Register June 26, 1986 (51 FR 23367-23393) and February 1, 1991 (56 FR 4134-4149).

USDA. 1986. Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or for Which There is Reason to Believe are Plant Pests. Federal Register June 26, 1986 (51 FR 23352-23366) and June 16, 1987 (52 FR 22892-22915).

Coulson, J. R., and R. S. Soper. 1989. Protocols for the Introduction of Biological Control Agents in the U.S. Chapter I, pages 2-35 In: Kahn, R. P. (ed.). Plant Protection and Quarantine. Volume III Special Topics. CRC Press, Inc., Boca Raton, Florida.

USDA, Office of Agricultural Biotechnology. 1988. USDA Guidelines for Research Outside the Laboratory Involving Biotechnology, also Federal Register June 26, 1986 (51 FR 23367-23313) and February 1, 1991 (56 FR 4134-4149).

International Guidelines and Protocols:

European Inland Fisheries Advisory Commission. 1988. Code of Practice and Manual of Procedures for Consideration of Introductions and Transfers of Marine and Freshwater Organisms. FAO. EIFAC. Occasional paper No. 23. 52 pages.

International Council for the Exploration of the Sea. 1982. Proposed Guidelines for Implementing the ICES Code of Practice Concerning Introduction and Transfer of Marine Species. 23-page manuscript.

Disease Related Guidelines and Protocols:

Anonymous. 1989. Operating Procedures for the Alma Quarantine Facility. Prepared for the Alma Research Station, Guelph, Ontario, Canada. 16 pages typewritten.

Horner, R. W., and R. L. Eschenroder. 1991. Protocols to Minimize the Risk of Introducing Salmonid Disease Agents with Importation of Salmonid Fishes. Draft manuscript. 11 pages. Prepared for Great Lakes Fish Disease Control Committee. Pages 27-37.

U.S. Department of Health and Human Services. 1984. Biosafety in Microbiological and Biomedical Laboratories. 1st Edition (March 1984). U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia 30333, and National Institutes of Health, Bethesda, Maryland 20892.

An additional 17 references on laboratory disease and pathogen control methods can be found listed in the Federal Register, May 7, 1986 (51 FR 16965).

Other Guidelines and Protocols:

Klingman, D. L., and J. R. Calcine. 1983. Guidelines for Introducing Foreign Organisms into the United States for Biological Control of Weeds. Bulletin of Entomological Society of America. Fall 1983:55-61.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Arthropod-Parasitic Nematodes into the United States for Biological Control of Arthropod Pests of Plants, Man, and Domestic Animals, and Vectors of Plant, Human, and Animal Pathogens, and for the Interstate Movement and Export of Foreign and Native Arthropod-Parasitic Nematodes for Research on Biological Control of Such Pests.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Microbial Pathogens (Fungi, Bacteria, Rickettsia Viruses, Protozoa) into the United States for Biological Control of Arthropod Pests of Plants, Man, and Domestic Animals, and Vectors of Plant, Human, and Animal Pathogens, and for the Export of Foreign and Native Arthropod Pathogens for Research.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Arthropods and Nematodes into the United States for Biological Control of Weeds, and for the Interstate Movement and Export of Foreign and Native Arthropod and Nematode Natural Enemies of Weeds.

Guidelines for the Importation, Interstate Movement, and Field Release in the United States of Foreign Microbial Pathogens for Biological Control of

Weeds, and for the Interstate Movement and Export of Foreign and Native Pathogens of Weeds for Research.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Beneficial Organisms (Microbial Pathogens and Antagonists) into the United States for Biological Control of Plant Nematodes and Plant Pathogens, and for the Export of Such Organisms (Foreign and Native) for Research.

Southeastern Cooperative Wildlife Disease Study. 1985. Model for State Regulations Pertaining to Captive Wild and Exotic Animals. University of Georgia, Athens, Georgia. 48 page manuscript. Prepared in response to Resolution #9. U.S. Animal Health Association, Milwaukee, Wisconsin 10/27-11/1/85.

Jennings, D. P., and J. A. McCann. 1991. Research Protocol for Handling Nonindigenous Aquatic Species. National Fisheries Research Center, U.S. Fish and Wildlife Service, Gainesville, Florida. 43-page manuscript.

Brown Tree Snake Protocol:

Pacific Basin Development Council. 1991. Recommended Protocol for Transport of Live Brown Tree Snakes (*Boiga irregularis*). Prepared for Plant Quarantine Branch, State of Hawaii Department of Agriculture and Biological Survey, and the U.S. Fish and Wildlife Service.

APPENDIX II

Definitions

Aquatic Nuisance Species - nonindigenous species in inland, estuarine and marine waters that presently or potentially: 1) threaten the diversity or abundance of native species; 2) impact ecological processes and stability, including other natural resources, of infested waters; 3) adversely affect commercial, agricultural, aquacultural, recreational or other activities dependent on such waters; or 4) adversely affect individuals, including health effects.

Biological Safety Officer (BSO) - an individual who is a member of the IBC who has the direct responsibility (after the PI) to ensure the activities and precautions stated in the research proposal are followed. See NIH guideline FR 51 No. 88, pg. 16963, for other roles and responsibilities.

Confinement - a term used primarily in the USDA guidelines meaning organisms restricted to research field facilities such as outside experimental pond areas and involving whole plants and animals.

Containment - a term used primarily in the NIH guidelines to mean restricted to laboratory environments and is usually in reference to micro-organisms, recombinant DNA molecules, or whole plants (Appendix P) or whole animals (Appendix Q).

Established - when used in reference to a species, this term means occurring as a reproducing, self-sustaining population in an open ecosystem, i.e. in waters where the organisms are able to migrate or be transported to other waters.

Institutional Biosafety Committee (IBC) - see NIH guidelines FR 51 No. 88, pg. 16962, for membership, roles, and responsibilities.

Nonindigenous Species - any species or other viable biological material that enters an ecosystem beyond its historic range, including any such organisms transferred from one country to another. Nonindigenous species include both exotics and transplants. [Note: Historic range is interpreted to mean the territory occupied by a species at the time of European colonization of North America.]

Pathogen - as defined in USDA guidelines, is a virus or micro-organism (including its viruses and plasmids, if any) that has the ability to cause disease in another living organism.

Principal Investigator (PI) - see FR 51 No. 88, pg. 16963, for roles and responsibilities.

Research Institution - means any public or private entity (including Federal, state, or local government agencies) conducting the research.

Research Protocol Committee (RPC) will be comprised of one or more representatives from each Federal Task Force agency who are qualified to evaluate nonindigenous species research proposals. Knowledgeable experts from other Federal, state, or private groups with different areas of expertise might be asked to assist the committee.

Surrounding Waters - means any free flowing or standing waters in the immediate vicinity of the research facility that are connected with public waters either directly or indirectly.

Survival - organism able to live in an ecosystem during its normal life span but not necessarily able to reproduce itself.

Unintentional Introduction - the release of nonindigenous organisms without any specific purpose. Such introductions, often unknowingly, occur as a result of activities other than the intentional introduction of a species such as the transport of nonindigenous organisms in ballast water or in water used to transport fish, mollusks or crustaceans for aquaculture or other purposes. The virtually inevitable escapement, accidental release, "aquarium dumping" or other improper disposal of intentionally introduced nonindigenous species do not constitute unintentional introductions.

Waters of the United States - the internal waters of the United States, including its territories and possessions, plus the Territorial Sea of the United States as established by Presidential Proclamation Number 5928 of December 27, 1988.

APPENDIX III

Membership of the Research Protocol Committee

Dr. James A. McCann, National Fisheries Research Center, U.S. Fish and Wildlife Service -- Chairman, May 1991-Present

Dr. Althaea Langston, Animal and Plant Health Inspection Service - Policy and Program Development, U.S. Department of Agriculture -- Member, May 1991-Present

Dr. David F. Reid, Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration -- Member, May 1991-Present

Dr. Edwin A. Theriot, Environmental Laboratory, Waterways Experiment Station, U.S. Army Corps of Engineers -- Member, August 1991-Present

APPENDIX IV

Other Legislation or Executive Orders Related
to the Nonindigenous Aquatic Species Act

Applicable State Laws, Regulations, Permit and Notification Requirements -
Must be determined on an individual basis by Principal Investigators and
Research Institutions.

Lacey Act of 1900 - 18 U.S.C. 42

Endangered Species Conservation Act of 1969 - 16 U.S.C. 668

Executive Order 11987 -- Exotic Organisms, May 24, 1977

Plant Quarantine Act of 1912 (7 U.S.C. 151 *et seq.*)

Federal Plant Pest Act of 1957 (7 U.S.C. 150aa *et seq.*)

Federal Noxious Weed Act of 1974 (Public Law 93-629, January 3, 1975,
7 U.S.C. 2801 *et seq.* and 21 U.S.C. 111 *et seq.*)

National Environmental Policy Act of 1969 (NEPA, 42 U.S.C. 4321 *et seq.*)

Occupational Safety and Health Act of 1970 - Federal Register April 12, 1984
(50 FR 14468), 29 U.S.C. *et seq.*

Outline of Information Required by Reference Guidelines

Identification of Species

Source of Research Specimens

Discussion of the Life History, Biology, Critical Environmental Factors, and Ecology of the Species

Biosafety Level Based on Risk Assessment and Possible Impacts

Diseases and Parasites

 Identification

 List of All Known Diseases and Parasites Found in Waters Where Species were Taken

 Quarantine Facilities/Procedures

Physical Containment

Biological Containment

Chemical Containment

Environmental Containment

Scope Limitations

Fate of Surviving Specimens - Close Out Procedures

Required Permits and Related Laws and Regulations

Shipping and Transportation

Training and Qualifications of Personnel

Security

Emergency Plan

Administrative Control, Roles, Responsibilities

Inspections, Monitoring

Compliance

Reporting

APPENDIX B: ASSESSMENT OF GENETICALLY MODIFIED FINFISH AND SHELLFISH WITH NON-DIOECIOUS MODES OF REPRODUCTION

Finfish, molluscs and crustaceans exhibit a wide diversity of modes of reproduction. One common mode is dioecy, in which male and female reproductive organs are in separate individuals and each individual is of one sex. Many species currently used in aquaculture are dioecious. Assessment of risks posed by accidental escapes of dioecious GMOs can be addressed by use of the existing Flowcharts.

Non-dioecious modes of reproduction are known to exist in fish, molluscan and crustacean species -- including in some important aquacultural species. While the modes of reproduction are quite variable, and often very complex, two general categories exist: hermaphroditism and parthenogenesis. Hermaphroditic organisms possess both male and female reproductive organs. Depending on the particular characteristics of their reproductive development and behavior, hermaphrodites may reproduce through cross- or self-fertilization. Parthenogenesis includes all modes of reproduction where one or more genomes are inherited clonally (Moore 1984). Reproduction in true parthenogens requires neither the presence of sperm to induce embryogenesis nor incorporation of DNA from a male for reproduction of viable progeny.

Considerable variation exists in the specific characteristics of non-dioecious reproduction in fish and shellfish. In order to assess the risks of releases of genetically modified non-dioecious GMOs, knowledge of the specific characteristics of their reproduction is essential. This Appendix provides:

- 1) background on non-dioecious modes of reproduction;
- 2) risk management guidance for non-dioecious GMOs of high concern; and
- 3) important guidance for use of the Flowcharts to evaluate non-dioecious GMOs other than those of high concern.

Background on Non-Dioecious Modes of Reproduction

Fishes

All known parthenogenetic fishes require at least physical stimulus of sperm to induce embryogenesis (Dawley 1989). Depending on the parthenogenetic species, males from the same or different species are involved in the reproduction. Consult Dawley (1989) for an explanation of two different modes of male involvement - gynogenesis and hybridogenesis. Families with species which are parthenogenic include *Poeciliidae*, *Atherinidae*, *Cyprinidae*, *Cobitidae* (Dawley 1989). Researchers working with fish in the family *Poeciliidae* may begin their

examination of the potential for parthenogenesis in their research organisms by consulting the following references: for *Poecilia formosa*, Dawley (1989), Monaco et al. (1984), and Scharl et al. (1995); for *Poeciliopsis spp.*, Dawley (1989) and Vrijenhoek (1984).

Currently, only a single species of self-fertilizing hermaphroditic fish is known: *Rivulus marmoratus* (Soto et al. 1992, Turner et al. 1992). Simultaneous and sequential hermaphroditic fish are known to exist among the *Sparidae* spp. (Buxton and Garrett 1990). Research involving GMOs within this family is likely because some species (i.e., the sea breams) are of economic importance in aquaculture production. It is critically important, to determine whether or not a particular GMO within *Sparidae* is hermaphroditic. Sequentially hermaphroditic fish may be protoandrous or protogynous (Sadovy and Shapiro, 1987, Debas et al 1990). In some sequential hermaphrodites, sex change results from social factors (Dawley 1989, Sunobe and Nakozono 1993).

Molluscs and Crustaceans

Among the 5600 known mollusc genera, 40% are either simultaneous or sequential hermaphrodites, including 9% of bivalves (Heller 1993). In bivalves, many types of hermaphroditism have been found (Peralta 1988). Hermaphroditism in bivalves has been thought to be limited to small, brooding species. In addition, those hermaphroditic species which spawn eggs for external fertilization are thought to self-fertilize inadvertently, or with less fit progeny resulting. However, selfed larvae of an aquacultural species, the bay scallop *Argopecten irradians*, have been found to grow as well as outcrossed larval cultures (Wilbur and Gaffney 1991). Increased incidence of hermaphroditism in triploid oysters has been documented through histological studies, though it is uncertain if such hermaphrodites yield functional gametes (Allen and Downing 1990).

Parthenogenesis is known to occur in strains of aquacultural crustacean species, such as *Artemia* (brine shrimp) (Triantaphyllidis et al. 1993) and *Daphnia spp.* (Hebert et al. 1993). These species are cultured and marketed to provide live foods for some aquacultural fish species. Results of a study of the freshwater crustacean, *Candonocypris novozelandiae* suggest that a sexual strain was displaced by a parthenogenetic strain in a disrupted habitat (Chaplin 1993). Two hermaphroditic individuals of the marine shrimp species (*Penaeus vannamei*) were found among broodstock on a shrimp farm and were suggested to be hermaphroditic as a result of environmental conditions encountered in captivity. (Perez-Farfante and Robertson 1992).

Risk Management Guidance: Non-Dioecious GMOs of High Concern

Compared to cross-fertilizing GMOs, organisms which reproduce by selfing can be particularly useful research organisms because of reduced variation in the genotypes of their progeny. However, accidental escapes of such organisms pose particularly high risks. The Flowcharts are not designed to fully evaluate the risks posed by research involving GMOs with either of two modes of reproduction by selfing: 1) self-fertilizing hermaphroditism; or 2) true parthenogenesis.

Accidental escape of a single GMO individual with either reproductive mode could result in the establishment of an entire population of GMO descendants. Following the precautionary principle, this high potential for population establishment demands that research with these types of GMOs employ the most stringent level of confinement possible.

- Non-Dioecious GMOs of High Concern - Proceed to Risk Management VI.B. Researchers using GMOs which are self-fertilizing hermaphrodites or true parthenogens should proceed to Risk Management VI.B. in the Flowcharts and manage for no/negligible accidental escapees. However, proceeding through the Flowcharts using the clarified terms below, will assist in identifying issues to consider in developing risk management. If one of the biological barriers used is induced sterility, permanence of sterility is essential.

Clarification of Flowchart Terms for Other Non-Dioecious GMOs

- Other Non-Dioecious GMOs - Proceed through Flowcharts. Researchers using other non-dioecious GMOs may proceed through the Flowcharts using the following clarifications as a guide to applying the questions to their research. The appropriate outcome will be based on responses to the Flowchart questions, as modified with the clarifications below.

Interbreeding and Hybridization. (Flowcharts II.A.1, II.B.1, , IV.A.1). In the case of parthenogenic GMOs, questions about "interbreeding" or "hybridization" refer to the presence of species in the accessible ecosystem with which the parthenogenic GMO can interact and reproduce. Specifically, it refers to the presence of species in which males have the capability to initiate embryogenesis in the parthenogen, regardless of whether or not the males actually contribute DNA to progeny.

In the case of hermaphroditic GMOs, "interbreeding" and "hybridization" refer to the potential for cross-fertilization of these GMOs with other hermaphroditic individuals, or with any other species.

Immediate potential for introgression. (Flowchart II.A.1). For parthenogens, the immediate potential for introgression and consequent effects (addressed in Flowchart IV.A) are generally not a concern. Instead, the primary concern is the potential establishment of a viable GMO population and any direct or indirect adverse effects on other organisms or ecosystem structure and processes (addressed beginning with Flowchart IV.B). Therefore, if the GMO is a parthenogen and Flowchart II.A.1 directs you to IV.A, you should proceed instead to Flowchart IV.B.

Hermaphroditic GMOs with potential for cross-fertilization do pose concern of immediate potential for introgression. Therefore, if the GMO is a hermaphrodite and you are directed to Flowchart IV.A, you should proceed as directed.

Interspecific hybrid involving Parthenogenic GMOs and Introgressive hybridization. (Flowchart II.C.1). Two cases of parthenogenic "hybridization" are presently known to exist. In one case, the male contributes DNA and sperm (hybridogenesis in *Poeciliopsis*). In the second case, the sperm usually only triggers embryogenesis (gynogenesis in *Poecilia*); Scharl et al. (1995) documented an exception where males of another species contributed microchromosomes to offspring of matings with the all-female *P. formosa*. In either case, the progeny should be considered interspecific hybrids for the purpose of these Flowcharts. In either case, if the males are a protected species, reproductive competition whereby too many males mate with parthenogenic GMO females of another species rather than with females of their own species is a greater risk than is introgressive hybridization.

Reproductively mature. (Flowchart III). Identification of reproductively mature individuals among sequential hermaphrodites should be considered carefully. Consult experts for advice on age at which individuals should be examined and proper methods to determine maturity.

Abiotic factors of accessible ecosystem. (Flowchart IV.B) For non-dioecious GMOs, in addition to considering whether or not abiotic factors might preclude reproduction, the possibility that abiotic factors might induce reproduction should also be examined.

In the case of GMOs which are thought to be dioecious, the potential for abiotic factors to trigger non-dioecious reproduction should also be considered, with consultation from experts. In one study, environmental factors in captive rearing conditions of an outdoor shrimp farm was suspected to have triggered a low incidence of hermaphroditism in the farmed population (Perez-Farfante and Roberston 1992).

When using Flowchart IV.B for non-dioecious GMOs, the following conditions must be met to allow for EXIT of the Standards at this point:

1. the accessible ecosystem lacks abiotic factors that trigger reproduction;
2. the accessible ecosystem has abiotic factors that *do* preclude reproduction;
and
3. the accessible ecosystem lacks conspecifics or closely related species that can trigger embryogenesis.

APPENDIX C: CHEMICAL STERILIZATION OF SEA WATER

Chlorine is the disinfectant most often used for water and waste water treatment. When chlorine is added to fresh water, hydrochloric and hypochlorous acids are formed.



In pure fresh water, the primary disinfectant and bleaching agent is hypochlorous acid (HOCl) or the ion, hypochlorite (OCl^-). In sea water, hypochlorous acid will react with naturally occurring bromide ion to produce hypobromous acid (HOBr). Ammonia in either fresh or salt water will in turn react with hypochlorite or hypobromite to form chlor- or bromamines (e.g. monochloramine (NH_2Cl) or monobromamine (NH_2Br)). All of these halogenated compounds contribute to the disinfectant and bleaching properties of the mixture.

Clorox, which is a 5.25% solution of sodium hypochlorite (NaOCl) is used as a source of OCl^- to disinfect sea water or containers. Clorox is readily available and safer to handle than other sources of hypochlorite.

Natural sea water can be passed through a filter (bag, cartridge, or sand filters) to remove large organisms and debris. Clorox is then added to sterilize the water before or after use as required. The water is left standing for at least 4 hours to insure sterilization. Since chlorine is toxic to embryos and larvae of marine organisms, the water must be dechlorinated. To dechlorinate, sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) crystals are dissolved in hot fresh water and added to the chlorinated water. The water should be aerated to insure uniform mixing and hence adequate dechlorination. A chlorine test kit of the type used to test swimming pools is suitable to verify the removal of all chlorine.

Table C1. Amounts of clorox and thiosulfate used to disinfect marine or estuarine water: examples for different sizes of tanks.

Container	vol (gal)	vol (l)	clorox (ml)	sodium thiosulfate (g)*
Carboy	5	19	5	0.2
Trash Can	40	113	20	0.5
Tank	100	380	67	1.7
Tank	400	1514	150	5.0
Tank	1000	3785	350	12.0

*Do not add sodium thiosulfate until 4 hours after the Clorox is added.

APPENDIX D: SCHEMATIC DIAGRAMS OF EXAMPLES OF MECHANICAL BARRIERS

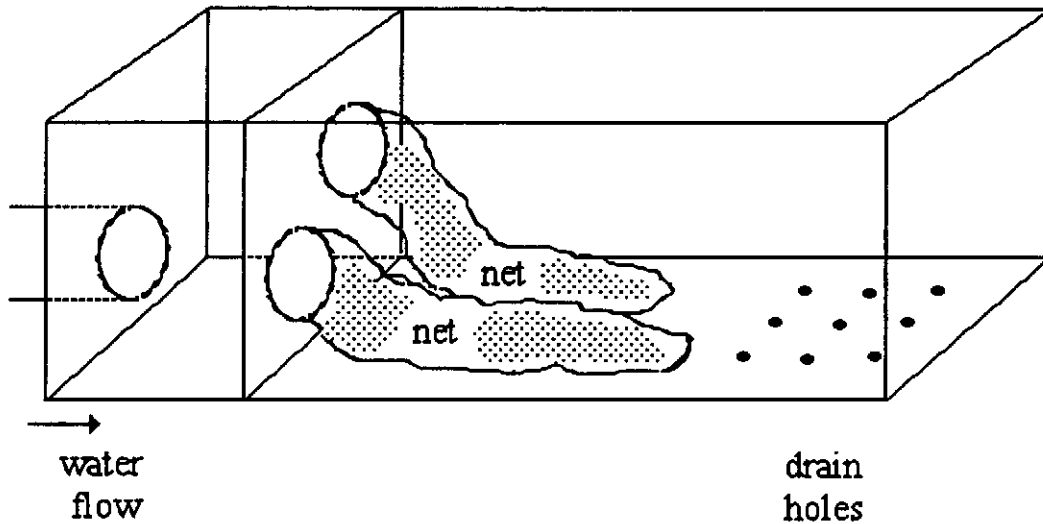


Figure D1. Sock filter trap. Effluent from any incubator or rearing tank holding fish embryos, larval fish, or small fish passes through such a trap. Any escaping organisms are trapped by a 0.3 mm mesh net. An overflow net will filter the effluent if the lower net should become occluded. Effluent discharged through the drain holes goes to the indoor laboratory's common effluent drain. Barriers for the common effluent drain are depicted in Figure D2.

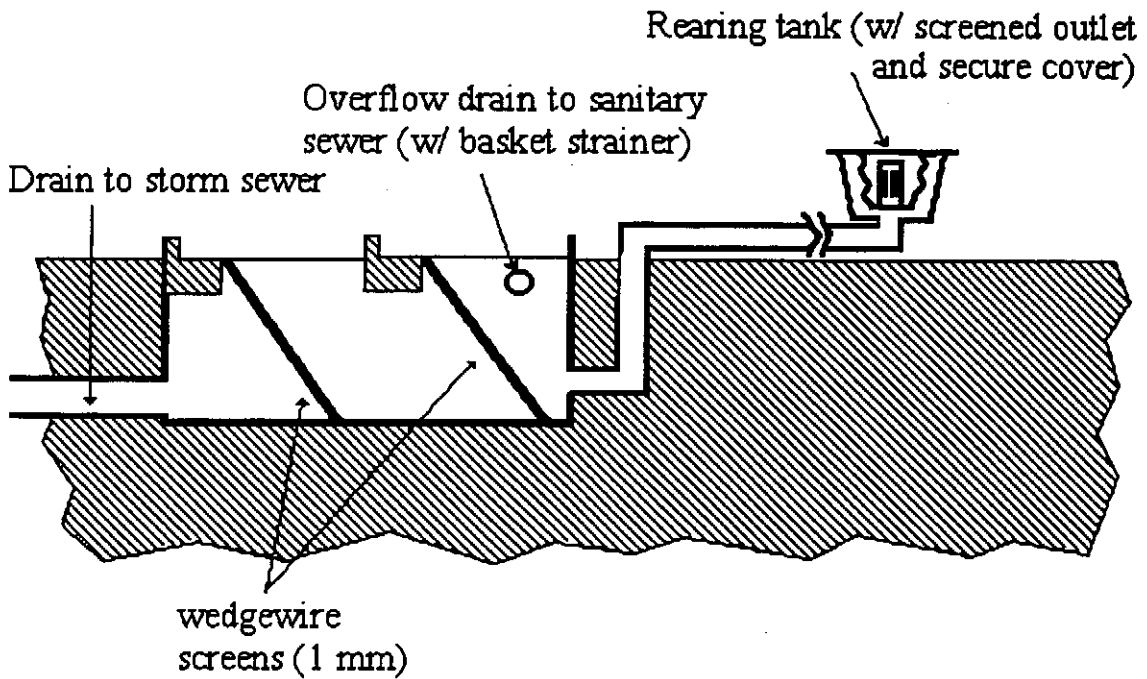


Figure D2. Two stainless steel wedgewire screens in series act as final barriers for the common effluent from flowthrough experimental units located in an indoor research facility for fish. There is a 1 mm wide gap between the stainless steel wedgewires. Effluent from any experimental units holding fish smaller than a total length of 2 cm must first pass through a sock filter trap (Fig. D1). Effluent from units holding fish at or above a total length of 2 cm (equivalent to a head diameter of 2 mm) goes directly to these screens. Empirical tests showed that the screens will clearly retain fish at or above this size.

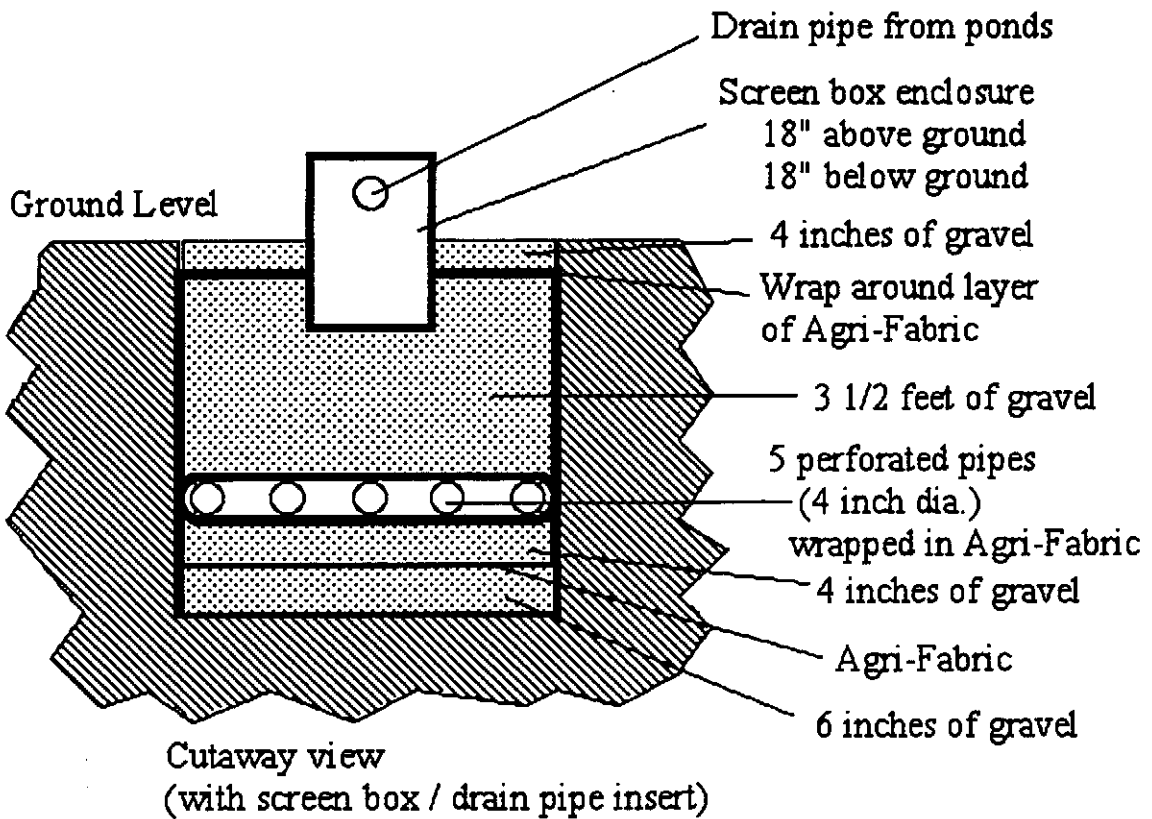


Figure D3. Schematic drawing of a French drain for outdoor fish ponds. Normally run as static systems, such research ponds might be drained partially during sampling or entirely at the end of an experiment. The French drain is designed to retain the smallest possible size of fish reared in the pond. Water discharged from this drain eventually reaches surface waters, (Adapted from Cooperative State Research Service 1990, as cited in Cooperative State Research Service 1990a.)

**APPENDIX E: ABRAC WORKING GROUP ON AQUATIC
BIOTECHNOLOGY AND ENVIRONMENTAL SAFETY**

1992-1994

ABRAC Members

Dr. Anne R. Kapuscinski (Chair)
Department of Fisheries and Wildlife
200 Hodson Hall
University of Minnesota
St. Paul, MN 55108-6124
Phone (612) 624-3019
Fax (612) 625-5299

Dr. Stanley Pierce of Counsel
Rivkin Radler & Kremer
7412 Mandarin Drive
Boca Raton, FL 33433
Phone (407) 487-7220
Fax (407) 477-1555

Dr. James Tiedje
NSF Center for Microbial Ecology
Michigan State University
East Lansing, MI 48824
Phone (517) 353-9021
Fax (517) 353-2917

Dr. William Witt
National Center for Toxicological
Research (HFT-240)
Food and Drug Administration
NCTR Drive
Jefferson, AR 72079-9502
Phone (501) 543-7949
Fax (501) 543-7576

Consultants

Dr. Howard Bern
Department of Integrative Biology
University of California
Berkeley, CA 94720
Phone (510) 642-2942
Fax (510) 643-6264

Dr. John Colt
Montgomery Watson Inc.
2375 130th Avenue, N.E.
Suite 200
Bellvue, WA 98005
Phone (206) 881-1100
Fax (206) 881-8937

Ms. Maryln Cordle
United States Department
of Agriculture/OAB
Room 1001-RPE
Washington, DC 20250-2200
Phone (703) 235-4419
Fax (703) 235-4429

Dr. Rex Dunham
Department of Fisheries
203 Seingle Hall
Auburn University
Auburn, AL 38649
Phone (205) 844-9121
Fax (205) 844-9208

Dr. Susan Ford
Haskin Shellfish
Research Laboratory
Rutgers University
RD1 Box B8
Port Norris, NJ 08349
Phone (609) 785-0074
Fax (609) 785-1544

Dr. Eric Hallerman
Dept. of Fisheries & Wildlife Sciences
211 Primrose Drive
Blacksburg, VA 24060
Phone (703) 231-3257
Fax (703) 231-7580

Dr. Harold Kincaid
National Fisheries R&D Laboratory
U.S. Fish & Wildlife Service
RD 4, Box 63
Wellsboro, PA 16901
Phone (717) 724-3322
Fax (717) 724-2525

Dr. Peter Moyle
Department of Fisheries and Wildlife
University of California
Davis, CA 95616
Phone (916) 752-6355
Fax (916) 752-4154

Dr. Roger Mann
P.O. Box 1303
Gloucester Point, VA 23062-1303
Phone (804) 642-7360
Fax (804) 642-7097

Dr. George Spangler
Department of Fisheries and Wildlife
University of Minnesota
200 Hodson Hall
St. Paul, MN 55108-6124
Phone (612) 624-9229
Fax (612) 625-5299

**APPENDIX F: PARTICIPANTS IN THE WORKSHOP ON PERFORMANCE STANDARDS
FOR RESEARCH WITH GENETICALLY MODIFIED FISH AND SHELLFISH**

August 18-20 1993, University of Minnesota, Minneapolis, MN

NOTE: Telephone numbers are listed first, followed by FAX numbers.

Craig Acomb
University of Minnesota
Dept. of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-3019/625-5299

Ira Adelman
University of Minnesota
Dept. of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-4228/625-5299

Standish Allen
Rutgers University
Haskins Research Laboratory
B-8, Rd #1
Port Norris, NJ 08349
(609) 785-0074/785-1792

Mike Amos
GalaGen, Inc.
4001 Lexington Ave. N.
Arden Hills, MN 55126
(612) 481-2932/481-2232

David Andow
University of Minnesota
Department of Entomology
219 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-5253/625-5299

David Attaway
National Sea Grant College Program
NOAA, R/OR 1
1335 East-West Highway
Silver Spring, MD 20910
(301) 713-2451/713-0799

Dale Baker
University of Duluth
Minnesota Sea Grant Program
208 Washburn Hall
Duluth, MN 55812
(218) 726-8108/726-6556

Donald Baltz
Louisiana State University
Coastal Fish Institute, CCEER
Baton Rouge, LA 70803
(504) 388-6512/388-6513

David Berkowitz
Food and Drug Administration
5600 Fishers Lane, Rm. 11-34, HF-6
Rockville, MD 20857
(301) 443-7573/443-7005

Howard A. Bern
University of California
Department of Integrative Biology
345 Mulford Hall
Berkeley, CA 94720
(510) 642-2940/643-6264

Chris Bidwell
Purdue University
Department of Animal Sciences
1026 Poultry Science Building
West Lafayette, IN 47907-1026
(317) 494-8016/494-9347

Vicki Blazer
National Fish Health Laboratory
Box 700
Kearneysville, WV 25430
(304) 725-8461/728-6203

Meryl Broussard
U.S. Department of Agriculture, CSRS
901 D Street S.W., Room 330
Washington, D.C. 20250-2200
(202) 401-4061

Charles Brown
U.S. Department of Agriculture
A.P.H.I.S.
6505 Belcrest Road
Hyattsville, MD 20782
(301) 436-8565/436-8669

David Brune
Clemson University
Agricultural and Biological Engineering
225 McAdams Hall
Clemson, SC 29634
(803) 656-4068/656-0338

Jane Burns
University of California, San Diego
School of Medicine
Basic Science Building, Room 4095
9500 Gilman Drive
LaJolla, CA 92093
(619) 534-4156/534-1087

Thomas Caceci
Virginia Polytechnic Institute and State
University
Department of Biomedical Sciences
College of Veterinary Medicine
Blacksburg, VA 24061-0442
(703) 231-7666/231-7367

Donald Campton
University of Florida
Department of Fisheries and Aquaculture
7922 NW 71st St.
Gainesville, FL 32606
(904) 392-9617/392-3462

Carolyn Carr
University of Minnesota
Conservation Biology Graduate Program
100 Ecology Building
1987 Upper Buford Circle
St. Paul, MN 55108
(612) 624-3476/624-6777

Thomas Chen
University of Connecticut
Biotechnology Center
184 Auditorium Road, U-149
Storrs, CT 06269-3149
(203) 486-5011/486-5005

Jack Christie
W.J. Christie and Associates
Ecological Consultants
RR #4
Picton Ontario, CANADA
K0K 2T0
(613) 476-1137/476-1137

John Colt
Montgomery and Associates
2375 130th Ave. NE, Suite 200
Bellevue, WA 98005
(206) 881-1100

MarylN Cordle
U.S. Department of Agriculture
Office of Agricultural Biotechnology
Room 1001, Rosslyn Plaza East
Washington, DC 20250-2200
(703) 235-1510/235-4429

James Crosson
U.S. General Accounting Office
441 G Street N.W. Room 5853
Washington, DC 20548
(202) 512-2636/512-2622

Kenneth Davis
Memphis State University
Department of Biology
Memphis, TN 38152
(901) 678-2594/327-8001

Robert Devlin
Department of Fisheries and Oceans
4160 Marine Dr.
W Vancouver, B.C., CANADA
V7V 1N6
(604) 666-7926/666-3497

Walton Dickhoff
Northwest Fisheries Science Center
2725 Montlake Blvd. E
Seattle, WA 98112
(206) 860-3234/860-3267

Rex Dunham
Auburn University
Dept. of Fisheries & Allied Aquaculture
Auburn, AL 36849
(205) 844-9121/844-9208

Charles Eirkson
U.S. Food and Drug Administration
Center for Veterinary Medicine HFV-152
7500 Standish Place
Rockville, MD 20855
(301) 295-8683/295-8207

Elliot Entis
A/F Protein
72 Bonad Rd.
Newton, MA 02165
(617) 576-8153

John Epifanio
Michigan State University
Department of Fisheries and Wildlife
Natural Resources Building, Room 13
East Lansing, MI 48824
(517) 336-3774/336-1699

Garth Fletcher
Memorial University of Newfoundland
Oceans Sciences Centre
St. Johns
Newfoundland, CANADA
A1C 5S7
(709) 737-3276/737-3220

Rebecca Goldberg
Environmental Defense Fund
257 Park Avenue S
New York, NY 10010
(212) 505-2100/505-2375

Mark Gross
University of Minnesota
Dept. of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-0744/625-5299

Perry Hackett
University of Minnesota
Department of Genetics and Cell Biology
1445 Gortner Ave.
St. Paul, MN 55108-1095
(612) 624-6736/625-5754

Eric Hallerman
Virginia Polytechnic Institute and State
University
Department of Fisheries and Wildlife
108 Cheatham Hall
Blacksburg, VA 24061-0321
(703) 231-3257/231-7580

Karen Heller-Taylor
North Carolina State University
College of Veterinary Medicine
4700 Hillsborough Box 8401
Raleigh, NC 27606
(919) 515-1962/829-4336

Choy-Leong Hew
University of Toronto
Hospital for Sick Children
Department of Clinical Biochemistry
100 College St. Room 351
Toronto Ontario, CANADA
M5G 1L5
(416) 978-3875/978-6505

Mark Hove
University of Minnesota
Dept. of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-3019/625-5299

Jan Husby
Directorate of Nature Management
Tungasletta 2, N-7005
Trondheim
NORWAY
47-7-580500/47-7-915433

Pung-Pung Hwang
Academia Sinica
Institute of Zoology
Nankang, Taipei, Taiwan
R.O.C
886-2-789-9521/785-8059

John Hynes
Minnesota Environmental Quality Board
300 Centennial Bldg
St. Paul, MN 55155
(612) 296-2871/296-3698

Ying Ji
Minnesota Department of Agriculture
Marketing Division
90 Plato Blvd.
St. Paul, MN 55117
(612) 296-5081/296-6890

Representative Phyllis Kahn
Minnesota House of Representatives
367 State Office Building
St. Paul, MN 55155
(612) 296-4257/296-9709

Anne Kapuscinski
University of Minnesota
Department of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108-6124
(612) 624-3019/625-5299

Harold Kincaid
U.S. Fish and Wildlife Service
National Fishery R & D Laboratory
RD 4 Box 63
Wellsboro, PA 16901
(717) 724-2233/727-2525

Helge Klungland
Agricultural University of Norway
Department of Food Science
PO Box 5036
N-1432 Aas
Ås, NORWAY
47-64-94-85-45/47-64-94-37-89

Christopher Kohler
Southern Illinois University
Fisheries Research Laboratory
Carbondale, IL 62901
(618) 453-2890/536-7761

Althaea Langston
U.S. Department of Agriculture - A.P.H.I.S.
6505 Belcrest Rd.
Hyattsville, MD 20782
(301) 436-8017/436-5899

Steven Laursen
University of Minnesota
250 NRAB
2003 Upper Buford Circle
St. Paul, MN 55108
(612) 625-9298/624-8701

Thomas Losordo
North Carolina State University
Dept of Zoology
Campus Box 7646
Raleigh, NC 27695
(919) 515-7587/515-5110

Jay Maher
University of Minnesota
Department of Fisheries and Wildlife
1980 Folwell Ave.
St. Paul, MN 55108
(612) 625-3183/625-5299

Christopher Mann
U.S. House of Representatives
Committee on Merchant Marine & Fisheries
547 Ford House Office Building
Washington, DC 20515
(202) 225-2808/225-6747

Roger Mann
Virginia Institute of Marine Sciences
College of William and Mary
Gloucester Pt., VA 23062
(804) 642-7360/642-7045

Joe Marcino
Minnesota Department of Natural Resources
500 Lafayette Road
St. Paul, MN 55155
(612) 296-3043/296-1811

Gonzalo Martinez
University of Minnesota
Dept. of Genetics and Cell Biology
250 Biological Sciences Tower
St. Paul, MN 55108
(612) 624-4206

John Matheson
Food and Drug Administration
Center for Veterinary Medicine, HFV 150
7500 Standish Place
Rockville, MD 20855
(301) 594-1679/594-1297

James McCann
U.S. Fish and Wildlife Service
National Fisheries Research Center
7920 NW 71st St.
Gainesville, FL 32606
(904) 378-8181/378-4956

Thomas McCormick
McCormick and Associates
Operations Manager
312 East Matilija Street #112-131
Ojai, CA 93023
(805) 640-1180/640-0165

Margaret Mellon
Union of Concerned Scientists
1616 P Street NW
Suite 310
Washington, D.C. 20036
(202) 332-0900/332-0905

Loren Miller
University of Minnesota
Dept. of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-0744/625-5299

Joan Mitchell
National Science Foundation
Division of Ocean Science
1800 G Street N.W. Room 609
Washington, D.C. 20550
(202) 357-9639/357-7621

Boaz Moav
Tel Aviv University
Department of Zoology
Tel Aviv, ISRAEL
69978
972-3-6409817/972-3-6409403

Jarle Mork
University of Trondheim
Biological Station
Bynesveien 46 N-7018
Trondheim
NORWAY
47-7-591589/47-7-591597

Peter Moyle
University of California-Davis
Department of Wildlife and Fish Biology
Davis, CA 95616
(916) 752-6355/752-4154

George Murphy
Taraval Associates
1220 University Drive Suite 204
Menlo Park, CA 94025
(415) 329-1044/329-1043

John Nickum
U.S. Fish and Wildlife Service
Division of National Fish Hatcheries
1849 C Street Northwest
ARLSQ 820
Washington, D.C. 20240
(703) 358-1878/358-2210

David Noakes
University of Guelph
Institute of Ichthyology
575 Gordon St.
Guelph, Ontario, CANADA
N1G 2W1
(519) 824-4120 ext. 2747/763-3906

Christine Noiville
IFREMER
24 Rue des Ecoles
75005 Paris
FRANCE
33-1-43-26-11-60/33-1-46-33-12-52

Nick Parker
Texas Tech University
Texas Cooperative Fish and Wildlife Unit
Lubbock, TX 79409-2125
(806) 742-2851/742-2280

Donald Pereira
Minnesota Chapter of
American Fisheries Society
1200 Warner Road
St. Paul, MN 55106
(612) 772-7962/772-7977

John Plumb
Auburn University
Fisheries and Allied Aquacultures
Auburn, AL 36849
(205) 844-4786/844-9208

Iola Price
Department of Fisheries and Oceans
Director, Aquaculture and Resource
Development Branch
Biological Sciences Directorate
Ottawa, CANADA
K1A OE6
(613) 990-0275/954-0807

Jeffrey Ram
Wayne State University
Michigan Sea Grant
Dept. of Physiology
Detroit, MI 48201
(313) 577-1558/577-5494

Robert Read
Wisconsin Department of Natural Resources
P.O. Box 7921
101 S. Webster Street
Madison, WI 53707-7921
(608) 266-2621/267-3579

William Reeves
Alabama Department of Conservation
and Natural Resources
64 N. Union St.
Montgomery, AL 36130
(205) 242-3886/242-3032

Phil Regal
University of Minnesota
Department of Ecology, Evolution and
Behavior
100 Ecology
1987 Upper Buford Circle
St. Paul, MN 55108
(612) 624-6751

Paul Sandifer
South Carolina Wildlife & Marine Resources
Marine Resources Division
P.O. Box 12559
217 Fort Johnson Road
Charleston, SC 29422-2559
(803) 762-5042/762-5001

Thomas Schwedler
Clemson University
Aquaculture Extension Specialist
308 Long Hall
Clemson, SC 29634
(803) 656-2810/656-0678

Scott Shore
North Carolina Dept. of Agriculture
P.O. Box 27647
Raleigh, NC 27611
(919) 733-6930/733-1041

Dan Siems
University of Minnesota
Dept. of Ecology, Evolution and Behavior
264 Ecology
St. Paul, MN 55108
(612) 624-6711

William Simco
Memphis State University
Department of Biology
Memphis, TN 38152
(901) 678-2594/327-8001

Peter Sorensen
University of Minnesota
Department of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-4997/625-5299

George Spangler
University of Minnesota
Department of Fisheries and Wildlife
200 Hodson Hall
1980 Folwell Ave.
St. Paul, MN 55108
(612) 624-9229/625-5299

Lee Stevens
Executive Director Sea Grant Association
2813 Lee Oaks Court #102
Falls Church, VA 22046
(703) 876-0121/(301) 314-9581

Katie Thompson
Chromaxome Corp
12991 Caminito Bodega
Del Mar, CA 92014-3818
619-792-5060/793-7630

Alice Tibbetts
Minnesota Sea Grant
new address:
2015 Iglehart Ave.
St Paul, MN 55104
(612) 647-1304

Thomas Vincent
University of Arizona
Department of Aerospace and Mechanical
Engineering
Tucson, AZ 85721
(602) 621-2325/621-8191

Percy Washington
Gaia Northwest, Inc.
10522 Lake City Way N.E. Suite C201
Seattle, WA 98215
206-367-2864/367-2865

Fred Wheaton
University of Maryland
Department of Agricultural Engineering
1124 Shriver Laboratory
College Park, MD 20742
(301) 405-1189/314-9023

Jack Wingate
Minnesota Department of Natural Resources
500 Lafayette Rd.
St. Paul, MN 55155-4012
(612) 296-0793/297-4916

William Witt
Food and Drug Administration
National Center for Toxicological Research
(HFT-240)
Jefferson, AR 72079-9502
(501) 543-7949/543-7576

Bill Wolters
U.S. Department of Agriculture-ARS
Catfish Genetics Research Unit
P.O. Box 38
Stoneville, MS 38776
(601) 686-5460/686-5462