Aquabyte is the Newsletter of the
Network of Tropical Aquaculture Scientists

Editorial

There is a growing international discussion on genetic engineering and the risks involved in the release of genetically modified organisms (GMOs) and exotic species, and the need for biosafety guidelines and policies. Many national governments are drafting their policies with regard to research in confinement and release of GMOs and exotics. This issue has an article on a computer software available for assessing and managing risks in experiments with GMOs. Pollution, leading to disease, has become a major problem in aquaculture operations. One of the articles in this issue details nitrogen pollution and how it can be reduced, while another deals with diagnostic kits available for shrimp diseases.

M.V. Gupta

A Computer Software Package for Assessing and Managing Risks Posed by Experiments with Genetically Modified Fish and Shellfish

Eric Hallerman, Doug King and Anne Kapuscinski

Abstract

Assessment and management of risk is needed for sustainable use of genetically modified aquatic organisms (aquatic GMOs). A computer software package for safely conducting research with genetically modified fish and shellfish is described. By answering a series of questions about the organism and the accessible aquatic ecosystem, a researcher or oversight authority can either identify specific risks or conclude that there is a specific reason for safety of the experiment. Risk assessment protocols with examples involving transgenic coho salmon, triploid grass carp and hybrid tilapia are described. In case a specific risk is identified, the user is led to consider risk management measures, involving culture methods, facilities design and operations management, to minimize the risk. Key features of the software are its user-friendly organization; easy access to explanatory text, literature citations and glossary; and automated completion of a worksheet. Documented completion of the Performance Standards can facilitate approval of a well designed experiment by oversight authorities.

Introduction

Biotechnology has developed many new powerful techniques for the propagation of aquatic and marine organisms. Application of biotechnology benefits aquaculture and fisheries management by improving production efficiency (e.g., transgenic coho salmon) and reproductive sterility (e.g., triploid grass carp), and combining valued traits of the parental species (e.g., hybrid tilapia). However, hazards posed by aquatic biotechnology have been predicted on the basis of ecological and genetic principles (Kapuscinski and Hallerman 1990, 1991; Hallerman and Kapuscinski 1992, 1993; Hindar 1993; OECD 1995). For example, maladaptive traits could be introduced into natural populations through the reproduction of transgenic fishes. Triploid males of some species produce viable sperm; should a large number of triploid males mate with normal diploid females, loss of the resulting interploid broods could pose a demographic risk to the natural population. Backcrossing of interspecific hybrids to either of the parental species poses the risk of introgression of one parental species' genes into the gene pool of the other. Recent empirical studies (Dunham 1996; Muir et al. 1996; Devlin et al. 1997) have begun to quantify some of these hazards.

It is against this *background* that the aquaculture community has discussed the issue of how to maximize benefits while minimizing risk associated with aquatic biotechnology (Pullin 1994; OECD 1995). Policymakers also seek to strike an acceptable balance between promoting and regulating the development of biotechnology. One useful approach to safe development of a new technology is application of risk assessment/risk management principles (Hallerman and Kapuscinski 1995). This article describes a tool for assessing and managing risk posed by experiments with genetically modified aquatic organisms (aquatic GMOs), and illustrates its use with three relevant examples.

Need for and Development of the Performance Standards

Development of the risk assessment/risk management framework is perhaps best understood within its historical and regulatory context. The first request for field testing of an aquatic GMO in the United States was controversial. It involved the proposed release of transgenic common carp (*Cyprinus carpio*) at Auburn University. The permit request was approved on an *ad hoc* basis, but U.S. Department of Agriculture's Office of Agricultural Biotechnology decided that a defensible policy on experiments with aquatic biotechnology should be developed. A Working Group on Aquatic Biotechnology and Environmental Safety was organized and mandated to develop a framework to facilitate biotechnology research which would have no significant ecological effects. The framework was to guide researchers on ecological safety assessment of a proposed experiment with genetically modified fish or shellfish. It would include a case-by-case assessment of the parental organism, the modified organism, and the accessible ecosystem(s). The outcome of risk assessment was to identify specific risk or reason for safety of the proposed experiment. Should any risk be identified, the decisionmaking framework should help the researcher identify a series of options to confine the proposed experiment effectively, such as different culture methods, facilities and operational safeguards.

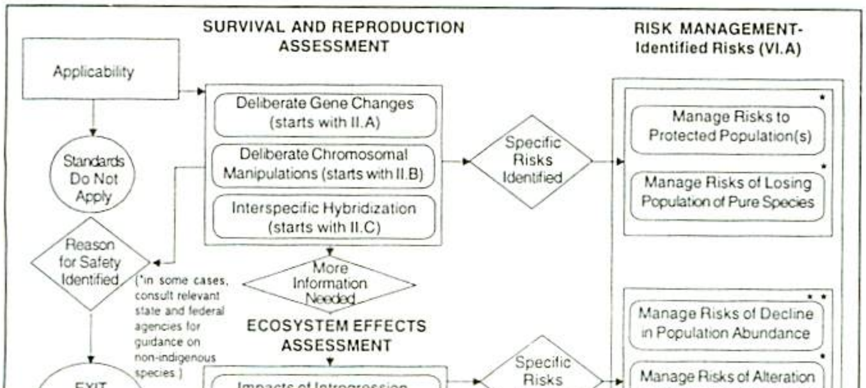

Fig. 1. Overview flowchart for Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish.

These guidelines were not set to prescribe design standards on how to achieve confinement but to help the researcher determine what degree of confinement is appropriate for a given experiment. Hence, the guidelines were to be designed as "performance standards."

Draft performance standards were distributed to a wide range of stakeholders in the outcome of the process, including aquaculturists, researchers, fisheries management agencies, federal regulatory agencies, and environmentalists. The risk assessment/risk management framework evolved through a workshop, notices in journals, revisions and discussions in three public meetings of the USDA's Agricultural Biotechnology Research Advisory Committee (ABRAC). The outcome of this process was Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish (ABRAC 1995a, 1995b). The Performance Standards have two parts (Fig. 1): addressing assessment and risk management. In February 1996, the USDA adopted the performance standards as voluntary guidelines for research with aquatic GMOs.

Risk Assessment

In the risk assessment section of the Performance Standards, the investigator answers several preliminary questions to determine the applicability of the Performance Standards to the experiment in question. The Performance Standards then request information on the fish and shellfish subject to gene transfer, chromosome set manipulation or interspecific hybridization. Depending on whether risk is identified, the investigator is either directed to the risk management portion or to exit the Standards. Should no summary finding be reached, the investigator proceeds to the assessment of ecosystem effects. Potential impact pathways assessed include the impacts of introgression, nonreproductive interference, reproductive interference (Fig. 1), and effects on ecosystem structure and process. Should no risk pathways be identified, the researcher concludes that there is reason to believe in the safety of the modified organism, and exits the Standards. Should there be a specific risk identified, or insufficient information to reach a judgment, the investigator proceeds to risk management.

Perhaps the best way to grasp the workings of the risk assessment framework is by examining a range of relevant examples. Examples of findings reached through the use of the Performance Standards to assess risk posed by hypothetical experiments with transgenic coho salmon and hybrid tilapia are presented in Table 1. We emphasize that there will be differences in the outcome of risk assessment in different locations and with or without the use of reproductively sterile GMOs.

Risk Management

Should any risks be associated with a proposed experiment, risk management measures which examine culture methods, facilities design and operations management are proposed. The investigator is presented with a series of confinement measures, including physical or chemical barriers, mechanical or biological barriers posed by the biology of the GMO itself and reduction of the scale of the experiment. Guidance is given for development of a written operational plan.

To seek institutional or agency approval for a proposed experiment, an investigator might want to document the pathway through the risk assessment/risk management process, showing justification for key findings and proposed confinement protocols. A worksheet included in the Performance Standards package provides a ready means of communicating key aspects of the proposed experiment, the outcome of the risk assessment process, the effectiveness of the chosen risk management protocol, and the process of gaining approval for the experiment by the institutional biosafety committee and any state or other supervisory agencies. To help an investigator complete the worksheet, the Performance Standards include sample worksheets for hypothetical experiments involving transgenic channel catfish, triploid Pacific oyster and hybrid striped bass. The worksheet also includes a brief text explaining the intended utility of the Performance Standards to a reviewing committee or agency. Submission of the completed Performance Standards worksheet should facilitate the approval of a well-designed experiment by an institutional biosafety committee, by a granting agency or by a fisheries management agency.

Development of the Computer-assisted Decision Support Framework

The USDA Office of Agricultural Biotechnology supported the development of a computerized version of the Performance Standards by Information Systems for Biotechnology (ISB). The program (ISB 1996) took all the information presented in the two-volume printed copy and condensed it into an easy-to-use package which can be run on any DOS-based microcomputer with a 386 or later processor. ISB chose the DOS platform so that the program could be used across the widest range of computers, especially for international users. The program can be run within DOS, Windows, or Windows 95.

A key feature of the software package is the availability of a wide range of supporting material. Options for navigating the program or access to supporting materials are shown at the top of the screen and are available at any time while using the program. A help screen shows users how to navigate the program and there is also a one-page, interactive tutorial. Text supporting consideration of issues relevant to questions asked by the Performance Standards is accessible from a menu item on-screen. Key technical terms in a given question are highlighted in yellow. Access to the glossary definition of the terms is available from the pull-down menu. A library, which includes several items not in the hard copy of the Performance Standards, is part of the software package. Relevant scientific and public policy articles are referenced in the "Literature Cited" section. The sample worksheets for transgenic channel catfish, triploid Pacific oyster and hybrid striped bass are included in the software package as well as other support material on risk assessment protocols for common carp and northern pike. Completion of the Performance Standards worksheet is automated. The completed worksheet can be printed as is, or exported to a word processor for further editing.

Electronic copies of the system are available from the authors at no cost. To download the software

Table 1. Pathway for Performance Standards-guided risk assessment for hypothetical experiments with aquatic GMOs.

Question or finding	Response
1. Transgenic coho salmon	
Description of proposed experiment: Performance of transgenic coho salmon expressing an introduced growth hormone gene will be evaluated in an on-shore tank culture system in British Columbia, Canada.	
Applicability of the Performance Standards:	
Are the research organisms finfish, crustaceans or molluscs?	Yes
Do the research organisms have a non-dioecious form of reproduction?	No
Are the organisms derived solely by intraspecific breeding or captive breeding?	No
Are the organisms modified solely by interspecific hybridization or by selective breeding of an interspecific hybrid?	No
Finding: Survival and reproduction assessment-necessary.	
Survival and reproduction assessment:	
Does the GMO result from deliberate changes of genes?	Yes
If containment is removed, does the GMO have direct access to suitable natural ecosystem(s)?	Yes
Is/are accessible ecosystem(s) isolated from other aquatic ecosystems and of low enough concern that killing of all fish/shellfish in the event of a GMO escape would be possible and practical?	No
Finding: Assess impact of deliberate gene changes.	
Impact of deliberate gene changes:	
Is the only gene change a gene deletion and/or an addition of a marker sequence, neither of which has any ecologically relevant phenotypic effects?	No
Do(es) the accessible ecosystem(s) contain conspecifics, or other closely related species with which the GMO could interbreed?	Yes
Are the GMOs permanently sterile?	No
Are the natural populations with which the GMO could interbreed threatened, endangered or of special concern?	No
Finding: Accidentally escaped GMOs may establish a viable population of GMOs with immediate potential for gene introgression into natural populations. Go to ecosystem effects assessment.	
Ecosytem effects - Deliberate gene changes:	
Does the gene modification produce intentional or unintentional changes in one or more ecologically relevant phenotypic traits?	Yes
Findings: (1) Lacking information about the reproductive potential, gene flow and fitness in a GMO population, as well as the structure and processes of the accessible ecosystem, proceed to Risk Management - Insufficient Information. (2) In the absence of information to evaluate risk, the goal of Risk Management must be no/negligible accidental escape.	
2. Hybrid tilapia	
Description of proposed experiment: The performance and commercial potential of a new strain of hybrid tilapia (*O. niloticus x O. aureus*) will be evaluated in the Philippines, an area in which the species are not native, but have become naturalized.	
Applicability of the Performance Standards:	
Are the research organisms finfish, crustaceans or molluscs?	Yes
Do the research organisms have a non-dioecious form of reproduction?	No
Are the organisms derived solely by intraspecific breeding or captive breeding?	No
Are the organisms modified solely by interspecific hybridization or by selective breeding of an interspecific hybrid?	Yes
Is the interspecific hybrid widespread in the accessible ecosystem(s)?	Yes
Is there clear documentation that the presence of the interspecific hybrid has shown adverse effects on the accessible ecosystem?	No
Findings: (1) If the GMO is a non-indigenous organism, consult relevant state and federal agencies before proceeding with the research project. (2) Standards do not apply.	

or to seek technical support, ISB's website can be visited on the Internet at http:/www.nbiap.vt.edu. An interactive Internet version is under development.

International Perspective

Although the Performance Standards were developed within the context of biotechnology oversight in the United States, the approach should be applicable internationally. A number of countries, e.g., Norway (Ministry of Environment 1993), Canada (Fisheries and Oceans 1994), UK (Department of the Environment 1994) have adopted policies which emphasize the development of environmentally safe aquatic biotechnology. Performance Standards should prove useful within a range of international regulatory contexts. Outcomes of the risk assessment or management processes will vary from country to country due to differences in their ecosystems and human values. Development and commercialization of aquatic biotechnology are ongoing in a number of countries where there are no biotechnology monitoring policies. In this context, it may be noted that the goal of sustainable use of biotechnology has been adopted by countries that are signatories of the Biodiversity Treaty signed at the Earth Summit in Rio de Janeiro (UNCED 1992). We hope that the Performance Standards will prove to be a useful tool in promoting safe and profitable development of aquatic biotechnology.

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Can Nitrogen Pollution from Aquaculture Be Reduced?

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Abstract

Nitrogen is essential for the normal growth of fish. It is an important ingredient in fish feed but is very expensive. There is evidence that nitrogen loading from feeding and metabolic activities of fish can cause pollution of the receiving waters. This paper reviews nitrogen losses and nitrogen retention in fish and suggests ways of reducing nitrogen loading to the environment for a sustainable aquaculture program.

Introduction

Proteins are the main source of nitrogen and essential amino acids, and also the most expensive energy source (Pillay 1990). To maximize the nutrient utilization and minimize the solid and soluble waste load, it is essential to provide cultured fish with the optimum level of protein (Cho 1993). Generally, nutrients absorbed in excess of requirements may be excreted as ammonia and urea (Beveridge and Phillips 1993). When food wastage is high and the nitrogen retention and assimilation are poor, a major portion of nitrogen is added to the culture system which may ultimately pollute the environment (Handy and Poxton 1993). The aim of aquaculture should be to provide sufficient nitrogen for good growth through balanced feed. Nitrogen pollution from aquaculture can occur in three ways, namely: (i) overfeeding of fish or feeding of fish at a time when they are not growing; (ii) feeding unstable and highly soluble diets; (iii) providing a diet of poor absorption and nitrogen retention efficiency (Handy and Poxton 1993).

Nitrogen which has not been absorbed by the fishes' gut and retained in the tissues may be excreted through the gills or through fecal loss (Fig. 1), leading to nitrogen pollution of the receiving waters (Handy and Poxton 1993). This article examines general issues of nitrogen pollution from aquaculture with particular reference to nitrogen losses and nitrogen retention, and suggests possible ways for reducing nitrogen loading to the environment.