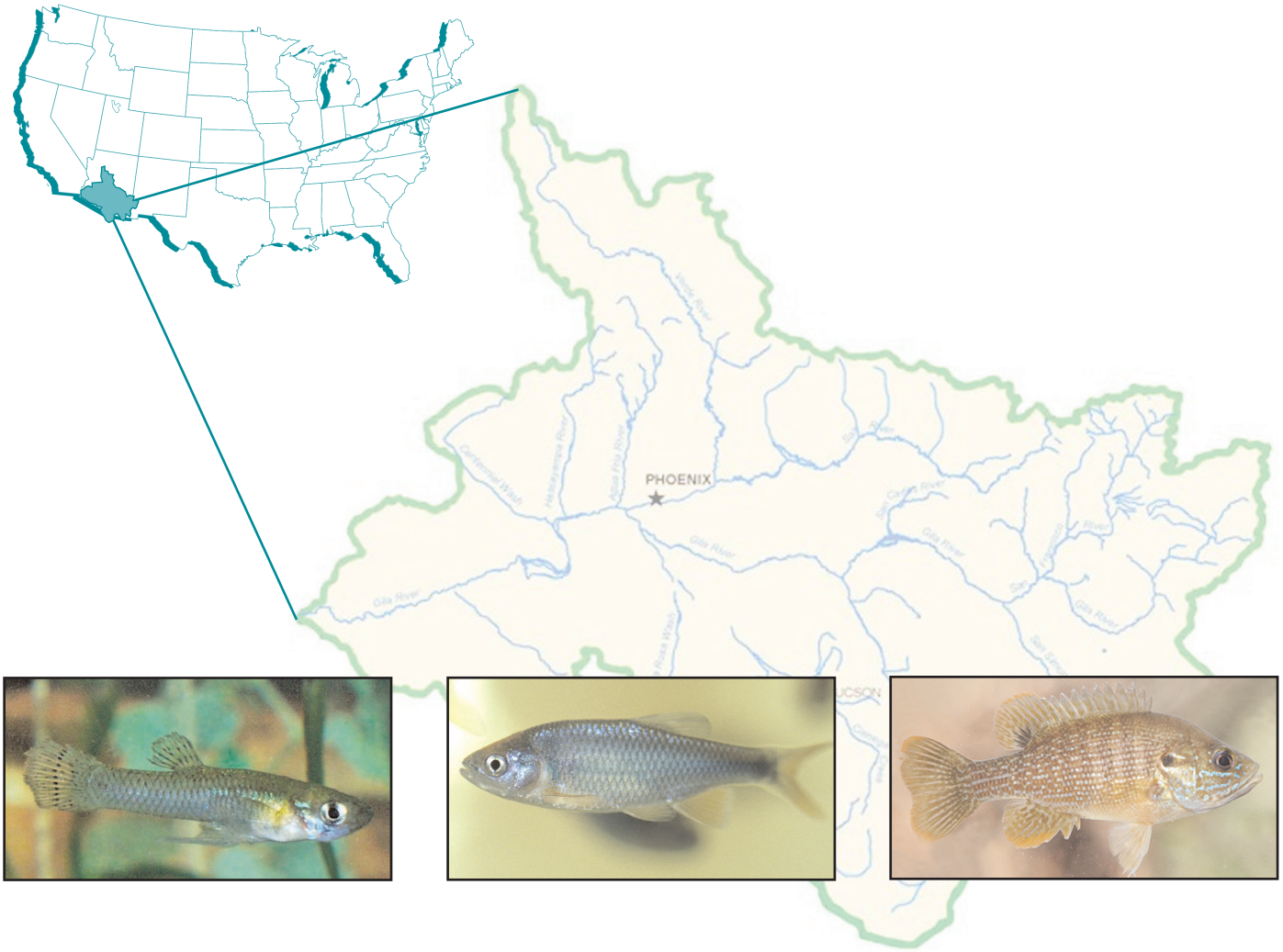
Genetic Methods for Biological Control of Non-Native Fish in the Gila River Basin

Final Report to the U.S. Fish and Wildlife Service

Anne R. Kapuscinski and Timothy J. Patronski



Sea Grant Minnesota

College of Natural Resources University of Minnesota

ISEES

A suggested citation for this report is:

Kapuscinski, A. R. and T. J. Patronski. 2005. Genetic methods for biological control of non-native fish in the Gila River basin: Development and testing of methods, potential environmental risks, regulatory requirements, multi-stakeholder deliberation, and cost estimates. Contract report to the U.S. Fish and Wildlife Service (USFWS agreement number 201813N762). University of Minnesota, Institute for Social, Economic and Ecological Sustainability, St. Paul, Minnesota. Minnesota Sea Grant Publication F 20.

Cover photographs provided by U.S. Bureau of Reclamation, Phoenix area office (map of Gila River basin), Roman Slaboch (western mosquitofish), JJ's Photographic Library, Johnny Jensen (red shiner), and Samford University (green sunfish).

Genetic Methods for Biological Control of Non-Native Fish in the Gila River Basin

Development and Testing of Methods, Potential Environmental Risks, Regulatory Requirements, Multi-Stakeholder Deliberation and Cost Estimates

Final Report to the U.S. Fish and Wildlife Service

Principal Investigator:

Anne R. Kapuscinski

Department of Fisheries, Wildlife, and Conservation Biology
Minnesota Sea Grant College Program
and
Institute for Social, Economic and Ecological Sustainability (ISEES)
University of Minnesota

Co-author:

Timothy J. Patronski

Graduate Program in Science, Technology and Environmental Policy
and
Institute for Social, Economic and Ecological Sustainability
University of Minnesota

Assistance by:

Ronny Millen (Policy and Regulatory Considerations)

Graduate Program in Conservation Biology
and
Institute for Social, Economic and Ecological Sustainability
University of Minnesota

Pouya Najmaie

Project Research Assistant
Institute for Social, Economic and Ecological Sustainability
University of Minnesota

September 2005

Contents

Acknowledgements

We thank the following individuals for reviewing the final draft of this report: Paul Barrett, U.S. Fish and Wildlife Service; Robert Clarkson, U.S. Bureau of Reclamation; Eric Hallerman, Virginia Polytechnic Institute and State University; Charles Krueger, Great Lakes Fishery Commission; Kristen Nelson, University of Minnesota and other anonymous reviewers in the Gila River basin. Valuable comments on earlier versions of this report were also provided by Paul Barrett; Robert Clarkson; and Jeff Humphrey, U.S. Fish and Wildlife Service.

We also thank all of the individuals listed in Appendix 1 whose meetings with us informed our research for this report. We especially benefited from extensive discussions with Ron Thresher, Keith Hayes, Nic Bax, Peter Grewe, and Jawahar Patil at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Marine Research laboratory in Hobart, Australia; and Tony Peacock and Brad Tucker of the Cooperative Research Centre for Pest Animal Control in Canberra, Australia.

Support for Anne Kapuscinski while preparing this report came in part from the U.S. Fish and Wildlife Service, a fellowship awarded by the Pew Fellows Program in Marine Conservation, a biotechnology-biodiversity interface grant from the United States Agency for International Development, and the Minnesota Sea Grant College Program supported by the National Oceanic and Atmospheric Administration (NOAA) National Sea Grant Office (NSGO), U.S. Department of Commerce.

Support for Tim Patronski while preparing this report came from the U.S. Fish and Wildlife Service, the Mark G. and Judy Yudof Fellowship for interdisciplinary work in science policy and ethics from the Graduate School at the University of Minnesota and the Walter H. Judd International Graduate and Professional Fellowship from the Office of International Programs at the University of Minnesota.

 This report is Minnesota Sea Grant Publication Number F20.

Executive Summary

Non-native fish, habitat degradation and water development have combined to become major stressors on the health of native fish and their habitats in the U.S. Southwest. In recent years, the impact of these stressors has led to the precipitous decline of many native fish species endemic to this area. Biologists have been searching for more effective ways to reduce the negative impact of undesirable non-native fish. Improved biological control of non-native fish could help address this complex challenge.

This report addresses the feasibility of using genetic methods as a new approach for biological control of non-native fish within the Gila River basin. This feasibility study was sponsored by the Central Arizona Project Funds Transfer Program.

The report reviews the status of existing genetic methods including chromosome set manipulations and recombinant DNA techniques; takes a preliminary look at potential ecological and human health risks; outlines policy and regulatory considerations; stresses the need for and presents an approach for multi-stakeholder deliberation; provides general cost and time estimates; and suggests integration of these considerations into a multi-component research and development program.

As of the writing of this report, no transgenic animal has been purposefully released into the environment in the United States. A future proposal to release a transgenic fish for biological control in the Gila River basin could end up being the first involving intentional environmental release of a transgenic animal in the United States.

Major Findings and Recommendations

Chapter 2 - Review of existing genetic methods for biological control of non-native fish

- The potential efficacy, strengths and weaknesses of genetic methods for biological control of invasive fish are poorly understood at present. Current understanding suggests that no one method will be adequately effective alone and achieving desired levels of control may require adapting the approaches of integrated pest management to the case of non-native fish.

- Triploid sterilization techniques are farther along in development than other genetic methods. It is unclear whether releasing sterile fish would substantially reduce the population size of a target non-native species. Table 2.1 summarizes our review of triploid sterilization.

- Transgenic techniques designed to produce sterile fish or spread deleterious transgenes to a target non-native species are in the very early stages of research. Although they offer a variety of powerful approaches for reducing non-native populations, numerous challenges such as achieving stable transgene integration and reliable long-term expression need to be overcome. Tables 2.1 and 2.2. summarize our review of transgenic techniques.

Chapter 3 - Addressing ecological and human health risks

- Ecological and human health risk assessment of any proposal to release triploid or transgenic fish for genetic biological control of non-native fish

should follow a systematic and publicly transparent process of distinct steps (Table 3.1), using information from multiple disciplines, to reach verifiable conclusions of safety or risk. Risk assessment should include explicit analysis of uncertainties.

- Risk management of any approved release should also involve transparent design and implementation of pre- and post-release steps (Table 3.1), using input from multiple disciplines, to allow adaptive learning about effectiveness and unexpected problems of the biocontrol effort.

- At present, environmental biosafety science is not well equipped to reliably predict the ecological effects of intentional releases of transgenic fish. Scientists are much better equipped to assess the ecological effects of intentional releases of triploid sterilized fish.

- Preliminary identification of hazards posed by releasing triploid or transgenic fish for biocontrol in the Gila basin revealed a range of potential ecological and human health hazards and consequent harms that would need to be fully assessed on a case-by-case basis (Table 3.2).

Chapter 4 - Policy and regulatory considerations

- Relatively few policies and regulations specifically apply to research and development or release of a triploid sterile fish or a transgenic fish for biological control (Tables 4.1 and 4.2).

- Regulation of transgenic animals is an evolving area of public policy. At present, it is not clear whether the U.S. Food and Drug Administration (FDA), another federal agency, or a state agency would have lead authority over environmental release of a transgenic fish for biological control purposes.

- To date, the FDA has claimed lead authority over food safety and environmental regulation of transgenic fish, applying statutes with secrecy provisions that allow blocking public review of draft environmental assessments and environmental impact statements before the agency would make a final regulatory decision.

- The U.S. Fish and Wildlife Service has a window of opportunity to develop a lead role in regulatory oversight of different genetic strategies for biological control of invasive fish and wildlife. The Service could also convene an interagency dialogue to sort out regulatory responsibilities in this area.

- Any biological control effort involving genetic methods would require the lead entity to comply with relevant federal, state, and tribal environmental policies and regulations.

Chapter 5 - Multi-stakeholder deliberation

- Any decision on the use of genetic methods for biological control of non-native fish must be based both on good science and meaningful deliberation among the potentially affected and interested parties within the Gila River basin. A preliminary list of relevant entities to bring to the table includes federal agencies, state agencies, non-governmental organizations, Native American reservations, and universities (Table 5.1). It should also include relevant additional groups represented on the Arizona Invasive Species Advisory Council established in 2005.

- Traditional approaches to risk communication in natural resources management have been much less participatory and accessible than is necessary to gain durable and broad public trust in decisions on proposed uses of genetic biocontrol. We strongly recommend using an analytic-deliberative approach that is transparent, equitable, legitimate, and science-driven. Table 5.2 outlines one such approach that has shown

promise for addressing other controversial genetic technologies.

- Any decision to further explore the use of genetic methods for biological control of non-native fish in the Gila River basin should include a strategy for substantive multi-stakeholder deliberation. Deliberation should be convened a number of times to inform go/no-go steps (decision points) in the research, development, assessment and regulatory review of a genetic biocontrol method.

Chapter 6 - Preliminary roadmap of programmatic activities and general cost estimates

- A multiple component program is the best strategy for pro-actively addressing the scientific, social and regulatory needs of any project to develop a transgenic fish for biological control. Components include (Figure 6.1): research to develop transgenic lines, testing their efficacy as control agents, risk assessment, modeling to further predict efficacy and risks, data gathering on target species ecology, multi-stakeholder deliberations, and seeking regulatory approval.

- We recommend temporal staging of work on different programmatic components and addressing key questions at specific go/no-go steps to decide whether to proceed to next steps in each component (Table 6.1). These go/no-go steps should help ensure that necessary multi-stakeholder support, information and resources exist for moving to the next phase of the program.

- We estimate that development, risk assessment including multi-stakeholder deliberation, efficacy testing, and seeking regulatory approval of a triploid sterile fish for biological control would cost 3-5 million dollars and take approximately five years (Tables 6.2 and 6.4).

- We estimate that development, efficacy testing, risk assessment including multi-stakeholder deliberation, and seeking regulatory approval of a transgenic fish for biological control would cost 15-20 million dollars and take approximately 20 years (Tables 6.2 and 6.3).

Overall Advisability

Any future effort to further develop the potential use of genetic methods for biological control of non-native fish within the Gila River basin or elsewhere would raise some difficult social and ecological questions. This is especially true for any proposed application of transgenic fish for biological control. Genetic biocontrol of non-native fish is a potentially powerful new tool to help recovery of precipitously declining native fish but may also be controversial. We strongly encourage following this report's recommendations for scientifically sound analysis of efficacy and risks, trusted multi-stakeholder deliberation, and a coordinated staged program. It would also be desirable to strengthen the base of scientific information regarding whether a specific non-native fish is indeed substantially impeding recovery of one or more native fish species.

Current understanding suggests that genetic methods alone will not be a panacea to the challenge of controlling non-native fish in the Gila basin. We therefore advise moving forward with a research effort to fully explore development, efficacy, and potential risks of various genetic biocontrol methods only if: (1) this is pursued as part of a multi-component research and development program along the lines presented in this report; and (2) the program is implemented as part of a broader, basin-wide integrated pest management strategy that might also include mechanical and chemical control methods and pheromone attractants to improve control efficiencies.

Chapter 1
Introduction

The native fish fauna of rivers in the U.S. Desert Southwest has declined through much of the last century and some endemic species are now extinct. Vast transformations of rivers and in-stream flow have been major triggers of these declines. A host of other human-induced changes has further contributed to the problem. Recent precipitous declines suggest that the nation is running out of time to save these unique fish communities for future generations to enjoy. This serious concern has increased attention on non-native fish which have swamped native fish fauna in many rivers (Rinne and Minckley 1991; Warren and Burr 1994; Tyus and Saunders 2000). Growing evidence of harmful effects of non-native fish on native stream fishes suggests that the presence of non-native fish impedes efforts to recover native fish communities; Tyus and Saunders 2000; Marsh and Pacey 2005).

Non-Native Fish Control and Native Fish Recovery in the Gila River Basin

The Gila River basin of Arizona and New Mexico, a part of the Colorado River basin, is a quintessential example of the disturbing trends described above (Desert Fishes Team 2003, 2004). Accordingly, biologists searching for solutions to save near-endangered or endangered species in the Gila basin are calling for control and removal of undesirable non-native fish as "the most urgent and overriding need" (Desert Fishes Team 2003, 2004).

The technical and policy committees of the interagency Central Arizona Project Fund Transfer Program recently became interested in genetic technologies as potential tools for controlling non-native fish in the Gila River basin. The reasons and sense of urgency propelling this interest were articulated in the statement of work that led to this report, as follows:

> Many fishes in the region already are federally listed as threatened or endangered and the Desert Fishes Recovery Team has recommended most others for listing. Primary causes of this condition have been habitat degradation and water development, exacerbated by the presence of non-native biota. Indeed, non-native fishes are implicated as the single most important deterrent to conservation and recovery of the native fauna. Relatively few practical and effective alternatives are available for dealing with non-native biota. Examples include chemical or other removal or depletion of undesirable exotics, construction of barriers to protect intact or repatriated faunas, and other control measures. These approaches are variously successful, even when carefully planned and effectively executed. All are logistically difficult and costly, and must account for effects on non-target biota. The status of the imperiled native fish fauna is one of continuing deterioration in spite of conservation efforts. New management tools and strategies are desperately needed if the fauna is to be saved from extirpation, or worse, extinction.

Potential Genetic Methods for Control of Non-Native Fish

It might be possible to control non-native fish through the application of two modern genetic techniques, recombinant DNA methods and chromosome set manipulations. Since the 1980's fish geneticists have applied these techniques primarily to improve desirable traits of fish for aquaculture production (Kapuscinski 2003, 2005). Some commercial aquaculture companies use chromosome set manipulations on a large scale to produce triploid sterilized fish. These sterile triploid fish divert more of their food energy into muscle growth (and none into gamete development), leading to improved yields on the fish farm. So far, only researchers in laboratory settings are applying

recombinant DNA methods to develop and study transgenic fish designed for faster growth, disease resistance and other traits of potential value in fish farming.

Purposefully releasing transgenic fish or triploid sterilized fish for biological control of non-native fish would be a very different and new way to apply these genetic tools. Researchers at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia were the first to seriously pursue transgenic fish for biological control. They are in early stages of developing several transgenic approaches for triggering steep declines in a nuisance non-native fish population. The idea of releasing sterile fish for biological control has been a topic of scientific discussion for at least two decades, inspired by successful control of pest insects by releasing sterile insects. Currently, release of sterile-male sea lamprey in the Great Lakes is the only invasive fish biocontrol program using this strategy. Relevant research for controlling other invasive fish is quite limited at present.

Scope of Report

This report examines the feasibility of developing and implementing genetic biocontrol technologies, including recombinant DNA methods and chromosome set manipulations, as mechanisms to control undesirable fish populations. The scope of work included (1) information gathering and review, (2) cost evaluation and (3) assessment of regulatory requirements. The report addresses scientific and technical, risk assessment and management, multi-stakeholder involvement, and regulatory components that would have to be part of any program for genetic biocontrol of non-native fish. After separately examining each of these components, the report presents an approach for integrating these individual components into a seven-part and staged program.

Although the report focuses on the Gila River basin, the principles and approaches discussed are more broadly relevant, as is much of the scientific and regulatory information reviewed. Parties wishing to explore the development of genetic biocontrol of non-native fish in other water bodies should therefore benefit from reading this report.

Outline of Chapters

Chapter 2 reviews the status of scientific understanding and technical development of genetic biocontrol methods. The review focuses on two general control strategies, sterile release and deleterious gene spread. Sterile release swamps the non-native fish population with either triploid or transgenic sterilized relatives to prevent successful matings by the non-native fish. Deleterious gene spread aims to move harmful transgenes from the released transgenic fish into the target non-native fish population. We review the scientific background and theory, status of development and methodological strengths and weaknesses for the sterile release and deleterious gene spread approaches. We also note other ecological, social and regulatory considerations that subsequent chapters address in more detail.

Chapter 3 introduces the major steps in risk assessment that should be applied case-by-case to address potential ecological and human health effects of each proposal to release triploid or transgenic fish for biological control. It also outlines risk management steps to pursue if a release goes forward. The chapter provides a preliminary list of potential ecological and human health hazards but does not contain a completed risk assessment. Instead, we outline a general approach for conducting a full risk assessment, stressing the importance of explicitly assessing scientific uncertainty and including risk communication. This chapter sets the stage for discussion of linking multi-stakeholder deliberation to technical analysis of a genetic biocontrol proposal (chapter 5) and integrating risk assessment into a coordinated program on genetic biocontrol (chapter 6).

Chapter 4 identifies the likely regulatory requirements for developing and releasing transgenic or triploid fish for biocontrol in the Gila River Basin. The chapter starts with a review of relevant U.S. federal environmental, biotechnology, and invasive species policy. It then examines specific regulatory issues for (1) research and development and (2) environmental release of triploid sterilized fish and transgenic fish for biocontrol. This regulatory review considers relevant federal, state, tribal and international jurisdictions. We also highlight the uncertain and evolving aspects of federal and state regulation of transgenic animals.

Chapter 5 explains the need to integrate multi-stakeholder and science-driven deliberations with technical analysis of genetic biocontrol proposals. It presents a preliminary list of stakeholder groups to involve in analysis and deliberation for proposals involving the Gila River basin. The chapter introduces a Problem Formulation and Options Assessment (PFOA) process that has recently shown promise in addressing another controversial kind of genetic technology, transgenic crops in agriculture. We outline steps showing how the PFOA process could be applied to multi-stakeholder deliberation of proposals for genetic biocontrol of non-native fish. The chapter also summarizes what we have learned about the risk communication strategy of Australia's daughterless carp program, which is in early stages of research to develop transgenic fish for eventual biocontrol of non-native carp.

Chapter 6 proposes seven key components for organizing a research and development program on genetic biocontrol of non-native fish. We lay out possible go-no go steps that could guide decisions on whether to proceed from early research stages to progressively more complex lab and field testing stages and finally to the stage of seeking regulatory approval. We also present general cost categories and estimates involved in developing transgenic fish or triploid fish for biological control. We developed all these recommendations from our main findings reported in chapters 2 through 5, our meetings with people in the Gila River basin, and what we learned about Australia's daughterless carp program.

Chapter 2

Review of Existing Genetic Methods for Biological Control of Non-native Fish

Introduction

Two genetic manipulation techniques—chromosome set manipulations and recombinant DNA methods—have been the focus of considerable research and development since the early 1980s to improve aquaculture production traits in fish (Kapuscinski and Hallerman 1991; Kapuscinski 2003; Pew Initiative on Food and Biotechnology 2003). Both techniques could be harnessed for biological control of invasive fish species.

Chromosome set manipulations (also called ploidy manipulations) enable production of fish whose chromosomes come entirely from the male or the female parent, or in which the number of chromosome sets is increased from the normal pair to either three sets or four sets (Thorgaard 1983). Induction of triploidy refers to inducing fish to bear three sets of chromosomes and, in some fish species, leads to varying degrees of sterility. Fish that are sterile but still enter into courtship behavior could offer one tool for biological control, as discussed further below. The idea of using triploid sterilized fish for biological control has been informally discussed within the fisheries community for at least 10 years but has not yet been applied in a field setting.

Recombinant DNA methods involve the transfer of novel genetic constructs (also called transgenes) into the fish genome, resulting in the development of a "transgenic" fish expressing a novel trait (Hew and Fletcher 1992; Hackett 1993; Donaldson and Devlin 1996; Houdebine 1996). Biologists have identified and refined techniques for chromosome set manipulations for many fish species and generally understand the associated strengths and weaknesses. The techniques of gene transfer via recombinant DNA techniques are not as fully developed and the strengths and weaknesses not as well understood. Purposefully releasing a transgenic fish expressing a deleterious transgene for biological control of harmful non-native fish species is a relatively new idea for applying recombinant DNA technology.

The potential efficacy, strengths and weaknesses of genetic methods for biological control of invasive fish are poorly understood at present. Ongoing research to anticipate the effectiveness and pitfalls of different genetic methods for biological control of fish suggests that in many cases no one method will be adequately effective alone and that achieving desired levels of biological control may require adapting the approaches of integrated pest management to the biological control of invasive fish (Sawyer 1980). An integrated pest management approach might combine genetic methods with mechanical or chemical control methods, as well as the release of pheromone attractants to improve the efficacy of these other methods (Dawson and Kolar 2003; Sorenson and Stacy 2004). As research in all the required areas moves forward (from genetics to population ecology), it will be important to periodically re-evaluate understanding of the potential effectiveness and pitfalls of different genetic methods. Meanwhile, we can draw on insights learned from recent research on several related topics: reproductive containment of fish for aquaculture and conservation purposes; integration of transgenes into established populations; risk assessment of transgenic organism release; and traditional biological control of other organisms, such as insects. Each genetic-based method may offer potential benefits that need to be considered in

light of the associated risks which must be carefully identified, assessed and managed (Kapuscinski 2002).

This review of existing genetic methods for biological control of invasive, non-native fish will focus on two general approaches: **sterile release and deleterious gene spread. Sterile release aims to prevent successful matings in the targeted non-native fish population. Deleterious gene spread aims to move deleterious transgenes throughout the targeted non-native fish population.** We review below the background and theory, development status, methodological strengths and weaknesses, and other ecological, social, and regulatory considerations for each approach.

Sterile Release[1]

It is possible to sterilize fish via two genetic methodologies: chromosome set manipulations for triploid sterilization and recombinant DNA methods for transgenic sterilization. Several longer known biological but non-genetic methods can also sterilize fish.

Background and theory

The Sterile Insect Technique (SIT), involving releasing sterilized insects of the same species as the target organism to competitively interrupt reproduction, has been used successfully by entomologists to control nuisance insect species (Whitten 1992). The traditional SIT approach involves the release of mass-reared and sterilized male insects to trigger many infertile matings with wild females, thus reducing the pest population size (Braig and Yan 2002; NRC 2002a; Wimmer 2003; NRC 2004). Pest reduction requires achieving high rates of sterile to fertile males. Preliminary evidence of effective sea lamprey control via release of male lamprey sterilized using bisazir, a chemical sterilant, in the Great Lakes (Twohey et al. 2003) suggests that releasing sterile fish may hold promise as a biological control method for other non-native fish; however, little if any formal research has been conducted on this topic. Further research would be worthwhile to explore the potential for release of sterile fish as a biological control method for non-native fish. Traditional techniques are well established and easy to apply; and there is also the potential of new transgenic approaches to induce sterility in fish.

Methods for making fish sterile

Triploid sterilization

Traditional induction of sterility via ploidy manipulations in fish involves application of a hydrostatic pressure, temperature or chemical shock at the appropriate number of minutes after sperm fertilization of an egg, in order to disrupt the egg's normal extrusion of a polar body containing a haploid set of maternal chromosomes; Figure 2.1 outlines this method. The resulting retention of the polar body leads to an embryo bearing two haploid chromosome sets from the female (instead of the normal one haploid set) and a third set from the male (NRC 2004). The presence of the odd set of chromosomes presumably causes mechanical problems involving pairing of homologous chromosomes during each cell division (Benfey 1999) and this disrupts the normal development of gametes to some extent. The resulting triploid condition differs from the normal diploid number of chromosomes. Tetraploid fish, containing four sets of chromosomes, are sometimes crossed with diploid fish to yield 100% triploid offspring (Hallerman and Kapuscinski 1993).

Transgenic sterilization

New transgenic methods theoretically could be used to induce sterility in fish. The most relevant research to date has involved a repressible sterility technique (Thresher et al. 1999) using interference RNA (RNAi) methods (Fire et al. 1998; Brummelkamp et al. 2002; Sui et al. 2002). The Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia is a world leader on developing RNAi techniques to produce inducible sterile fish (also called 'sterile ferals') (Thresher et al.

[1]The review in this section draws heavily on a related review by the National Research Council (NRC 2004).

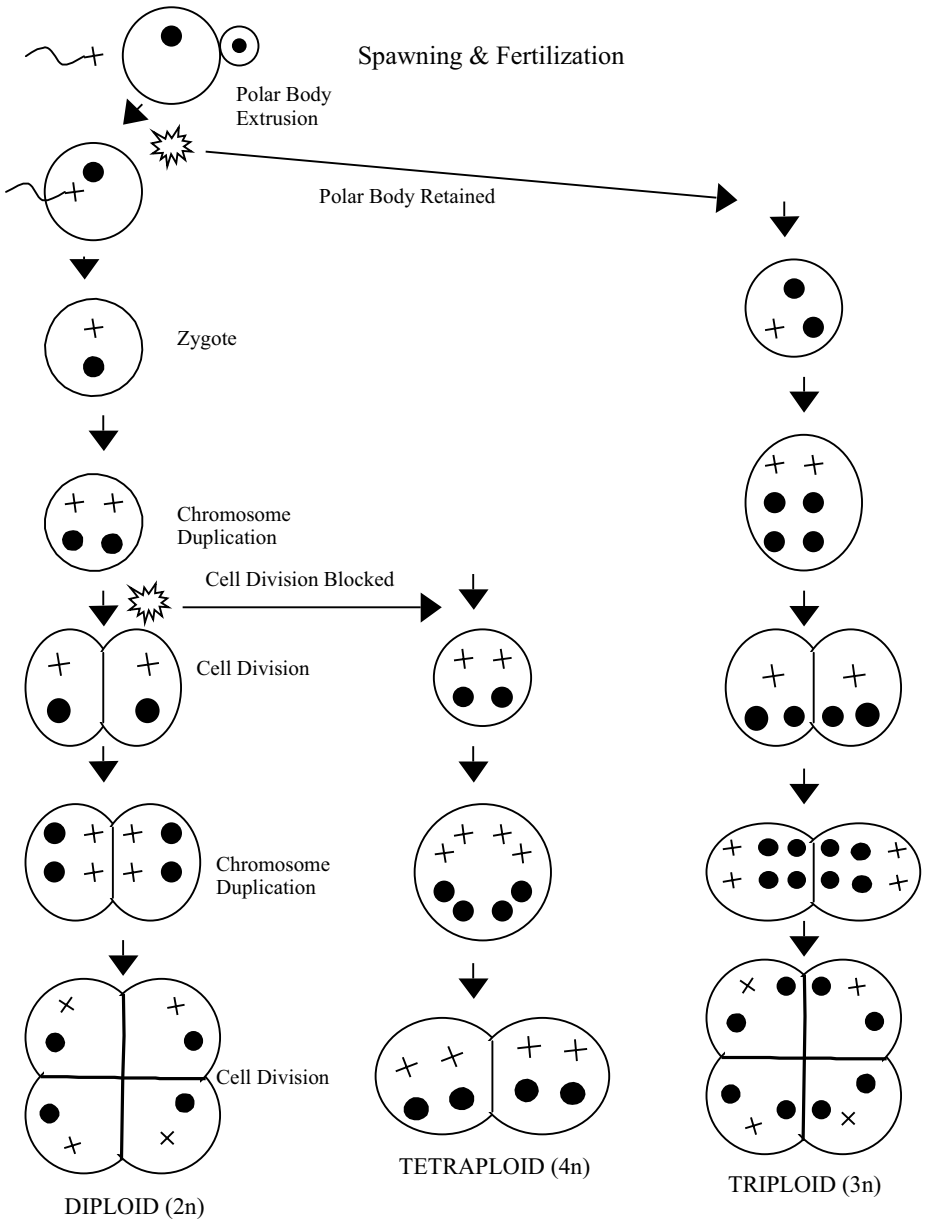


Figure 2.1. Normal steps in gamete fertilization and early cell division that lead to the development of a normal diploid (2*n*) fish or shellfish embryo. Induction of triploidy (3*n*) or tetraploidy (4*n*) occurs by temperature shock, chemical shock, or pressure at an appropriate time after fertilization. Symbols:

✸ denotes the point at which the shock is applied; • denotes one haploid chromosome set derived from the female parent; and **x** denotes one haploid chromosome set derived from the male. From NRC (2004), adapted from Donaldson, unpublished data.

1999). This approach involves inserting a transgene designed to block expression of an endogenous gene essential for development of viable gametes or embryos. The transgene includes a sequence for a blocker molecule (such as RNAi) that prevents expression or at least causes mis-expression of the targeted endogeneous gene. Expression of the blocker is under control of an inducible promoter, ideally inducible by the presence or absence of a compound that can be added to the food of captive animals. Other parts of the construct can be designed to allow reversible activation/repression of the inducible promoter and hence of expression of the blocker (NRC 2004). See the section on deleterious gene spread below for detail on methods for developing transgenic fish.

Radiation, chemical, or surgical sterilization

The longest-existing methods of rendering fish sterile are all non-genetic. They include radiation (Thorpe et. al 1987), steroid treatment (Yamazaki 1976) and surgical removal of gonads, i.e., gonadectomy (Donaldson et. al 1993). Some evidence indicates that administering high doses of steroids results in accelerated growth in the treated fish (Yamazaki 1976). If this accelerated growth leads to increased success in securing mates during courtship, it could increase the effectiveness of a steroid-sterilized fish as a biological control tool. Gonadectomy may be highly ineffective for biological control purposes because the loss of steriodogenic gonadal tissue could remove courtship and other reproductive behaviors of the fish (see next paragraph). Table 2.1 compares these non-genetic methods to the genetic methods that are the main focus of this report.

Courtship behavior of sterile fish

Released sterile fish will enable biological control only if they still enter into courtship behavior with relatives in the target population. There are huge information gaps regarding this issue. For triploid sterilization, little research has investigated the extent to which triploid adults of different species retain normal reproductive behavior (Inada and Taniguchi 1991; Kitamura et al. 1991; Donaldson et al. 1993; Hallerman and Kapuscinski 1993; ABRAC 1995; Cotter et al. 2000). In one of the few field tests of the behavior of triploid fish released into the natural environment, triploid adult Atlantic salmon migrated back from the ocean to natal freshwaters at a much lower rate than control salmon, thus reducing the numbers that could try to mate with wild fish of the opposite sex (Cotter et al. 2000). Some evidence indicates that sterilized triploid males, which retain functional gonadal steroidogenic tissue, still exhibit normal reproductive behavior (E. M. Donaldson, personal communication, February 2004; Dunham 2004). It is important to note that reproductive behavior differences between diploids and triploids will likely vary among species and methods of chromosome manipulation (Hallerman and Kapuscinski 1993). For transgenic sterilization, development of such transgenic fish lines is at too early a stage to include empirical tests of courtship behavior.

Strengths, weaknesses and other considerations

The strengths and weaknesses associated with the sterile approach vary for the two main genetic methodologies. We highlight main points below and provide a detailed list of strengths, weaknesses and other considerations for each method in Table 2.1.

Triploid sterilization methodology

Strengths of triploid sterilization include: zero risk of spread of the "sterility condition" to non-target populations and thus no risk of associated possible genetic harm; relatively low cost of applying the technology and relatively short time period required for research, development, and implementation. Ecological risks are limited to just the competition and predation posed by the triploid individuals themselves. Weaknesses include: difficulty of achieving 100% sterility in mass applications, thus requiring screening to cull fish that are still fertile; costs associated with multiple stockings of sterilized males; and current uncertainties regarding the level and nature of reproductive behavior exhibited by sterilized adults. The release of tetraploid females may hold promise for biological control purposes

Table 2.1. Overview of methods for the sterile release approach for biological control of non-native fish.

Method of Sterilization	Strengths	Weaknesses	Other Ecological, Social, and Regulatory Considerations
Triploid Sterilization via temperature, chemical or pressure shock to newly fertilized egg. (Also called chromosome set manipulation or ploidy manipulation.)	1. Methods well developed. 2. Limitations understood. 3. Ready to use for some species. 4. Relatively low cost. 5. Shorter research and development time than transgenic methods.	1. Triploid induction not 100% effective for treated eggs. 2. Requires individual screening to cull failures. 3. Potential for mosaic individuals (mix of diploid and triploid).	1. Do sterile individuals retain active reproductive hormone levels and normal courtship behavior? 2. How many modified fish would need to be stocked and at what frequency? 3. Would predation of and competition with native species outweigh benefits gained by stocking sterile non-native fish? 4. Need to adapt methods to biology of each species. 5. May be more socially acceptable and more feasible from a regulatory standpoint than transgenic methods.
Gamma Irradiation to disrupt development of normal gametes.	1. Ready to use for some species. 2. Shorter research and development time than transgenic methods.	1. Limitations not fully understood. 2. Sterilization not 100% effective. 3. Requires individual screening to cull failures.	1. See considerations 1-4 for triploid sterilization. 2. Some evidence indicates that irradiation does not prevent secretion of steroid sex hormones and development of secondary sex characteristics (Thorpe et al. 1987). 3. Potential social and regulatory concern if fishing leads to humans eating previously irradiated fish.
Steroid Treatment	1. Ready to use for some species. 2. Relatively low cost. 3. Shorter research and development time than transgenic methods.	1. See weaknesses 1-3 for gamma irradiation.	1. See considerations 1-4 for triploid sterilization. 2. Potential social and regulatory concern over negative effects of residual steroids in fish on food webs in the environment or on human health.
Chemical Treatment	1. Ready to use for some species. 2. Some experience with field application: the sterile male sea lamprey release in the Great Lakes employed bisazir, a chemical sterilant (Twohey et. al 2003). 3. Relatively low cost. 4. Shorter research and development time than transgenic methods.	1. See weaknesses 1-3 for gamma irradiation.	1. See considerations 1-4 for triploid sterilization. 2. Potential social and regulatory concern over negative effects of residual chemicals in fish on food webs in the environment or on human health.

Table 2.1. *Continued.*

Method of Sterilization	Strengths	Weaknesses	Other Ecological, Social, and Regulatory Considerations
Surgical Gonadectomy	1. Highly effective with experience. 2. Ready to use on most species. 3. Shorter research and development time than transgenic methods.	1. Costly due to labor-intensive and time-consuming need to treat fish individually. 2. Fish must reach minimum size before surgery. 3. Risk of infection during surgery could reduce efficacy for biocontrol. 4. Fish likely to lose courtship behavior due to loss of gonadal tissue. 5. Risk of gonad regeneration if removal is incomplete	1. See considerations 1-3 for triploid sterilization. 2. Probably inappropriate for biological control applications due to probable loss of courtship/reproductive behavior.
Transgenic Sterilization. (Involves recombinant DNA techniques; also called genetic modification or genetic engineering.)	1. Capability to control sterility expression via repressor or inducer molecules. 2. Can build in redundant sterilization methods by stacking transgenes that affect different stages of development.	1. Costly to develop. 2. Long research and development period for each transgenic line. 3. Limitations not fully understood. 4. Probable limits to gene stacking. 5. May require individual screening to ensure success.	1. See considerations 1-4 for triploid sterilization. 2. How stable is the transgene expression? 3. How complete is the induced sterility? 4. Unexpected presence of repressor molecule (e.g. tetracycline) in natural waters might repress expression of sterility genes. 5. May not be as socially acceptable as non-transgenic sterilization methods.

because they could mate with diploid males leading to the hatching of 100% triploid offspring (Hallerman and Kapuscinski 1993); however, tetraploid fish often have low survival rates and poor performance (Donaldson and Devlin 1996); which would reduce the efficacy of this method.

Transgenic sterilization methodology

Strengths of transgenic sterilization of fish include the potential for repressible on-off sterility expression and for building in sterility redundancy by "stacking" sterility-inducing genes. Also, if the transgenes do not disrupt steriodogenesis or other physiological processes that affect reproductive behavior, then the transgenic-sterile fish should be capable of normal courtship behavior, a plus for biological control. Weaknesses include the preliminary status of the technology, higher costs and long-term commitment associated with research, development, and implementation, costs associated with multiple stockings, and concerns about the stability of transgene integration into the fish genome and the reliability of transgene expression. Note, however, that the sterile feral technology, patented by the CSIRO of Australia, is sufficiently developed that other parties are negotiating licenses from CSIRO to use it.

Other considerations

Some important ecological questions regarding effectiveness and risks of the sterile release approach remain and would need to be further researched. These include the following. To what extent would sterile males attempt reproduction with females in the target fish population and thus successfully interrupt mating? How many sterile fish would need to be released and at what frequency and spatial distribution? Would predation or different kinds of

competition between the sterile fish and wild, non-target native fish species, pose ecological risks—due to stocking of additional invasive (sterile) fish? Would these risks outweigh the benefits of the intended biological control?

Social and regulatory considerations may also differ for the two main sterilization methodologies. A number of stakeholder groups and other interested parties could be more willing to accept the release of non-transgenic, triploid sterilized fish than transgenic-sterilized fish for biological control. However, decision makers need relevant empirical data to guide them on this issue, including data from regionally-specific social science research conducted by qualified social scientists; and stakeholder consultations at appropriate points in the process of developing a biological control program (see also chapter 5). Regulatory oversight may also be less complex for releases of triploid sterilized than transgenic-sterile fish (see also chapter 4).

Deleterious Gene Spread

Background and theory

Another potential genetically-based approach to controlling non-native fish populations is the release of transgenic individuals (of the same species as that targeted for control), bearing a deleterious genetic construct (transgene) designed to disrupt a specific aspect of the organism's life cycle or biology. A variety of genes could be targeted to control aspects of development, survival, or gametogenesis in offspring. Figure 2.2 illustrates various points of possible disruption via transgenic methods during the life cycle of a fish. For example, targeted genes could control for important aspects of body plan or gill development or function during the embryonic or larval periods, or gamete development during the juvenile period. In the future, improved understanding of gene function and regulation could conceivably identify many different genes that could be disrupted or regulated for biological control purposes. Ongoing multi-laboratory initiatives to map the genome of several fish species are likely to accelerate the identification of such genes. Table 2.2 lists some examples of fish genes and their functions

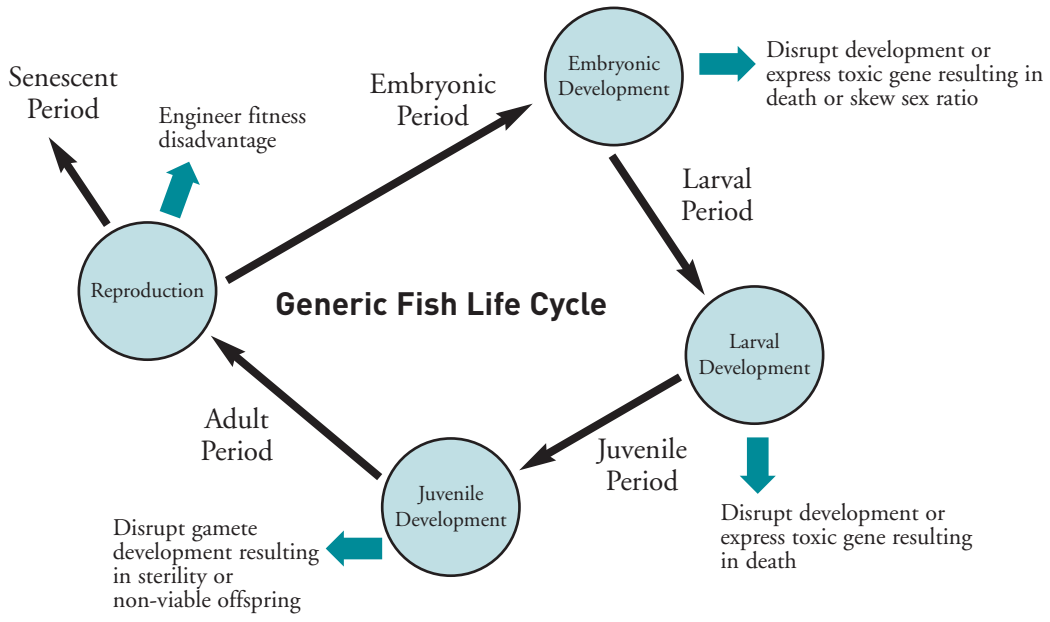


Figure 2.2. Examples of potential points of opportunity to disrupt a fish life cycle via transgenic methods.

Table 2.2. Examples of endogenous genes whose expression could be disrupted in transgenic fish designed for biological control of invasive fish species (The listing is adapted from Table 2 in Donaldson and Devlin 1996; NRC 2004).

Gene	Potential strategy for biological control	Reference
Aromatase (daughterless gene)	Block to produce all-male line	Thresher et al. (2002); Genebank cited by Donaldson and Devlin (1996)
Estrogen receptor	Sterilization	Genbank cited by Donaldson and Devlin (1996)
Gonadotropin releasing hormone	Delayed maturation	Genbank cited by Donaldson and Devlin (1996)
Gonadotropin subunits	Delayed maturation	Genbank cited by Donaldson and Devlin (1996)
Protamine	Sterilize males	Genbank cited by Donaldson and Devlin (1996)
Steroid 17-α mono-oxygenase	Block steriodogenesis	Genbank cited by Donaldson and Devlin (1996)
Vitellogenin	Sterilize females	Genbank cited by Donaldson and Devlin (1996)
Zebrafish, bone morphogenetic protein	Disrupt embryonic development ("sterile feral" technology)	Thresher et al. (1999)
Growth-regulating genes such as growth hormone I and II and insulin-like growth factor	Block to disrupt normal development	Genbank cited by Donaldson and Devlin (1996)

that could be targeted. Below we introduce different strategies involving transgenes for disrupting a given essential gene. Some of these strategies are still being developed conceptually, while others are being tested experimentally on insects and fish. Stocking of the transgenic fish would be necessary for each strategy although at different magnitudes and frequencies to be determined experimentally and via simulation modeling.

Sex ratio distortion

Sex ratio distortion involves spreading a transgene designed to alter the target population's sex ratio. For example, researchers at CSIRO are developing a "daughterless gene" construct which consists of a promoter that activates the daughterless gene to express only in females (Anonymous 2002; Thresher et al. 2002). Activated during early development, the gene inhibits production of aromatase, the key enzyme necessary for female development, and the fish defaults to a male. The daughterless gene encodes a piece of interference RNA (RNAi) that binds to the fish's native gene for aromatase, consequentially blocking synthesis of this enzyme. Releases of transgenic fish possessing a daughterless gene in appropriate quantities over time could drastically reduce a population's size or possibly eliminate it altogether.

Engineered underdominance

Engineered underdominance involves release of transgenic fish carrying 2 mutually suppressive transgenic constructs (each construct contains a unique lethal gene and a suppressor gene that prevents expression of the lethal gene on the other construct). After mating with targeted pest fish, 50% of the offspring die because they inherit only 1 transgenic construct and its lethal gene is now expressed. Another 25% of the offspring are also transgenic but survive because they inherit the 2 transgenic constructs. The deletion of 50% of offspring from such matings continues in every generation in which transgenic fish possessing both alleles mate with wild-type individuals. Davis et al. (2001) suggest that depending on environmental conditions, the proper level of transgene integration could be maintained in a population via periodic release of transgenic fish at 3% of the native population size.

Conditional lethality

Conditional lethality involves designing transgenes that are lethal only when transgenic individuals are exposed to specific environmental conditions. For example, the 'inducible fatality gene' concept involves the spread of a gene designed to induce death once a particular compound is released into the environment or fed to the fish (Grewe 1996). Only those fish possessing the deleterious transgene die, without harm to fish not carrying the transgene. It could be difficult, however to make this strategy work in a natural environment.

Engineered female-specific lethal

Engineered female-specific lethal involves interrupting an aspect of development leading to death of female offspring. It could be accomplished by targeting or silencing an important female-specific developmental pathway or it could involve expression of a toxin such as ricin (Peter Grewe, CSIRO Pest Marine Control Group, personal communication, May 2004) in female offspring. Males which carry but are unaffected by the lethal construct, pass it on as they mate with wild-type females. Schliekelman and Gould (2000) and Gould and Schliekelman (2004) suggest that release of males with multiple copies of the lethal construct is more effective than a release of similar size with a single copy or a similar-sized release of sterile males.

Engineered fitness disadvantage

Engineered fitness disadvantage can be achieved via at least two theoretical strategies: use of selfish genes and use of intentional Trojan genes.

Selfish genes are genes which naturally gain a transmission advantage relative to other components of an individual's genome. One type of selfish genetic element, homing endonuclease genes (HEGs), codes for sequence-specific endonuclease –a protein which cleaves DNA. The catalytic activity of genes such as HEGs has been characterized as super-Mendelian (Koufopanou et al. 2002) and has potentially played an important role in eukaryotic evolution and extinction (Hurst and Werren 2001). Burt (2003) suggests that the power of site-specific selfish genes to copy themselves into a defined target DNA sequence may be able to be engineered and harnessed to eradicate a target population.

The use of *intentional Trojan genes* (Muir and Howard 1999; Howard et al. 2004) involves insertion of a novel gene construct which simultaneously confers one advantage, such as a mating advantage, that drives the transgene into the target population and one disadvantage, such as reduced offspring viability, that triggers decline in number of fish. Analysis of Muir and Howard's research suggests that this approach needs to involve use of a transgenic construct that will ensure: 1) the proper balance between advantage and disadvantage; and 2) the stability of the Trojan gene effect over enough generations of fish reproduction to achieve the desired level of biocontrol.

Methods for developing a transgenic fish

All transgenic strategies for genetic biocontrol face a set of obstacles to getting transgenes stably integrated into the fish genome. Major methods for integrating transgenes include various types of microinjection and can be facilitated by use of retroviral vectors and transposons (Hew and Fletcher 1992; Hackett 1993; Donaldson and Devlin 1996; Houdebine 1996). Gene transfer to fertilized eggs can also be achieved by immersing eggs in a buffer solution containing foreign DNA and applying electric pulses. This technique, called electroporation, is being used with increasing success and has advantages over injection techniques because its feasibility is not limited by egg size or quantity (Inoue and Yamashita 1996). While microinjection and electroporation of newly-fertilized eggs have been used and refined since the 1980s, it remains difficult to use these methods for inserting transgenes into the genomes of live-bearing fish species due to their lack of externally released eggs.

Several lines of ongoing research are trying to develop other reliable ways of getting transgenes into fish genomes. Key examples include: genetic engineering of embryonic stem (ES) cell lines as a

pathway to integrate novel DNA into the genome of a fish embryo (Collodi 2003); in vitro genetic engineering of sperm followed by fertilizing eggs with the transgenic sperm (Kurita et al. 2004); and the generation of live transgenic fry from transplantation of transgenic primordial germ cells into the peritoneum of parental fish (Takeuchi et al. 2003). Any of these approaches could eventually facilitate development of a transgenic fish for biological control and might lead to more stable transgene integration. All of these lines of research will likely need 5-10 years of further research to reach practical application. Additionally, as described above in the section on transgenic sterilization, researchers are developing the capability to induce or repress expression of a target gene by using RNAi technology. This technology offers endless options for controlling the expression of both introduced and endogenous genes, a very interesting tool for genetic-based biological control.

Strengths, weaknesses and other considerations

Each transgenic strategy of deleterious gene spread has strengths and weaknesses based on its current status of development and function. We highlight main points below and provide a detailed list of strengths, weaknesses and other considerations for each strategy in Table 2.3.

Overall, the strengths associated with these transgenic strategies for reducing non-native fish populations include the novelty of a unique population-level approach and the potential to reduce populations over time at an efficiency not possible with traditional physical or chemical approaches. There could be opportunities for combining different strategies to make the deleterious gene spread even more effective such as use of RNAi techniques to build in a repressible capability or use of a mating advantage to drive transgenes into the population faster.

Weaknesses of all modes of transgenic deleterious gene spread include the preliminary status of the technology and the very high costs and long-term commitment associated with research and development, demonstrating sufficient efficacy and safety, and obtaining regulatory approval and implementation.[2] Another weakness of all transgenic methods is that one must expect some degree of instability in expression of the deleterious genes after transgenic fish cross with target fish (Davis et al. 2001). Generally, as transgene instability increases, efficacy for biological control would decrease while increasing the number of undesired, non-native fish in the natural water body. Some evidence suggests that insertion of transgenes at multiple sites improves integration and expression rates (Grewe 1996; Davis et al. 1999). Embryonic stem cell mediated gene transfer may offer a more reliable method for stable transgene integration and expression. This technology, however, is still in preliminary stages of development, and will likely take longer to develop than other transgenic techniques. Effectiveness of biological control could also be reduced by leakiness of promoters driving expression of the deleterious transgenes, as well as natural selection against transgenic individuals. In daughterless transgenic lines, for example, natural selection could go against the lack of females.

Some important ecological questions related to the effectiveness and risks of each kind of deleterious transgenic strategy remain and would need to be further researched. Examples of some of these questions include: How many transgenic fish would have to be released and at what frequency and spatial distribution? Would transgenic fish show heightened predation on or competition with wild, non-target native fish species? If so, would this raise ecological risks to a level that outweighs the benefits of the intended biological control? What risks are associated with a transgenic fish escape to areas of its native distribution and how could these risks be managed? Would there be significant risks to human health if a transgenic fish was caught and eaten? For further discussion of the need to assess potential risks, see chapter 3.

[2]See the related discussion in chapter 6 on Preliminary Roadmap of Programmatic Activities and General Cost Estimates

Purposefully releasing transgenic fish in order to spread deleterious transgenes into wild-type populations could precipitate social opposition and ultimately lead to regulatory disapproval. This could be stimulated, in part, by incomplete understanding of the environmental risks involved, impossibility of recalling transgenes after deployment (if unforeseen problems arise) and diverse attitudes of influential stakeholders and the general public towards genetically engineered organisms in general. Important considerations for addressing these concerns are presented in chapters 4 and 5.

Table 2.3. Overview of deleterious gene spread strategies for biological control of non-native fish.[3]

Method	Strengths	Weaknesses	Other Ecological, Social, and Regulatory Considerations
Sex Ratio Distortion: transgene disrupts key step in sexual development. 'Daughterless gene' best developed so far: disrupts expression of aromatase enzyme to produce all-male offspring.	1. Lab research furthest along of all transgenic biocontrol strategies for fish. 2. Less likely, compared to sterile-triploids, to disrupt mating behavior required to achieve biological control. 3. Potentially better transgene spread, thus better biological control, than female-specific lethality. 4. When method involves interference RNA, resulting transgenic fish would likely be safe for predators and humans to eat—but need rigorous testing to confirm.	1. Expensive (e.g., $15-20 million) to develop to point of showing adequate efficacy and safety, and seek regulatory approvals. 2. Effectiveness could be reduced by gene silencing, leakiness of promoters, or natural selection against transgenic individuals. 3. Could take long time to achieve desired level of biological control (e.g., 50-100 years preliminary estimate for common carp control in Australia). 4. Might require indefinite release of transgenic fish, depending on level of desired control and case-specific biology and ecology.	1. Ability to fully evaluate effectiveness and risks requires robust quantitative models of target species population dynamics and ecology that incorporate spatial distribution, stochasticity and uncertainty. 2. Likely less socially acceptable than non-genetic engineering methods. 3. Major investment in community awareness and involvement needed to gain public understanding and support. 4. Complex, uncertain and potentially high cost regulatory requirements. 5. Difficult to remove all transgenic fish from natural populations if unforeseen problems emerge. 6. Do sex-ratio distortion genes reduce fitness, thus reducing biocontrol? 7. Do sex ratio distortion genes increase aggression or competition with non-target fish species?

[3]Table entries based mostly on Davis et al. (2001), Muir and Howard (2002), Burt (2003), Gould and Schliekelman (2004) and personal communications with scientists at CSIRO Marine Pest Control group (Ron Thresher, Nic Bax, Peter Grewe, Keith Hayes, and Jawahar Patil), May 2004.

Table 2.3. *Continued.*

Method	Strengths	Weaknesses	Other Ecological, Social, and Regulatory Considerations
Engineered Underdominance: release transgenic fish carrying 2 mutually suppressive but individually lethal transgenic constructs. Mating with target fish kills off 50% of offspring inheriting only 1 transgene, while 25% inheriting both transgenes further spread the effect.	1. Accelerates spread of the deleterious transgenic construct, compared to other transgenic strategies. 2. Can discontinue release of transgenic fish once frequency of transgenic alleles attains a relatively low threshold. 3. Should be effective under realistic population conditions (overlapping generations, non-random mating, mild barriers to gene flow). 4. May be relatively insensitive to density-dependence (N. Bax, CSIRO, personal communication 2004). 5. Shows good biological control potential which deserves being explored (Gould and Schliekelman 2004)	1. See weaknesses 1-2 for sex ratio distortion. 2. Difficult to produce appropriate transgene constructs due to leakiness of promoters and incomplete action of suppressors. 3. Effect of spatial dynamics unclear; certain conditions could prevent wide spatial spread.	1. See considerations 1-5 for sex-ratio distortion.
Conditional Lethality: transgenic individuals die only when exposed to specific environmental conditions.	1. Transgenic fish with multiple copies of conditional lethal gene could enhance biocontrol; optimal number of insertions affected by characteristics of transgene, release population, target population and release strategy (Schliekelman and Gould 2000). 2. Offers ability to purge environment completely of transgenic individuals, assuming the construct works as intended.	1. See weaknesses 1-2 for sex ratio distortion. 2. Effectiveness may be reduced as fitness cost associated with conditional lethal alleles increases. 3. Could be difficult to make this work given spatial and temporal variability in natural environments. 4. Fish resistant to the lethality gene could persist in the population (Muir and Howard 2004). 5. Additional limitations not yet fully understood.	1. See considerations 1-5 for sex-ratio distortion. 2. Will lethal-inducing compound be effective under local water conditions? 3. Mass fish die-off could be socially objectionable and may adversely affect other species through disease, etc. (Muir and Howard 2004).

Table 2.3. *Continued.*

Method	Strengths	Weaknesses	Other Ecological, Social, and Regulatory Considerations
Engineered Female-Specific Lethal: release of a transgenic male carrying a female-specific lethal construct which is passed on during matings with wild-type females. Male offspring survive to continue passing on the lethal construct.	1. Release of transgenic males carrying multiple female-specific lethal genes could be more effective than sterile male release (assuming no density dependence and no reduced fitness to males). 2. Reduces population by killing females, while at the same time passing lethality to future generations.	1. See weaknesses 1-2 for sex ratio distortion. 2. If presence of lethal gene reduces male fitness, it may reduce effectiveness of strategy. 3. Maintenance of pre-release population could be difficult due to female lethality, but could be addressed by linking the lethal construct to a repressible promoter.	1. See considerations 1-5 for sex-ratio distortion. 2. Use of a toxin such as ricin may be unacceptable given public perception of potential harm to human health.
Engineered Fitness Disadvantage - selfish gene: release of transgenic fish with transgene engineered to copy itself into a species' DNA sequence- could be used to reduce fitness of target population.	1. Accelerates spread of deleterious transgenic construct compared to other transgenic strategies. 2. Only a few individuals would need to be released to eradicate a population in less than 20 generations (Burt 2003). 3. Offers prospect of enhanced transgene stability and reversibility via release of additional alleles. 4. Could be used as a mechanism to induce sex ratio distortion, female specific lethality, or sterility.	1. See weaknesses 1-2 for sex ratio distortion. 2. Least developed of all transgenic biocontrol strategies. 3. Consider potential for evolution of resistance to the selfish gene.	1. See considerations 1-5 for sex-ratio distortion.
Engineered Fitness Disadvantage - intentional Trojan gene: release of transgenic fish with an advantage such as a mating advantage, which allows spread throughout population, but also confers a disadvantage such as reduced offspring viability.	1. Mating advantage may serve to spread genes through population faster than other approaches via induced selection for transgenic individuals.	1. See weaknesses 1-2 for sex ratio distortion. 2. Must have proper balance between advantage and disadvantage for desired effect to occur.	1. See considerations 1-5 for sex-ratio distortion.

Chapter 3

Addressing Ecological and Human Health Risks

Introduction

Current scientific understanding indicates that ecological risk or safety of genetically engineered fish is case-specific and depends on the genes inserted, the altered traits expressed in the modified fish, the intended use of the fish and the characteristics of the accessible ecosystems (Kapuscinski and Hallerman 1991; Scientists' Working Group on Biosafety 1998; NRC 2002, Pew Initiative on Food and Biotechnology 2003; NRC 2004). The environmental release of a specific genetically engineered fish line, thus, could pose lesser, equal or higher risk to native fish biodiversity than release of traditionally bred or wild-type fish of the same species. Although one might expect the release of triploid sterilized fish to pose less risk to native fish than release of fertile traditionally bred or wild-type fish, such proposals should also be scrutinized for possible ecological risks (ABRAC 1995; Scientists' Working Group on Biosafety 1998). Ecological risk assessment and management (also called environmental biosafety assessment and management) requires identifying hazards, analyzing risks, managing the major estimated risks, and monitoring (post-release) for unforeseen problems, on a case by case basis (Kapuscinski 2002; NRC 2002a; NRC 2004; Kapuscinski 2005).

This chapter introduces the major steps in risk assessment and management, including the importance of addressing scientific uncertainty, and then presents a preliminary list of the potential hazards related to use of transgenic or triploid fish for biological control in the Gila River basin. The chapter does not present a complete risk assessment. Rather, it outlines a general approach for conducting a full risk assessment in the future. Chapter six further discusses how to integrate the conduct of a risk assessment into a seven-part research and development program on genetic biocontrol of non-native fish. Such integration is absolutely essential for the risk assessment to utilize empirical data gathered from pivotal lab, field and modeling studies.

Risk Assessment and Management

Ecological risk assessment and management of transgenic or ploidy-manipulated fish for genetic biocontrol requires integration of methods and knowledge from multiple fields such as genetics, physiology, evolutionary biology, population biology and ecology, community ecology, ecosystem ecology, and system safety science. There is a long-standing body of principles and processes of risk assessment and risk management, developed for many mature technologies, to assess and verify the safety of various technologies (NRC 1996; Aldrich 1997; McIntyre 2000; Amendola 2001; Kapuscinski et al. 2003). Ecological risk assessment and management of proposed genetic biocontrol of non-native fish should follow a similar systematic process of distinct steps that build upon each other and lead to verifiable conclusions of safety or risk.

Major steps

Table 3.1 summarizes the systematic steps in risk assessment and management. Risk assessment involves hazard identification and risk analysis; risk analysis includes estimating exposure to and likelihood of the hazard, risk of harm given exposure and severity of harm. Risk assessment should also involve evaluating how well established is the knowledge used for each of these steps. Deciding what constitutes an environmental harm and assessing the severity of potential harms may involve economic and social, as well as biological, considerations. The steps in risk management include risk reduction, risk monitoring and remedial

Table 3.1. Systematic steps in risk assessment and management. (From NRC 2004 and Kapuscinski 2005 as adapted from Kapuscinski 2002).

Step	Key Questions
RISK ASSESSMENT	
Hazard identification	What event posing harmful consequences could occur?
Risk analysis	Estimate hazard exposure: how likely is the hazard?
	What would be the harms from realization of the hazard, and how severe are they, taking into account social values?
	How likely is the harm, given hazard exposure?
	Compile quantitative risk assessment conclusions, for instance, as a matrix of risk (likelihood of harm) plotted against severity of harm? Each cell of the matrix should be accompanied by a qualitative assessment of the response and a quantification of assurance needed to reduce harm if the cell's conditions were to occur.
	How well established is the knowledge used to identify the hazard, estimate its risk, and predict harms?
RISK MANAGEMENT	
Risk reduction planning and implementation	What can be done (including bioconfinement and other confinement) to reduce risk, either by reducing the likelihood or mitigating the potential harms? Are there steps that can be taken to prepare for remediation?
Risk tracking (monitoring)	How effective are the implemented measures for risk reduction?
	Are they as good as, better than, or worse than planned?
	What follow-up, corrective action or intervention will be pursued if findings are unacceptable?
	Did the intervention adequately resolve the concern?
Remedial action	What remedial action should be taken? What assurance is there that the action itself will not cause another environmental problem?
RISK COMMUNICATION	
Transparency and public participation	How transparent should the entire process be? How much and what type of participation should there be in all steps above by the public at large, by experts, and by interested and affected parties?

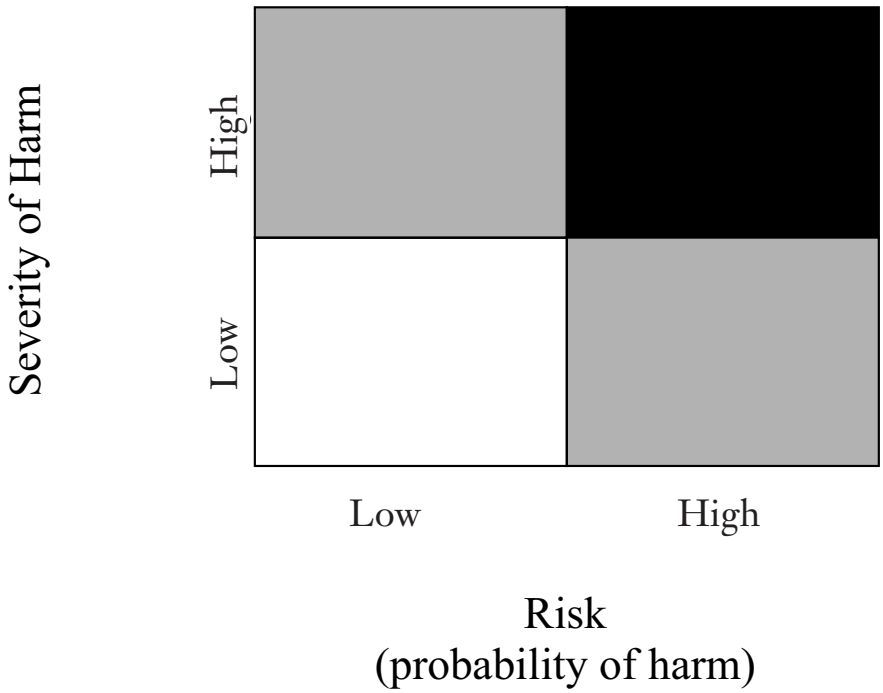


Figure 3.1. Schematic of a risk assessment matrix. Hazards of greatest concern are those with high probability of occurrence and high severity of consequences (black area). Social and economic considerations will influence the priority given to hazards in the gray areas. Adapted from Miller et al. (2004).

action. Finally, considerations of transparent decision making and public participation apply to all steps in risk assessment and management (discussed in more detail in chapter 5).

We can summarize the results of conducting a risk assessment as a matrix of risk (probability of harm) plotted against severity of harm. Figure 3.1 depicts a simplified risk assessment matrix (NRC 2004; Miller et al. 2004). Depending on the quality of available information, the axes of a real risk matrix could consist of continuous values or more discrete categories. Social, economic and ecological considerations influence the estimation of environmental harm and its severity.

Status of science for risk assessment and risk management

Presently, environmental biosafety science is not very well equipped to reliably predict the ecological effects of different kinds of transgenic fish. Virtually no effort has yet been made to predict ecological effects of transgenic fish that would be released into the environment for biological control purposes. The focus instead has been on assessing effects of unintentionally escaped fish that would be farmed in contained or confined aquaculture systems. In risk assessment, the science is best developed to identify hazards; lacks a confirmed methodology to estimate exposure to the hazard; and is least developed for assessing risk and severity of harm, given exposure to the hazard (Kapuscinski 2005). In risk management, discussions have focused on reducing risk via a mix of confinement methods and largely ignored planning for risk monitoring and remedial action (Kapuscinski 2005). Recent scientific reviews of the status of methods and information for assessing ecological risks of transgenic fish appear in Kapuscinski (2005, 2005a), NRC (2002a) and NRC (2004). The status of methods and information for assessing human health effects of transgenic fish is reviewed in a report from an expert consultation convened by the Food and Agriculture Organization

and the World Health Organization (FAO/WHO 2004); and some relevant material also appears in a recent scientific review on assessing the safety of genetically engineered foods (NRC 2004a).

Scientists are much better equipped to assess the ecological risks posed by intentional releases of triploid sterilized fish. The knowledge base is more solid because this biocontrol approach raises fewer potential ecological hazards. Also, there is a larger database of relevant information on the traits of chromosome-manipulated fish. Recent scientific reviews of methods and issues to consider in a risk assessment include reports by the Scientists' Working Group on Biosafety (1998) and the National Research Council (NRC 2004).

Uncertainty

Despite efforts to account for as many potentially relevant hazards and resulting harms as possible in both quantitative and qualitative terms, uncertainties exist within the risk assessment process (NRC 1996) and must be taken into account. The two general categories of uncertainty as described by Burgman (2005) are epistemic uncertainty and linguistic uncertainty. Epistemic uncertainty reflects incomplete knowledge as a result of measurement error, systematic error, natural variation, model uncertainty and subjective judgment; while, linguistic uncertainty reflects differences in language and can involve vagueness, context dependence, ambiguity, indeterminacy, and underspecificity. Uncertainties can also arise from lack of knowledge about risk-generating processes (NRC 2004). Thus, a proper risk assessment should include an analysis (identification and characterization) and explicit disclosure of all identified uncertainties.

A variety of approaches exist for identifying, characterizing and reducing uncertainties involved in risk assessment (Burgman 2005). One example involves addressing uncertainties by building stochasticity, often through use of Bayesian analytical methods (Malakoff 1999), into existing predictive models. Including uncertainty analysis in risk assessment can reduce chances of making type I or type II statistical errors. The risk assessment should include a publicly transparent process in which information is gathered from and made available to interested and affected parties.

Type I statistical errors are ones that conclude an action caused an adverse effect when, in fact, it did not. Type II errors conclude that an action did not cause an adverse effect when, in fact, it did. For instance, a type I error would occur if one decides not to release a transgenic fish for biological control because of predicted (but unrealized) risks; while a type II error would occur if one decides to release a transgenic fish and this leads to ecological harms that were not predicted. Although uncertainty exists to some degree in all fish management decision making, any future proposal to control non-native fish by releasing transgenic fish or triploid sterilized fish should involve a comprehensive risk assessment that includes uncertainty analysis to minimize type II error. It may be more important to minimize type II error than type I error in situations where unforeseen but realized environmental harms are impossible to reverse or technically difficult and expensive to remediate (Dayton 1998; Scientists' Working Group on Biosafety 1998; NRC 2004). Any future proposal to control non-native fish via release of transgenic or triploid sterilized fish should also assess the public's level of acceptability of type II versus type I error with methodologies from the social sciences (Susskind et al. 2000; Wondolleck and Yaffee 2000; Nelson et al. 2004). It is important to realize, however, that 'acceptability' is a social decision that cannot be dictated by science itself.

In fact, public understanding and acceptance of uncertainty is more complex than one might think. A recent study on public perceptions of agricultural biotechnologies in Europe (Marris et al. 2001) showed that participants did not express strong opinions for or against agricultural GMOs. Instead, their responses showed a more sophisticated understanding that both benefits and risks exist. Participants did not ask decision-makers to assure 'zero risk' or full certainty with respect to the

impacts of agricultural GMOs. They recognized that science could never fully predict all impacts of a new technology and felt that these inherent uncertainties should be acknowledged by experts and used to inform public decisions. Further, they considered the denial of uncertainty by experts and institutions to be disconcerting and untrustworthy. Two critically important lessons from this study relevant to genetic biocontrol of invasive fish are that: (1) experts and managers should not assume that they fully understand the perceptions of stakeholders and the public toward release of a genetically engineered fish for biological control and (2) they should make every effort to explicitly disclose uncertainties to inform decision making (see further discussion in chapter 5 on Community Awareness and Involvement).

Preliminary Hazard Identification for Genetic Biocontrol in Gila River Basin

We have taken a first look at identifying the potential ecological and human health hazards posed by the potential release of transgenic or triploid-sterilized fish for genetic biocontrol in the Gila River basin. Table 3.2 and the discussion below do not constitute either a full hazard identification or a full risk assessment. If agencies decide to go forward with a genetic biocontrol program, they will need to carry out a full hazard identification process employing one or a variety of recognized hazard identification techniques (Hayes 2002, 2002a; Hayes et al. 2004; Burgman 2005) followed by all the other steps in risk assessment (Table 3.1). Risk assessment, risk management, and risk communication would need to be conducted as an iterative process to identify and respond to new hazards that may develop over the life of the project. Post-release monitoring and communication with the public are important components necessary to inform decision making. Chapter 5 discusses risk communication as part of multi-stakeholder deliberations and introduces a promising process for such deliberations.

The discussion below addresses potential hazards posed by a fish genetic biocontrol program in the order of decreasing likelihood based on a preliminary and rough qualitative assessment. A formal risk assessment should reconsider the relative likelihoods of each of these hazards.

Density-dependent compensation

Density-dependent compensation refers to the ability of certain fish populations to increase their numbers, via improved survival or reproduction, in response to some external factor that decreases the numbers of individuals in a certain segment of the population. Such a density-dependent population increase could continue for an extended period of time, until the cumulative effect of the external factor reaches a critical point in decreasing a segment of the population–the point at which this decrease is sufficient to counterbalance the compensatory effect and thus sufficient to achieve a lasting decrease in population size. If the target non-native fish population exhibited such a compensatory response to an introduction of a transgenic or triploid fish for biological control, this could greatly increase the risk of additional harm to and potential extinction of the native species before the target population reaches this critical point of steady decline.

Failure of intended trait change

The complexity of natural aquatic systems makes it difficult to develop reliable predictions of the behavior and other traits of transgenic or triploid fish after they are released for biological control purposes. If these released fish do not perform as intended due to failure to exhibit the intended trait in natural ecosystems, then their release would simply add more non-native fish to the system. This would likely pose additional harm to the native species.

Transgene side effect on trait that enhances predation or alters another non-target behavior

In some cases, insertion of a novel gene construct into a fish genome may alter other traits beyond the intended trait change. This "side effect" phenomenon is called pleiotropy and refers to a single gene which affects a number of seemingly unrelated traits. A released transgenic fish expressing a "side effect" of enhanced predation or competition for example, could inflict additional harm to the native fish before the biological control effect prevails. Unintended pleiotropic effects have already been found in other existing transgenic fish lines (e.g., Howard et al. 2004).

Pest replacement

Sometimes when one pest population is removed from or depressed within a system, another pest population can take its place. This sort of "pest replacement", as described by Elher (2000), should be expected when the non-target pest is without natural enemies and is being held in check by the target species. One example of this is the invasion of reed canary grass after purple loosestrife is removed from an area via biological control with beetles (Thompson et al. 1987). If a transgenic or triploid fish was used to successfully remove the target non-native fish, the level of interspecific competition between the two species would be an important factor in determining whether "pest replacement" would occur (George Heimpel, University of Minnesota, personal communication, November 2004). If released from competition, the other pest could pose additional harm to native species.

Transgene spread to native range of species

Escape of a transgenic fish released for biological control in the Gila River basin to its native range could lead to transgene spread beyond the target population and possibly inflict harm to the fish within its native range. This is a serious concern because of the high frequency of human-facilitated and natural movement of aquatic species among systems (OTA 1993; ABRAC 1995) and the relative close proximity of many non-native species in the Gila River system to their native distributions. Three non-native species in the Gila River which have been discussed as possible targets for biological control: mosquitofish (*Gambusia affinis*), red shiner (*Cyprinella lutrensis*), and green sunfish (*Lepomis cyanellus*) have native distributions in adjacent states. See Appendix 2 for further detail on proximity of non-native fish in the Gila River to their native ranges and suggested considerations for further evaluation of this hazard.

Transgene spread to closely related species by hybridization

Many species hybridize with closely related species, raising the possibility of transgene spread to closely related non-target species both within and outside of the Gila River basin. The magnitude of this hazard and resulting harms is related to the proximity of closely related species, the hybridization potential of the transgenic fish with those species, and the viability and fertility of any offspring that are produced. Of the three species mentioned above as potential targets for biological control, the red shiner (family Cyprinidae) and the mosquitofish (family Poeciliidae) are from the same family as one or more Gila River native fish. See Appendix 2 for further detail on the hybridization potential of these species and suggested considerations for further evaluation of this hazard.

Transgene spread to fish caught for eating

If a transgenic fish was caught for human consumption and it expressed some level of toxicity or allergenicity –beyond that normally found in the tissues of wild-type fish –it could present a human health hazard. Upon initial review, the likelihood of harm from this hazard seems slim; however, as with all other identified hazards, this must be fully evaluated as part of a comprehensive risk assessment.

Analysis of this harm would likely involve food safety tests including: characterization of the novel transgenic construct and the protein produced; and an allergenicity and toxicity evaluation (FAO/WHO 2004). See Appendix 6 for further information on considerations and costs related to conducting food safety assessments.

Horizontal gene transfer to non-target species

Horizontal gene transfer refers to non-reproductive movements of genetic sequences consisting of small pieces of DNA, possibly via ingestion, to non-target species (Syvanen 1994). It is more likely a concern for genetically engineered microorganisms, such as bacteria, than it is for fish. Although current knowledge suggests that transgenic fish for biocontrol are unlikely to pose this hazard, a full risk assessment should nonetheless explicitly consider this hazard.

Table 3.2. Preliminary identification of hazards associated with the use of transgenic or ploidy manipulated fish for genetic biocontrol. Hazards listed in order of roughly estimated decreasing likelihood, based on initial and incomplete brainstorming. Shaded cells list hazards that apply only to transgenic fish.

Decreasing likelihood (in general)

Hazard	Potential Harm
Density-dependent compensation for X years	Wipe out endangered fish before biocontrol effect prevails
Failure in intended trait change	Increased number of fit non-natives increases disruption of native fish
Transgene side effect on trait that enhances predation, competition or alters another non-target behavior	Increases disruption of native fish before biocontrol effect prevails
Pest replacement, once the target species is removed	Another pest species may be released from competition/predation and become a greater pest to native fish
Transgene spread to native range of species	Depress or extirpate native populations
Transgene spread to closely related species via hybridzation	Harm to non-target species and communities
Transgene spread to fish caught for eating	Harm to human health
Horizontal gene transfer to non-target species	Depress populations of non-target species

Chapter 4
Policy and Regulatory Considerations

Introduction

The goal of this chapter is to help anticipate some of the important regulatory issues that might arise from potential future implementation of genetic methods to biologically control non-native fish species in the Gila River basin. It is helpful to consider these legal and regulatory issues within the larger context of public policy. This chapter first reviews relevant aspects of U.S. federal environmental, biotechnology, and invasive species policy. It then looks more closely at specific regulatory issues related to: (1) research and development of triploid sterilized and transgenic fish for biocontrol, and (2) release of triploid sterilized and transgenic fish into the environment. Where appropriate, the discussion focuses on relevant jurisdictional levels: federal, state, tribal, and international. The chapter does not address legal issues of liability and redress if post-release events result in environmental damage or harm to human health. This chapter should be treated as an initial scoping document for more in-depth legal analysis by appropriate legal experts if and when any party decides to move forward with a program of genetic biocontrol of non-native fish.

Federal Environmental Policy

Two statutes have played a key role in shaping U.S. national policy regarding the environment and protection of biodiversity: (1) the National Environmental Policy Act of 1969 (NEPA), and (2) the Endangered Species Act of 1973 (ESA). National policy under NEPA is to create and maintain conditions under which man and nature can exist in productive harmony. 42 U.S.C. § 4331(b). NEPA requires each federal agency to incorporate this overarching policy within its planning and decision-making process and to consult with other federal agencies having jurisdiction or special expertise with respect to the environmental impact of proposed major federal projects.

National policy under the ESA is that all federal agencies should seek to conserve endangered and threatened species. 16 U.S.C. § 1531(c)(1). Section 7 of the Act requires federal agencies contemplating projects in an area containing listed species to consult and take steps to prevent jeopardy to the species or harm to its habitat. The rationale for any future proposal for genetic biocontrol of non-native fish could be the mandate of the ESA to conserve threatened and endangered species. Scrutiny of such a proposal should address whether the genetic biocontrol could unintentionally increase jeopardy to potentially affected listed species and their habitats (see chapter 3).

Federal Biotechnology Policy

The framework for federal regulation of biotechnology is set forth in the 1986 Coordinated Framework for Regulation of Biotechnology. 51 Fed. Reg. 23302. The policy question underlying the Coordinated Framework was "whether the regulatory framework that pertained to products developed by traditional genetic manipulation techniques was adequate for products obtained with the new techniques." The Administration at that time decided that existing laws were adequate to regulate products developed through new techniques of genetic recombination (also called genetic engineering).

To date, under the Coordinated Framework, lead jurisdiction for products developed through genetic engineering has been distributed among four agencies: (1) the Food and Drug Administration and (2) the Occupational Safety and Health Administration, both within the Department of Health and Human Services; (3) the Department of Agriculture, via its Biotechnology Regulatory Service within the Animal Plant Health Inspection Service, and (4) the Environmental Protection Agency.

Where oversight responsibility for a particular product falls to more than one agency, the policy establishes a lead regulatory agency. It remains unclear, however, which agency under the Coordinated Framework would have primary regulatory responsibility for applications of transgenic fish for biocontrol purposes, as discussed further below.

Federal Invasive Species Policy

The two main sources of federal invasive species policy have been: (1) the 1990 Nonindigenous Aquatic Nuisance Prevention and Control Act (NANPCA) which established the Aquatic Nuisance Species Task Force and (2) President Clinton's 1999 Executive Order 13112 which established the National Invasive Species Council.

Aquatic Nuisance Species Task Force

A national policy toward aquatic invasive species first emerged in the late 1980s in response to the unintentional introduction of the zebra mussel into the Great Lakes through discharge of ballast water. In 1990, Congress passed the Nonindigenous Aquatic Nuisance Prevention and Control Act (NANPCA). 16 U.S.C. §§ 4701-4741. The Act set up an Aquatic Nuisance Species Task Force and charged it with developing a broad program to prevent introductions of aquatic nuisance species.

Section 1207 of NANPCA addresses intentional introductions of non-indigenous species. It requires the Task Force, in consultation with state fish and wildlife agencies, to review current policies toward intentional introductions and identify approaches for reducing the risk of adverse consequences. Congress required the Task Force to report their findings and recommendations for addressing the problem. The report, produced in 1994, provides one of the foundational national policy documents regarding intentional introductions of nonindigenous species (Aquatic Nuisance Species Task Force 1994).

In 1996, NANPCA was reauthorized and amended by passage of the National Invasive Species Act (NISA). 16 U.S.C. §§ 4701-4751. The NISA established a Western Regional Panel to report to the Task Force on activities among western states relating to aquatic nuisance species prevention, research, and control. In addition, NISA authorized state governors to submit to the Task Force a state management plan for environmentally sound prevention and control of aquatic nuisance species. A team of researchers has drafted such a management plan for Arizona setting forth recommendations for dealing with invasive non-native fish (Fitzsimmons 2002). On April 1, 2005, the Governor of Arizona issued Executive Order 2005-09 announcing the establishment of the Arizona Invasive Species Advisory Council and charging it with submitting "recommendations to the Governor for a statewide invasive species strategic plan by June 30, 2006" (Napolitano 2005). Implementing a comprehensive state plan would help to provide an integrated management approach under which relevant parties could assess whether and how to pursue genetic biocontrol of an invasive fish species in the Gila River basin.

National Invasive Species Council

In addition to the Aquatic Nuisance Species Task Force, the other major federal policy-making body dealing with invasive species issues is the National Invasive Species Council. This interdepartmental council, created in 1999 by President Clinton's Executive Order 13112, has issued a National Management Plan (National Invasive Species Council 2001). The Plan contains probably the closest approximation to a national policy on invasive species.

An effective management strategy to control non-native fish in the Gila River basin should embody a few key recommendations, based on the National Management Plan:

1. Use interdisciplinary research to develop and apply technologies, and incorporate these advances into management and policy decision-making.

2. Strive for control methods that are socially, culturally, and ethically acceptable and that provide the desired effect while minimizing the negative impact on the environment.

3. The United States should aim to provide global leadership in managing invasive species and sharing information and technologies.

This report outlines an approach consistent with these recommendations. In chapter 6, we recommend a coordinated program that would integrate development and risk analysis of genetic biocontrol methods, multi-stakeholder deliberations at key steps in an entire program, and information exchange of various kinds.

Regulatory Framework

A different set of laws and regulations apply to each of two stages in the development and use of genetically modified fish as biocontrol agents, including: (I) research and development of a genetically modified fish; and (II) introduction of a genetically modified fish into the environment. The key feature distinguishing the first and second stages is whether the activities are carried out under confinement conditions or whether they involve release of the genetically modified fish into the environment. Although both sets of activities are constrained by laws and regulations, the legal context shifts significantly when one moves from the laboratory or confined field trials to deliberate introduction into the environment.

Regulation of Research and Development

This subsection examines international agreements, federal and state government laws and regulations and institutional policies and rules regarding: (1) humane care and use of research animals, including fish, and (2) laboratory research involving recombinant DNA molecules and transgenic animals, including fish. A summary of relevant regulations, policies, guidelines and standards is presented in Table 4.1.

Animal care and use

Laws and regulations regarding care and use of animals provide standards for the humane treatment of animals primarily in laboratory but also in field research. These regulations apply whether laboratory procedures involve use of recombinant DNA techniques to produce transgenic fish or use of triploid induction to sterilize fish. There are three concurrent levels of governance: (a) international, (b) federal, (c) university or institutional, in addition to other fish-specific guidelines.

Federal Animal Care and Use Regulations

Federal authority over the use of fish in laboratory research is exercised primarily by the Public Health Service (PHS) of the Department of Health and Human Services and the Department of Agriculture via its voluntary performance standards for genetically modified fish and shellfish. If the Food and Drug Administration decides to assert regulatory authority over transgenic fish for biocontrol uses, a possibility discussed later in this chapter and in Appendix 3, the agency's Good Laboratory Practices for securing regulatory approval would apply.

University and Institutional Guidelines

The *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy), implemented by the Office of Laboratory Animal Welfare at the National Institutes of Health (NIH),

applies to the use of live vertebrate animals in any activity conducted or supported by the Public Health Service, including the NIH and the Food and Drug Administration. This PHS policy on laboratory animals applies both to institutions that receive PHS support for any research—that is, to all research projects at an institution receiving PHS support—as well as individual researchers who receive any such support. Consequently, compliance with this policy is *de facto* mandatory for most animal research in the U.S.A. At the core of the PHS Policy is the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (OSTP 1985), which provide the foundation for humane care and use of laboratory animals in the United States.

Before conducting activities involving animals, an institution must submit an Animal Welfare Assurance document promising to comply with the PHS Policy. The Assurance describes the institution's program for the care and use of animals under the PHS activities, which must follow the *Guide for the Care and Use of Laboratory Animals* (NRC 1996a). The Policy also requires each institution to set up an Institutional Animal Care and Use Committee (IACUC) to oversee the institution's animal program, facilities, and procedures. In addition to their duties under the PHS Policy, research institutions generally have their own policies and rules.

Other Fish-Specific Care and Use Guidelines

The Animal Welfare Information Center at the National Agricultural Library of the U.S. Department of Agriculture maintains a database that includes a wealth of information on fish welfare resources (Animal Welfare Information Center 2003). One of the authoritative guidelines in the database is the American Fishery Society's 2004 *Guidelines for the Use of Fishes in Research* (AFS 2004). A number of the topics covered would apply to work with genetically modified fish for biocontrol, including: statutory requirements and regulatory bodies, animal welfare consideration, activities with wild fishes, and laboratory activities with fishes. The committee of biologists who developed the AFS Guidelines recommended that they be adopted and adapted by state and federal agencies with regulatory responsibilities for fish as well as by universities and research institutions.

Laboratory research involving recombinant DNA molecules and transgenic animals

As with research animal care, multiple layers of regulations and guidelines govern laboratory research involving recombinant DNA (rDNA) molecules and transgenic animals. Most regulations originate at the federal level supplemented by rules at the individual research institutions.

Federal Regulations and Guidelines

There are three main sources of federal guidelines relevant to laboratory research involving recombinant DNA molecules and transgenic animals. They include the National Institutes of Health's *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*); the U.S. Department of Agriculture's (USDA) *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (ABRAC – Agricultural Biotechnology Research Advisory Committee 1995); and the Federal Food Drug and Cosmetic Act, specifically the provisions for investigative new animal drugs.

The NIH Guidelines are applicable both to institutions that receive NIH support for any rDNA research as well as individuals who receive any NIH support. In addition, individuals receiving NIH support for rDNA research must be affiliated with an institution that assumes the responsibilities of implementing the NIH Guidelines (NIH 1986). Compliance with the NIH Guidelines is *de facto* mandatory for most rDNA research in the United States, even though it is technically voluntary. Section IV of the NIH Guidelines describes the roles and responsibilities of institutions conducting

experiments with rDNA molecules (NIH 1986). Each institution is expected to establish an Institutional Biosafety Committee (IBC) and appoint a Biological Safety Officer whose duties include inspecting laboratories and reporting violations of the NIH Guidelines, as well as providing technical advice to researchers. Most U.S. public and private institutions engaged with rDNA research have their own rules that specify duties of their IBC and Biosafety Officer.

Section III of the NIH Guidelines describes the various categories of experiments to which the NIH Guidelines apply, including those intended to produce transgenic animals. The USDA developed the Performance Standards because the animal provisions of the NIH Guidelines were designed with terrestrial organisms in mind and are poorly suited to fish and other aquatic animals. The Performance Standards are designed to fit with requirements and the IBC oversight process of the NIH Guidelines. The Secretary of Agriculture adopted the Performance Standards as voluntary guidance for assessing environmental hazards and implementing corresponding confinement of research receiving USDA funding.

A number of researchers conducting laboratory or confined research pond experiments with transgenic fish have voluntarily applied the Performance Standards to comply with the NIH Guidelines. Specifically, under section III of the NIH Guidelines, the Principal Investigator is responsible for making the initial determination of appropriate physical and biological containment levels. The Performance Standards help to address this responsibility by outlining steps for taking special care in evaluating the appropriate containment conditions given the possibility for production of undesirable traits in the host animal. All transgenic animal experiments require the Principal Investigator to submit a registration document to the IBC, which must be approved before experimentation can begin. The IBC has a responsibility to make an independent assessment of the containment levels required by the NIH Guidelines.

Research on transgenic biocontrol fish might also have to comply with the new animal drug provisions of the Federal Food Drug and Cosmetic Act (FFDCA), for reasons discussed below and in Appendix 3. Specifically, researchers would have to apply for an Investigative New Animal Drug (INAD) Permit if the fish species involved in the research is commonly eaten by humans or incorporated into animal feeds. The FDA Center for Veterinary Medicine sent a letter to Land Grant Universities with research projects on transgenic animals informing them of the applicability of the INAD provisions to such research (CVM 2003). The main concern is to keep transgenic research animals out of the animal feed or human food supplies. For instance, this would apply to any future effort to produce a transgenic fish for biological control that is also caught by anglers for human consumption, such as the green sunfish.

Table 4.1. Anticipated regulations, policies, guidelines and standards related to research and development of triploid sterilized and transgenic fish. Shaded cells only apply to transgenic fish.

Jurisdiction	Regulation, Policy, Guidelines or Standards	Required Compliance Action	Responsible Authority Action
U.S. Federal	Public Health Service Policy on Humane Care and Use of Laboratory Animals	Submit Animal Welfare Assurance Document Establishment of Institutional Animal Care and Use Committee (IACUC)	Affirm compliance with policy
	USDA Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish	Voluntary (unless entity receives grant support from USDA)	n/a
	NIH Guidelines for Research involving Recombinant DNA Molecules	Technically voluntary but de-facto mandatory Establishment of Institutional Biosafety Committee and Biological Safety Officer	Affirm compliance with guidelines
	Federal Food Drug and Cosmetic Act	Investigative New Animal Drug (INAD) application	FDA issuance of permit for INAD for studies under containment
Other	AFS Guidelines for the Use of Fishes in Research	Recommended adoption by federal, state and other institutions	n/a

Regulation of Introduction into the Environment

This section reviews the laws and regulations pertaining to the environmental introduction of the two types of genetically modified fish that might be considered for biological control of a non-native fish species: triploid sterilized fish and transgenic fish. Environmental release is regulated at three main levels: federal, state and international. In addition, federal Indian law could apply when developing a transgenic fish biocontrol program, as discussed in a sub-section below. A summary of relevant regulations, policies, guidelines and standards is presented in Table 4.2.

Regulation of introduction of triploid sterilized fish

State and federal government oversight of any future release of triploid fish for biocontrol purposes would likely build on approaches currently used to regulate triploid grass carp (*Ctenopharyngodon idella*), a non-indigenous species that was introduced into the United States for biological control of aquatic weeds (Lewis 1999). This introduction raised concerns that fertile grass carp could establish naturally reproducing populations, spread into waterbodies where it is unwanted, and cause ecological damage because its prodigious feeding ability could destroy vegetation that supports wetlands and waterfowl breeding grounds (Lewis 1999). This worry can be mitigated by rendering the grass carp sterile via induction of triploidy combined with pre-release testing to verify the triploid status of each individual.

A number of states have restrictions on stocking triploid grass carp for aquatic weed control and these are complemented by a federal inspection program for certifying triploidy of fish before release. Any future effort to release triploid-sterilized fish for biocontrol of a non-native fish species similarly could combine government regulation with an inspection program to assure sterility of each

released fish. An inspection program would serve two important needs. First, it would assure maximum efficacy of the biocontrol program by confirming that each released fish is indeed sterile and thus capable of disrupting successful reproduction in the target population (as explained in chapter 2). Second, it would prevent accidental release of fertile individuals of the non-native species which would simply increase the numbers of the target species that the biocontrol program is trying to reduce.

States that regulate stocking of grass carp allow purchase of triploid fish only from a licensed, permitted dealer who has to provide certification that each fish sold is triploid. When such dealers are out-of-state, some states also require a permit to import triploid grass carp. Appendix 4 reviews the regulation of triploid grass carp in Arizona, California, and New Mexico. California is included because of its close proximity to Arizona and its connectedness to the Gila River via the Colorado River. Appendix 4 also summarizes the Triploid Grass Carp Inspection Program operated by the U.S. Fish and Wildlife Service.

Potential regulation of introduction of transgenic fish for biocontrol

Several federal and state laws would govern the introduction of a transgenic fish for biocontrol in the Gila River basin. Federal laws may be classified into two groups depending upon whether they are (1) cross-cutting environmental statutes which impose requirements on federal agencies authorizing, funding, or carrying out the introduction; or (2) specific statutes, invoked under the Coordinated Framework for Regulation of Biotechnology, to regulate environmental release of transgenic biocontrol fish. Several international agreements may also apply in situations involving transboundary movements of transgenic fish for biological control.

National Environmental Policy Act

NEPA section 102(C) imposes strict *procedural* or administrative requirements to assess the potential impacts of any new federal action—such as environmental introduction of a transgenic fish for biological control—on the human environment. The act does not prescribe a certain standard of environmental protection, thus does not impose any *substantive* requirements on agency decision-making. *Vermont Yankee v. NRDC*, 435 U.S. 519 (1978). The NEPA process starts with preparation of an environmental assessment (EA) and, depending on the findings of the EA, may require preparation of an environmental impact statement (EIS). An EIS contains, among other things, a detailed analysis of the environmental affect of the proposed action and alternatives to the project. Before making the statement, the action agency is required to consult with any federal agency having jurisdiction by law or special expertise with respect to the environmental impacts. Copies of the statement, along with comments and views of the appropriate federal, state, and local environmental agencies, must be made available to the public as provided by the Administrative Procedure Act, and must accompany the proposal through the agency review process. 42 U.S.C. § 4332(C), *Calvert Cliffs Coordinating Committee v. Atomic Energy Commission*, 449 F.2d. 1109 (D.C. Cir. 1971).

Applicability to effects within U.S. waters. If a party comes forward with a proposal to release a transgenic fish for biocontrol, the critical early question will be whether the action meets the statutory threshold for preparation of an EIS as "a major federal action significantly affecting the quality of the human environment". 16 U.S.C. § 4332(C). For projects involving unique or unknown environmental risks—which would most probably include a transgenic fish biocontrol project—the Interior Department manual specifies that the action would require the preparation of environmental documents, either an environmental assessment (EA) or a full-blown EIS (USDOI 2001). Where appropriate, such an EA or EIS could be a

programmatic document addressing a group of similar or related actions, perhaps having cumulative impacts. However, programmatic documents do not absolve the agency from the responsibility to prepare site-specific environmental documents (USDOI 2001). Initial stages of EIS preparation require public participation in a scoping process to identify significant issues, the needs for the action, potential impacts, and a range of alternatives (USDOI 2001). NEPA also calls for public review of a draft EA or draft EIS before the agency makes a final decision.

Applicability to transboundary effects beyond U.S. borders. The Council on Environmental Quality, which administers NEPA, has advised agencies that, "based on legal and policy considerations," the NEPA process should analyze federal actions taking place in the United States that may have transboundary effects extending across the border and affecting another country's environment (CEQ 1997). Because one of the major tributaries to the Gila River basin—the San Pedro River—has its headwaters in the Sonoran highlands of Mexico and because of the potential for transboundary movement of fish from the Gila River to Mexico via the Colorado River, the scoping process should seek to identify and include such potential transboundary effects in the EIS. Addressing these potential effects will likely require collaboration between the U.S. Fish and Wildlife Service and agencies in Mexico with similar expertise.

Endangered Species Act

Two provisions of Section 7 of the Endangered Species Act (ESA) are particularly relevant to a potential proposal to introduce biocontrol transgenic fish into interstate waters, such as the Gila River basin. Section 7(a)(1) requires all federal agencies to work pro-actively for the conservation of listed species. 16 U.S.C. § 1536(a)(1). It is not clear what positive conservation duties, if any, Section 7(a)(1) imposes on federal agencies (Stanford Environmental Law Society 2001). This might become an important question if a federal agency proposed to use its traditional authorities for conservation programs as the legal basis for implementing a transgenic fish biocontrol project, in order to reduce the threat to listed native species posed by non-native fish.

Section 7(a)(2) requires all federal agencies authorizing, funding, or carrying out an action that may jeopardize the existence of a listed species or may destroy or adversely modify its critical habitat to consult with the appropriate expert agency before acting. 16 U.S.C. § 1536(a)(2). If the Fish and Wildlife Service serves as the action as well as the expert agency, Section 7 consultation would proceed according to the internal consultation guidelines (USFWS 1998). Intra-service consultations could focus either on individual actions or on a Service program as a whole, such as a genetic biocontrol program. A programmatic analysis—including a survey of the range of genetic biocontrol methods, and the general feasibility and significant risks of each method—could be used as a framework under which site-specific assessments of individual actions might be tiered. During the consultation, the action agency and the applicant may not make an irreversible or irretrievable commitment of resources that would preclude implementation of any reasonable and prudent alternatives to the activity or program. 16 U.S.C. 1536(d).

Once the consultation is completed, the Fish and Wildlife Service would issue a biological opinion on the question of jeopardy and/or adverse habitat modification. 16 U.S.C. § 1536(b). Each biological opinion contains an Incidental Take Statement critical to avoiding liability under the ESA. If the project is not likely to jeopardize the continued existence of a listed species or adversely modify its habitat, the Incidental Take Statement may authorize the project to proceed subject to certain terms and conditions. 16 U.S.C. § 1536 (b)(4). If a jeopardy/adverse modification opinion is received, the biological opinion will specify certain reasonable and prudent alternatives which avoid jeopardy or adverse modification of habitat. A transgenic fish biocontrol project description would have to be modified to implement these alternatives. Section 9

of the ESA prohibits the unauthorized taking of individual members of listed species. Without authorization provided by an Incidental Take Statement, a federal agency or permittee would be responsible for any take, whether directly or indirectly caused, by permitting, funding, or other activities associated with a transgenic fish biocontrol project.

Lacey Act

Should an environmental review reveal ecological hazards posed by transgenic fish, the Fish and Wildlife Service may be able to invoke the regulatory powers of the Lacey Act. Of its two separate parts, one seems like a poor fit for regulating transgenic fish developed for biocontrol. Specifically, the original Lacey Act of 1900 as amended confers upon the Department of Interior authority to prohibit importation and transportation of certain species of wildlife, including fish, determined to be "injurious to human beings, to the interests of agriculture, horticulture, forestry, or to wildlife or the wildlife resources of the United States." 18 U.S.C. § 42. It is unclear whether transgenic forms of natural fish species could be considered as "species" under the terms of the Lacey Act. The Department of Interior has indicated that it is investigating whether Congress intended transgenic fish to be included within the scope of the Act (CEQ-OSTP 2001); but the status of this investigation is unclear. From a practical perspective, none of the proscribed fish species listed in the Fish and Wildlife Service regulations is currently a realistic candidate for genetic engineering for biocontrol purposes. 16. C.F.R. 15.13(a)(2).

Potentially more relevant to a proposed transgenic fish biocontrol project are the Lacey Act Amendments of 1981 which prohibit trade in "tainted" fish or wildlife. 16 U.S.C. §§ 3371-3378. Taint arises when the fish or wildlife is taken, possessed, transported, or sold in violation of a wildlife-related federal, state, tribal, or foreign law or regulation. Showing a violation of the underlying law—the "predicate" violation—constitutes the first step in proving a trafficking violation. The second step involves showing an overlying violation of the Lacey Act's list of prohibited acts: import, export, transport, sale, receipt, acquisition or purchase of the tainted fish or wildlife. When the underlying law violated is a state law or regulation, the prohibited acts must involve interstate or foreign commerce. 16 U.S.C. § 3372(a)(2). In this context, state laws regulating possession of transgenic fish may become more common in response to the recent introduction of transgenic GloFish into the U.S. ornamental fish market without any federal regulatory action (Weiss 2003). As of 2004, California had banned the sale of these transgenic ornamental fish within the state (Pollack 2004).

Coordinated Framework for Regulation of Biotechnology

The Coordinated Framework does not clearly indicate which agency would lead the oversight of production and releases of transgenic animals in general, let alone releases of transgenic fish for biocontrol. Federal agencies have issued little guidance on whether and how they intend to regulate transgenic animals (NRC 2002a; Pew Initiative on Food and Biotechnology 2004). The Food and Drug Administration (FDA) previously indicated it would assume the role of lead agency for regulating transgenic animals including fish, although it has not issued any formal policy or guidance document to this effect (CEQ-OSTP 2001; NRC 2002a).

FDA claim of lead regulatory authority. The FDA stepped up first—in the early 1990s—to claim a lead role when a company developing transgenic salmon for eventual commercial fish farming sought advice on how to apply for federal approval (CEQ-OSTP 2001).The agency stated it would exercise its oversight responsibility through the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA). The FDA claimed that this statute's definition of a new animal drug applies to the inserted transgenes and expressed proteins in a transgenic animal.

Numerous analysts have pointed out two major problems with regulating transgenic fish under the new animal drug provisions (Kapuscinski 2002; NRC 2002a; Pew Initiative on Food and Biotechnology 2003, 2004; Kelso 2004). First, the animal drug provisions require that the FDA conduct a secret review of drug applications, which the FDA has interpreted to apply even to conducting an environmental review under NEPA. Second, the FFDCA is not an environmental statute and does not contain an environmental safety standard for making decisions. This was underscored when the FDA declined to regulate the first transgenic fish marketed in the U.S.A., an ornamental GloFish, because these fish posed "no threat to the food supply" (USFDA 2003). By declining to regulate these fish, the FDA avoided taking a federal action that would have required it, under NEPA, to prepare an environmental assessment or an environmental impact statement before making a regulatory decision. Appendix 3 reviews the agency's exercise of authority, to date, over transgenic fish being developed for aquaculture and the limits of this authority for regulating environmental safety of transgenic fish.

Other possibilities for lead federal agencies over transgenic animals are being explored (Pew Initiative on Food and Biotechnology 2004). For example, the USDA Biotechnology Regulatory Service recently began to review other statutory options for regulating transgenic animals (Bob Rose, USDA APHIS, personal communication, November 2004), although it is unclear whether this review includes consideration of transgenic fish for biocontrol.

The U.S. Fish and Wildlife Service would have an important opportunity if and when the first application comes forward for purposeful release of a transgenic fish for biocontrol in federal waters. Should such an effort go forward, it would be advisable for the Service to convene an interagency dialogue during the initial planning stages to sort out the regulatory responsibilities. One possible result might be that, given its expertise in fisheries science and aquatic ecology and its oversight of fish resources in federal waters, the Service would emerge with lead authority.

Conflict between Secrecy Requirements of FFDCA and Public Review under NEPA. If the FDA were to exert a lead role in regulating the purposeful release of transgenic fish for biological control purposes, the secrecy provisions of the Trade Secrets Act and the FFDCA may supercede the normal public involvement mandated by NEPA. To date the FDA has acted on the premise that these secrecy provisions preempt NEPA provisions for public involvement (Appendix 3). This issue is quickly becoming more socially controversial as transgenic fish gain public attention and is likely to be tested through the courts, perhaps after FDA issues its first commercial approval for a transgenic fish.

These secrecy requirements for new animal drug approvals invert the usual NEPA procedures by precluding public comment, including submission of supplemental information, which might influence the agency's determination during the environmental review (Kelso 2004). Public comment is possible only after approval of a transgenic fish, placing potentially affected parties, independent experts, and other interested citizens in a reactive position. Their options are to submit a citizen petition at any time or sue the FDA within a time limit to revoke action already taken. This approach goes against the recommendations of the National Academy of Science regarding how to gain durable public trust in risk decision-making (NRC

1996): when making risk-based decisions, involve from the outset all potentially affected and interested parties in the deliberative phase of an iterative analytic-deliberative process. The Academy recommended against leaving out public participation until after decisions are made (see also Gibbons 1999).

State Law

A small but growing number of states have laws or regulations specifically targeting transgenic fish. Federal authority over transgenic organisms under the Coordinated Framework is limited to interstate commerce, including federal waters, and would not directly apply to uses and effects of transgenic fish in state waters. Furthermore, states have considerable authorities over activities—such as releases of transgenic fish—that could affect fish and wildlife, either under delegated federal laws, independent police powers, or the public trust doctrine (Kelso 2000).

States from the East coast to the West coast have passed laws or regulations pertaining to transgenic fish. Minnesota first passed a law regulating environmental releases of genetically engineered organisms including transgenic fish (Sec. 28. MN Statutes 1990, 116C.91-95 and MN Laws 1991, chapter 250). In 2001, Maryland passed a five-year moratorium prohibiting "introduction of transgenic species or any genetically altered species into any waterway of the State that flows into any other water body" (House Bill 189, chapter 54). In Michigan, recent amendment of the Natural Resources and Environmental Protection Act gives authority to the state Department of Natural Resources to regulate general release of genetically engineered fish. M.C.L. § § 324.41301 et seq. The Michigan Aquaculture Development Act, as amended in 2003, also gives the state department of agriculture authority to regulate aquaculture of genetically engineered fish (M.C.L. § § 286.871 et seq.). The Indiana Department of Natural Resources regulations on exotic fish (Ind. Admin. Code 312 9-6-7) include 'genetically altered' fish and its Aquatic Nuisance Species Management Plan discusses genetically engineered fish (Indiana Department of Natural Resources 2003). California, one of Arizona's neighbors, also regulates transgenic aquatic organisms, as detailed below.

In connection with a proposal to use transgenic fish as a biocontrol agent in the Gila River basin, the most relevant state laws to consider are those of Arizona; California, which borders Arizona along the Colorado River (into which the Gila River flows); and New Mexico, from whose western mountains arise the headwaters of the Gila River. Although Arizona does not explicitly regulate transgenic fish, a number of aquatic wildlife regulations could be applied to regulate potential use of transgenic fish for biocontrol. Like Arizona, New Mexico does not specifically regulate transgenic aquatic wildlife but could apply certain existing fish and wildlife statutes and regulations to oversight of a transgenic biocontrol fish program. California has much more restrictive regulations than Arizona and New Mexico and explicitly regulates transgenic fish. Appendix 5 reviews the relevant and potentially relevant regulations in Arizona, California and New Mexico.

Federal Indian Policy and Law

U.S. courts have long recognized a special relationship between the federal government and Indian tribes based upon the tribes' unique legal status as "domestic, dependent nations" (Mazurek 1998). After many shifts, the federal government has settled upon a policy of self-determination which encourages tribes to plan and carry out their own service programs—including fish and wildlife management—while recognizing the historic trust responsibilities of the federal government. In the 1990s, Indian tribes and the Department of the Interior agreed to put aside their legal disputes over the Endangered Species Act and began forging a hopefully more effective working relationship based on principles arrived at through government-to-government negotiation. The process of developing and implementing a plan to manage non-native fishes in the Gila River basin offers an opportunity

to strengthen this new approach to a working relationship. Moreover, cooperation is the most likely way to achieve the conservation purposes of such a plan.

This subsection introduces the (1) federal policy of self-determination, (2) legal doctrine of trust responsibility, and (3) newly emerging working relationship between tribes and the federal government and their relevance to any future program to control non-native fish in the Gila River basin. Tribal lands in the Gila River basin most relevant to consider belong to the tribes whose reservations lie adjacent to or encompass sections of the Salt, the Gila, and the Verde Rivers.

Although this discussion focuses on federal government relations with the tribes, any potential program of biocontrol of invasive fish should also consider relationships of states with the tribes. For example, over the past thirty years, the Arizona Game and Fish Department has developed a strong working relationship with a number of tribes through cooperative agreements and memoranda of understanding (Arizona Commission of Indian Affairs 2003). It would be important for any proponents of a transgenic fish biocontrol project for the Gila River basin to build upon those partnerships.

Tribal self-determination and self-governance. The Indian Self-Determination and Education Assistance Act of 1975 first established a process for tribes to administer federal programs to the Indian community. Amendments in 1988 and 1994 solidified tribal self-governance compacts which function something like block grants. Compacts are written agreements between a tribe and a federal agency setting out the legal responsibilities of each and are accompanied by an annual funding agreement or protocol for the transfer of funds directly to the tribe. The Interior Department's Office of Self-Governance administers the negotiation and contracting/compacting process, and agencies such as the Fish and Wildlife Service have been encouraged to use the process in working together to achieve common conservation goals.

If the U.S. Fish and Wildlife Service were to pursue a program of biocontrol of non-native fishes in Arizona, it could build upon cooperative programs with tribes in the Gila River basin. A recent example of cooperation is the White Mountain Apache Game and Fish Department working with the Arizona Game and Fish Department, the U.S. Fish and Wildlife Service, and the U.S. Forest Service to restore one of Arizona's most famous native fish: the Apache trout. The Service could explore ways of establishing a non-native fish management program under the general framework provided by the Indian Self-Determination and Education Assistance Act and the Interior Department's Self-Governance Program.

Trust responsibilities. Any future federal effort to undertake a transgenic fish biocontrol program in the Gila River basin could be affected by trust responsibilities of federal agencies to individual tribes. The doctrine of trust responsibility imposes legal duties on federal agencies, enforceable in the courts. The extent to which trust responsibilities effectively restrain executive branch action was called into question by Supreme Court decisions on two cases in the 1980s, commonly known as the Mitchell cases. Whether these decisions would limit trust responsibilities viz a viz an invasive fish biocontrol program is a complex legal issue that should be taken up by appropriate legal experts.

One might look at how courts have interpreted trust duties in scenarios resembling those most likely to occur in a transgenic fish biocontrol program in the Gila River basin. Some releases of transgenic fish for biocontrol might occur in streams on federal lands adjacent to Indian reservations. Depending upon the barriers to fish movement, some of these fish could migrate into streams and lakes on a reservation, where they could for various reasons, including cultural ones, be viewed as unwelcome intruders. A breach of trust action based on the common law

doctrine of federal trust responsibility to protect Indian waters from such fish would, in many ways, resemble a nuisance or trespass claim at an inter-sovereign level (Wood 1994). However, unlike other sovereign entities such as states which would find it difficult to prevail in a civil liability action, Indian tribes have a special legal relationship with the federal government. Courts may listen more carefully to tribal arguments that the trust responsibility provides a basis for enjoining federal actions.

In *Northern Cheyenne Tribe v. Hodel* (1985), the District Court of Montana concluded that the government's trust responsibilities to the Northern Cheyenne Tribe required the Interior Secretary to carefully consider the social, economic, and cultural impact on the tribe from coal leasing on public land adjacent to the reservation. To buttress its breach of trust argument in the suit, which was filed shortly after the first of the *Mitchell* cases, the tribe included two statutory claims, one of which was the agency had failed to take a "hard look" at the environmental consequences of its actions as required by the judicial interpretation of NEPA. When an environmental impact statement (EIS) is required because the proposed action affects the natural or physical environment, the EIS must consider economic or social effects related to the natural impacts. 40 C.F.R. § 1508.14. The court found no evidence in the EIS that the Department had recognized the Northern Cheyenne Tribe reservation as a culturally distinct entity within the region.

This ruling that agencies must consider the potential impacts of their actions on tribal culture extends the scope of the preliminary review beyond questions of scientific and technical feasibility. It means, in short, that agencies must make a meaningful effort to learn about the ways of life of their Indian neighbors. It may also mean that where modern technology conflicts with these ways, at least from the Indian point of view, agencies must be prepared to look for alternative means to accomplish their conservation goals.

A new federal-tribal working relationship. Indian tribes in the Gila River basin and the U.S. government now have a more cooperative framework (than courtroom battles) for addressing fish management issues. In the 1990s, the White Mountain Apache Tribal Chairman Ronnie Lupe and the Director of the U.S. Fish and Wildlife Service Mollie Beattie felt that legal wrangling over application of the Endangered Species Act was getting in the way of the Tribe and the Service developing a practical working relationship that could achieve some of their common conservation goals while not burdening tribal rights to economic development. They met in a neutral outdoor site (Getches et al. 1998) and ultimately signed an agreement, *Statement of the Relationship between the White Mountain Apache Tribe and the U.S. Fish and Wildlife Service* (Statement of Relationship), which became more than a pact between a single tribe and a field office in Arizona. It has become a model for similar negotiations at the national level. The 1997 (Interior) Secretarial Order on *Tribal Rights, Federal-Tribal Trust Responsibilities, and the Endangered Species Act* resulted from a similar decision to avoid legal wrangling in favor of seeking a working relationship whose principles could guide agency actions in the field.

In essence, the Statement of Relationship calls for the Tribe to develop its own management plan in accordance with Tribal values. The Tribe and the Service pledge to communicate with each other, and to cooperate in developing management practices based upon identified threats to sensitive species. In ESA Section 7 consultations regarding sensitive species on tribal lands, the Tribal Management Plan will generally serve as the basis for Reasonable and Prudent Measures and Alternatives. Adoption and implementation of the Tribal Management Plan will normally mean that no additional special management considerations or protection for sensitive species will be needed.

As one of the first steps in planning a future non-native fish biocontrol project in the Gila River basin the tribes and the Fish and Wildlife Service could

find a suitable place to meet. The purpose of meeting would be to formalize an agreement that will clarify the substantive responsibilities of the tribal governments and the United States government. Even getting all the parties to the table likely will not be easy and negotiators would do well to read the account of the negotiations leading to the Secretarial Order on the Endangered Species Act (Wilkinson 1998).

International Law

International law arising from bilateral and multilateral agreements and treaties may be relevant to a biocontrol project involving transgenic fish in the Gila River system. International law enters the picture because of the potential movement of transgenic fish into Mexico via the San Pedro River, a headwater tributary of the Gila River, or via the Colorado River into which the Gila River flows. The San Pedro River arises in the state of Sonora in Mexico. Protecting it is a shared responsibility between the states of Arizona and Sonora in Mexico, as well as among a number of non-governmental organizations involved in conservation efforts.

Certain international agreements to which either the United States or Mexico or both nations are parties may have legal consequences for a biocontrol project involving transgenic fish in Arizona. The discussion below examines the potential regulatory significance of (1) the Cartagena Protocol on Biosafety, (2) the North American Agreement on Environmental Cooperation, and (3) the Agreement on Sanitary and Phytosanitary Measures (SPS).

Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity regulates transboundary movements of living modified organisms. Article 3 of the Protocol defines living modified organisms in a way that clearly includes transgenic fish (but not triploid sterilized fish) developed for biological control purposes. Although the Protocol primarily regulates *intentional* transboundary movements (i.e., imports and exports), Article 17 on *unintentional* transboundary movements and emergency measures addresses issues of inter-state cooperation, preventative measures, and imposes a notification requirement on Parties. A Party to the Protocol must give notification when the release involves the potential transboundary movement of a living modified organism "that is likely to have significant adverse effects on the conservation and sustainable use of biological diversity, taking also into account risk to human health." Cartagena Protocol, 17.1. Article 17 would apply if an intentional introduction of a living modified organism into the environment of a Party gives rise to an unintentional transboundary movement of the organism to another State or if accidental release (e.g. from a contained use facility) lead to unintentional transboundary movement (Mackenzie et al. 2003).

The United States is presently a non-party to the Cartagena Protocol and thus is not bound by the notification requirements. However, Mexico, which is a party, may be obligated to notify the United States of releases within Mexico which might lead to potentially harmful transboundary movements into U.S. territory. This obligation arises from Article 24 which states that transboundary movements of living modified organisms between Parties and Non-Parties shall be consistent with the objectives of the Protocol. Cartagena Protocol, 24.1.

North American Agreement on Environmental Cooperation

The North American Agreement on Environmental Cooperation (NAAEC) is the environmental side agreement to the North American Free Trade Agreement (NAFTA). NAAEC set up the Commission for Environmental Cooperation to facilitate regional environmental cooperation between the United States, Canada, and Mexico.

NAAEC imposes no international environmental law obligations upon the parties beyond the requirement that each party "effectively enforce its environmental laws and regulations through

appropriate governmental actions". NAAEC 5.1. Perhaps the most significant provisions here are Articles 6 and 7 on private access to remedies and procedural guarantees. These would permit, for example, citizens of Mexico to sue violators of United States environmental laws within the United States court system.

Article 13 allows the Secretariat to prepare reports on environmental matters related to the treaty. A series of documents on the Upper San Pedro River basin have been prepared as part of the Commission's Upper San Pedro River Initiative seeking to advance economic and environmentally sustainable strategies for preserving and enhancing the riparian ecosystem (Commission for Environmental Cooperation 1999). To be feasible, any plan to use transgenic fish for biocontrol in the San Pedro River would probably need to harmonize with this continuing Initiative.

Sanitary and Phytosanitary Agreement

Article 20 of the General Agreement on Tariffs and Trade provides that governments may enact trade measures to protect human, animal, or plant life or health provided they are not applied in a manner which would constitute arbitrary or unjust discrimination or a disguised restriction on international trade. GATT 1947, Article XX(b). The World Trade Organization Agreement on Sanitary and Phytosanitary Measures (SPS) Agreement, hammered out in GATT 1994, includes basic rules for adopting measures relating to safe trade of animals. These would presumably apply to international trade of transgenic fish developed for biocontrol uses, if such trade develops in the future. In case such trade does arise in the future, the discussion below summarizes potential application of the SPS Agreement.

International trade could entail exports and imports of fish gametes, embryos, or other life stages for use in biocontrol in the importing countries. The SPS Agreement recognizes the World Organization for Animal Health (OIE - Office International des Épizooties) as the reference organization responsible for the development and promotion of international animal health standards, guidelines and recommendations to ensure safe trade in live animals and animal products. Although the OIE has not yet developed guidelines for transgenic animals, it recently began exploring the scientific basis of the issue (OIE 2005).[4]

The human health aspects of the SPS Agreement could come into play if the fish species targeted for genetic engineering for biological control purposes is also a species eaten by humans. The key issue would be whether or not proponents can prevent transgenic biocontrol fish from becoming unintentionally co-mingled with live or processed fish in international food trade. The SPS Agreement recognizes the Codex Alimentarius Commission as the reference organization for food safety standards in international trade. In 2003, Codex issued principles and guidelines for safety assessment of foods derived from recombinant-DNA plants and foods produced using recombinant-DNA microorganisms (Codex Alimentarius Commission 2003, 2003a, 2003b). As a first step towards developing similar Codex guidelines for animal-derived foods, the FAO and WHO convened an expert consultation in 2003 on safety assessment of foods derived from genetically modified animals including fish (i.e., transgenic animals). The consultation concluded that assessing the safety of foods derived from transgenic animals would follow largely the same steps and case-by-case approach as Codex has already established for foods derived from recombinant-DNA plants (FAO/WHO 2004). At present this entire issue is a remote concern because there is no serious interest in intentionally trading any transgenic fish that was developed for biological control of non-native fish in the Gila River basin.

[4]The OIE also has an Aquatic Animal Health Code governing the sanitary safety of international trade in aquatic animals (OIE 2004).

Table 4.2. Anticipated regulations, policies, guidelines and standards related to U.S. environmental release of triploid sterilized and transgenic fish for biocontrol. Shaded cells only apply to transgenic fish.

Jurisdiction	Regulation, Policy, Guidelines or Standards	Required Compliance Action	Responsible Authority Action
U.S. Federal	NEPA	Preparation of EA/EIS; Consultation with Mexico on transboundary effects	Final Environmental Impact Statement (FEIS)
	Endangered Species Act	Section 7 Consultation	FWS issuance/denial of Incidental Take Statement
	Lacey Act	Secure approval for interstate commerce for any state-prohibited transgenic fish	FWS enforces federal trafficking laws where state wildlife laws are violated
	Federal Food, Drug, and Cosmetic Act	New Animal Drug Application to FDA	FDA approval/disapproval of New Animal Drug Application
State	Arizona	Application for Stocking Permit to Arizona Game and Fish Dept. (AFGD)	AGFD issuance/denial of stocking permit
	California	Consultation with California Fish and Game Department	California may enforce state laws prohibiting transgenic fish in state waterways; Consultation may require public participation
	New Mexico	Consultation with New Mexico Department of Game and Fish	NMDGF issuance/denial of stocking permit
Tribal	Relevant Tribal Policies and Interests	Consultation with appropriate Tribes	May need to negotiate agreement with affected Tribes
International	North American Agreement on Environmental Cooperation	Consultation with Mexico	Mexican citizens have private access to remedies in U.S. courts for violation of U.S. environmental laws
	GATT Sanitary and Phytosanitary Agreement	Possible obligations regarding safe trade of animals; No obligations regarding safe trade of human food (revisit if transgenic methods applied to fish eaten by humans)	World Trade Organization reviews and rules on any international trade dispute brought forth by a party against another party
	Cartagena Protocol	No obligations unless and until U.S.A. becomes a party	No obligations unless and until U.S.A. becomes a party

Chapter 5
Multi-Stakeholder Deliberation

Introduction

Any decision on the use of genetic methods for biological control of non-native fish must be based both on science and deliberation among the potentially affected and interested parties within the Gila River basin. Good science and its application to issues raised in chapters 2 and 3 *is necessary and indispensable but not sufficient* to reach sound and widely supported decisions regarding proposals to apply genetic biocontrol (Kapuscinski 2002). The most effective way to arrive at broadly trusted decisions is to use an iterative *analysis* and *deliberation* process (NRC 1996). Linking deliberation to analysis will increase public trust in conclusions drawn about the potential risks of genetic biocontrol and its potential to meet a core need. Analysis is the process of using rigorous, replicable methods, evaluated under the agreed protocols of an expert community, to arrive at answers to factual questions. Deliberation among multiple stakeholders is the process for communication, raising and collectively considering issues, increasing understanding and arriving at substantive decisions.

There are three compelling rationales for incorporating broad participation and deliberation among interested and affected parties in risk characterization: normative, substantive and instrumental (Fiorino 1990, NRC 1996). The normative rationale suggests that governments should obtain the consent of the governed. Under this rationale, citizens have the right to participate meaningfully in public decision making and to be informed about the basis for government decisions. The substantive rationale admits that scientists in the public and private sectors and public officials simply do not hold all the relevant wisdom; thus participation by people with diverse experience will provide key information and insights to risk analysis. The instrumental rationale affirms that broad participation enhances the chances of reducing conflict and increasing general trust in risk decisions made by government agencies. Incorporating analysis and deliberation within an open risk characterization and decision process, in which all parties have access to all the key information, can lead to reaching a greater degree of agreement among scientists, governments and interested and affected social groups. The use of an analytic-deliberative process should ultimately lead to actions which are best for both human communities and the environment.

Numerous studies have shown that a strong link between analysis and legitimate deliberation is absolutely essential to winning durable and broad acceptance of decisions about proposed uses of a technology that presents both potential benefits and harms to the environment or human health (Susskind et al. 2000; Wondolleck and Yaffee 2000; NRC 1996). The lack of sufficiently accessible and participatory deliberation during development and pre-market analysis of the first generation of genetically engineered crops helped to fuel polarized conflict after they entered the marketplace (Sagar et al. 2000; Louet 2001; Marris et al. 2001). Consequently, there is a growing call for embracing an analytic-deliberative process for making future decisions about uses of gene technologies (Gibbons 1999; Sagar et al. 2000; NRC 2002; NRC 2004). There are some promising initial efforts to take such an approach for transgenic organisms (Kapuscinski et al. 2003; Nelson et al. 2004; Capalbo et al. 2005).

Table 5.1. Preliminary list of relevant entities to include in community awareness and involvement activities within the Gila River basin. Note: this list is not comprehensive and is offered as a suggested starting point. A full stakeholder analysis would need to be conducted if a decision was made to further investigate the use of genetic methods for biological control of non-native fish.

U.S. Federal Agencies	State Agencies	Non-Governmental Organizations	Native American Groups	Universities	Other
U.S. Fish and Wildlife Service	Arizona Game and Fish Department	Audubon Society	Gila Bend Indian Reservation	Arizona State University	Arizona Riparian Council
U.S. Bureau of Land Management	Arizona Department of Environmental Quality	Trout Unlimited	Salt River Pima-Maricopa Indian Reservation	University of Arizona	Gila Monster Watershed Council
U.S. Environmental Protection Agency	Arizona Department of Water Resources	American Fisheries Society	Ak-Chin Indian Reservation	Northern Arizona University	Upper San Pedro Partnership
U.S. Geological Survey	New Mexico Department of Game and Fish	Society for Conservation Biology	Cocopah Indian Reservation	New Mexico State University	Lower Gila River Citizens Advisory Council
U.S. Natural Resources Conservation Service	New Mexico Environment Department	Sierra Club	San Carlos Indian Reservation	University of New Mexico	New Mexico Riparian Council
U.S. Forest Service		Desert Fishes Council	Gila River Indian Reservation	Western New Mexico University	
U.S. Army Corps of Engineers		Center for Biological Diversity	Colorado River Indian Reservation		
U.S. Department of Agriculture – Southwest Watershed Research Center		Animal Defense League of Arizona	Tonto Apache Reservation		
		People for the Ethical Treatment of Animals	Fort Yuma Indian Reservation		
		Anglers United	Tohono O'odham Nation		
		Federation of Flyfishers	Fort McDowell Indian Reservation		
		Gila Fish and Gun Club	San Xavier Indian Reservation		
		Gila Resources Information Project	Camp Verde Indian Reservation		
		Gila Watch	Pascua Yaqui Indian Reservation		
		Gila Wildlife Rescue	Yavapai-Apache Indian Reservation		
			White Mountain Apache (Fort Apache Reservation)		

Integrating Deliberation with Analysis of Genetic Biocontrol Proposals in the Gila River Basin

The purpose of *analysis* in a genetic biocontrol program for the Gila River basin is to generate the knowledge needed to inform deliberation. Findings from analysis and deliberation both inform decision-making by project-executing agencies and regulatory bodies. Analysis would primarily involve geneticists developing the fish lines (transgenic or triploid sterilized); fisheries scientists, ecologists and public health scientists assessing the possible environmental and health risks; and legal and regulatory experts analyzing the evolving regulatory framework. They would generate necessary data and synthesize relevant existing information regarding potential efficacy, possible risks, and regulatory requirements of deploying a particular fish line for biocontrol purposes. Analysis would build upon and update the information and issues outlined in chapters 2, 3 and 4 of this feasibility study.

The purpose of *deliberation* among multiple stakeholders in a genetic biocontrol program for the Gila River basin is to develop a shared understanding of knowns and unknowns and get their input at key decision points from early stage research through seeking regulatory approval. Deliberation would involve parties who may be affected or interested in the proposed biocontrol program because of their livelihoods, direct interactions with fish resources in the affected waters, cultural and social practices, or some other legitimate stake in the issue. A preliminary stakeholder list (Table 5.1) and organizations represented on the newly-formed Arizona Invasive Species Advisory Council (Napolitano 2005) provide a starting point to assemble the proper scope of inclusion in deliberation. Deliberations should be structured to obtain input at go/no-go steps (decision points) in the program, as laid out in chapter 6. Structured deliberations would be staged to inform decisions on whether to proceed to next steps in research and development, efficacy testing, risk assessment, and seeking regulatory approval (Table 6.1 in chapter 6).

Traditional approaches of risk communication in natural resources management have been much less participatory and accessible than the analytic-deliberative approach (NRC 1996). Prevailing approaches have relied primarily on one-way communication from the experts to the public during early stages of developing a new management program. They have delayed more in-depth public input—usually as public comment processes—until close to final decision-making by the responsible government agency. This prevailing approach has often failed to win broad and durable public support, as reviewed in NRC (1996) and elsewhere. It often has fueled social resistance and conflict because stakeholders felt disenfranchised from key prior decisions that led to the final decision point. Social resistance, in turn, has fueled reactive behaviors by project proponents and regulatory bodies. To reduce chances of these pitfalls, we recommend taking a more pro-active analytic-deliberative approach to multi-stakeholder involvement in any future genetic biocontrol program.

Problem Formulation and Options Assessment – A Promising Option for Deliberation of Genetic Biocontrol Proposals

A multidisciplinary team, led by a natural resource sociologist, developed the process of Problem Formulation and Option Assessment (PFOA) to guide deliberations on proposed uses of genetically modified organisms (www.gmo-guidelines.info; Nelson et al. 2004). The team built PFOA based on the insights gained by many social scientists and practitioners in testing different deliberation techniques in environmental planning and environmental risk assessment. This deliberation process is designed to be "transparent, equitable, legitimate, and data driven when possible" (Nelson et al. 2004, Susskind et al. 2000). The team has

conducted three trial runs of PFOA, each involving a different genetically modified organism proposed for use in a different country (Nelson et al. 2004; Nelson et al. in preparation; Capalbo et al. 2005). Nelson et al. (2004) present a critique of the PFOA methodology using a trial run of PFOA to address a proposal to approve genetically modified corn for farming in Kenya. Participants in trial runs concluded that PFOA is essential for assessing proposals to release genetically modified organisms, particularly useful for encouraging constructive dialogue and potential agreements, and worthy of incorporation into the policy and regulatory decision-making process. The PFOA methodology was further evaluated and improved in similar workshops in Brazil and Vietnam. In each case, scientists and regulators considered the PFOA methodology and modified it to fit their unique national conditions (Nelson et al. 2004; Capalbo et al. 2005; Nelson et al. in preparation).

A PFOA process involves brainstorming, discussion and analytical components (Table 5.2). In the context of transgenic fish for biological control, it focuses deliberation on: what societal need will the application of genetic biocontrol satisfy, and at what risk? It starts with formulating the problem and then applies a comparative approach to risk assessment. If the multi-stakeholder group agrees that non-native fish pose a serious enough problem to merit analysis of options to control them, they then examine the range of future alternatives for controlling non-native fish. Alternatives "for solving the problem are compared in relation to their attributes, potential ability to address the problem, changes required to implement the option, and potential adverse effects" (Nelson et al. 2004). The PFOA process is science driven in that "[q]uestions are answered with data, impacts are assessed with valid indicators, and the limits of our understanding are clearly delineated by a research agenda or procedures for taking uncertainty into account" (Nelson et al. 2004).

Problem Formulation and Options Assessment could be used iteratively to inform different decision points in an entire genetic biocontrol program (see chapter 6). That is, it can inform go/no-go decisions made along the way from early phases of research to seeking regulatory approval (Table 6.1 and Figure 6.2). A PFOA conducted at earlier phases of research and development identifies the nature of multi-stakeholder support and concerns and clarifies important areas of uncertainty. It helps to anticipate the issues that will matter the most to stakeholders if and when the program reaches the phase of seeking regulatory approval. This helps scientists to prioritize studies that should be conducted in order to improve understanding about these issues before reaching the phase of seeking regulatory approval. It is particularly important to conduct a PFOA at the later research phase (isolated field studies to test efficacy and safety) and as part of seeking regulatory approval. Here, PFOA develops a shared understanding of the knowledge gained by this point in the program.

Table 5.2 represents a preliminary step in adapting PFOA to the issues relevant to a proposal to develop or implement genetic methods to control non-native fish in the Gila River basin. Any future effort to apply PFOA to this issue should be led by qualified experts from the social sciences (e.g. sociology, conflict management), who ideally have experience in guiding deliberations on environmental or natural resource problems. Such experts would hopefully carefully consider the unique needs and characteristics of the stakeholders that should be engaged. Finally, Problem Formulation and Options Assessment is likely to evolve and improve with further testing and implementation. It would be wise to monitor lessons learned from further applications of PFOA to inform any future decision to undertake a non-native fish genetic biocontrol program.

Table 5.2. Problem Formulation and Options Assessment (PFOA) Process (Nelson et al. 2004): Applied as a possible approach for multi-stakeholder deliberation in a genetic biocontrol program.

Initiating Proposal:

A1. Proposal to Develop a Genetic Method to Control a Specific Non-Native Fish:

A PFOA would determine multi-stakeholder support and concerns early in the program (Table 6.1): Phase 1 Contained Lab Tests –question 1e; and Phase 2 More Complex and Confined Tests – question 2d. At this point, less data will be available to inform the deliberation than will be available later on. The PFOA can identify and prioritize issues that scientists and other analysts should address in order to build the understanding that stakeholders will care most about if and when the program reaches Phase 4 (Final Application for Regulatory Approval).

OR

A2. Proposal to Implement Genetic Biocontrol of a Specific Non-Native Fish

A more in-depth PFOA would be initiated by the request that environmental release of a specific line of transgenic fish or triploid sterilized fish would be a beneficial alternative to the way things are currently being done to control undesired non-native fish. The PFOA will determine multi-stakeholder support and concerns at later phases of the program (Table 6.1): Phase 3 More Complex Tests in Isolated Stream Reaches – question 3b; and Phase 4 Final Application for Regulatory Approval – question 4b.

B. Decision by Project-Executing Agency:*

Is there merit to moving forward to develop a genetic biocontrol method (proposal A1) or evaluate the fully developed method (proposal A2) as a possible option or is the initiating proposal premature? Yes/No

****Or possibly by regulatory body if the proposal is to implement a fully developed method.***

****

PFOA process: questions to be answered by all representatives of affected and interested parties and shared in the deliberative process

Step 1: Problem Formulation

Formulation of Problem:	Natural Resource Needs	Interests
An unmet need that requires change	Fishing, viable native fish populations	A stakeholder group's values, goals and perspectives

A. Whose problem is it? Whose problem should it be?

1. What needs of the people are not being met by the present situation?
2. What aspects of the present situation must be changed to meet the needs?

Table 5.2 *Continued.*

Step 2: Prioritization and Scale

A. Is this problem a core problem for the people identified?

1. Do the people recognize the problem as important to their lives?
2. What are the potentially competing needs of these people?
3. How do the identified needs rank in importance to these other competing needs?

B. How extensive is the problem?

1. How many people are affected?
2. Where are these people located in relation to the Gila River basin?
3. How large an area is affected by the problem?
4. How severe is the problem (local intensity)?

Step 3: Problem Statement:

A statement of the shared understanding of the unmet need and its relative importance for a particular group of people

Step 4: Recommendation by Project-Executing Agency*:

Do we move forward to identify options and conduct an options assessment?

Or possibly by regulatory body if the proposal is to implement a fully developed method.

****

Option Identification and Assessment

Option Identification and Assessment Chart

Step 5 Options	Step 6 Characteristics	Step 7 Changes	Step 8 Effect on the system	
Future Alternatives	For problem solving	Required/Anticipated	Internal	External
			(Social, environmental, economic)	
Option A Option B Option C Etc.				

Step 5: Option Identification:

Brainstorm possible future alternatives to solve the identified problem, with one option being release of a line of genetic biocontrol fish.

This step can be completed by the multiple stakeholder group for the initial identification of options. The multi-stakeholder group can do Steps 6-8, or a technical committee can develop a report that covers Steps 6-8 and the multi-stakeholder group can use the document to begin their evaluation of options and modify the assessment.

Step 6: Assessment of the Options in Relation to the Problem:

Assessing capability of potential solution to solve problem.

1. What are the characteristics of each "technology" option? For instance, address transgenic fish, triploid sterilized fish, genetic biocontrol combined with species-specific pheromone attraction, large-scale capture and removal of non-native fish, etc.
2. What is the range of the target non-native fish and what is the geographic region in which the option is likely to be used in or have an effect?
3. What is the efficacy of the "technology" on the target?
4. What are the costs of deploying the technology within the target water-body?

Table 5.2 *Continued.*

5. What barriers to use of each technology option exist? For instance, can the potential solution be integrated into present fisheries and river basin management; can the executing agency and its potential partners afford the potential solution?
6. How might the use of the option change fishing, fisheries management and use of other natural resources in the river basin? What useful practices are reinforced by the potential option?
7. What information is needed to show that the changes are likely to occur? Baseline data associated with the diversity of present practices should be used if they are available.
8. How will anticipated changes in natural resource use and management affect the needs identified in Steps 1 and 2?

Step 7: Changes Required and Anticipated for a Specific Option:

1. What changes in *land-owner* practices might contribute to the solution?
2. What changes in the *local community* might contribute to the solution?
3. What changes in *government support* for river basin users might contribute to the solution?
4. What changes in the *structure of natural resource use and management* might contribute to the solution?
5. What other changes would likely be *needed to facilitate use and efficacy* of this option?

Step 8: Adverse Effects:

Potential adverse consequences from this option. Potential beneficial effects can be considered "negative" adverse effects.

1. How might the potential solution affect the structure of natural resource use and management?
2. How might the potential solution reinforce poor natural resource practices or disrupt useful practices?
3. What are the potential adverse effects of these changes internally and externally to the river basin?
4. How will its use affect other accessible natural resource systems (can its use be restricted to targeted regions of the Gila River basin)?
5. Are any of these changes difficult to reverse, once they occur?

Step 9. Recommendation

The multiple stakeholder group should present its problem formulation and option assessment to the appropriate decision making body.

Stakeholder Involvement in the Daughterless Carp Program in Australia

Any future effort to undertake a non-native fish genetic biocontrol program should also examine the progress and lessons learned in an Australian daughterless carp program, mentioned earlier in this report. This is an ongoing program to develop and test transgenic methods as part of an integrated pest management strategy to control an invasive fish. An aim of the program is to control non-native common carp in the Murray-Darling River basin. Researchers are presently at the early stage of laboratory research to develop specific transgenic methods. Even at this early stage, the program has developed a communication strategy (Murray Darling Association 2003). The purpose of this strategy is "to plan, promote and coordinate effective communications, practical engagement strategies and meaningful relationships for all stakeholders involved in the daughterless carp technology project." The comprehensive plan outlines: 1) communication partners within the basin and desired relationships; 2) key communication messages; 3) communication objectives, targets and actions; 4) timeframe and responsibilities for implementing the plan; and 5) consistency with other plans. This plan does not explicitly call for the kind of pro-active, analytic-deliberative process that we recommend. Our meetings with the program's scientists and communications experts (see Appendix 1), however, suggested they are committed to adaptive learning and flexibility regarding how to best engage stakeholders.

Interestingly, the proceedings of a National Carp Control Workshop held in Canberra, Australia (Lapidge 2003) reported that people in the community wanted to be involved from early on in research and development of a carp control program. It reported that involvement of community members has been valuable for drawing upon the wealth of wisdom, skills and knowledge of people residing along waterways in the Murray-Darling basin. The proceedings also admitted that involving the community is not simple or easy, but rather takes time, patience and resources. The daughterless carp program operates within the broader context of the Murray-Darling Basin Commission's Native Fish Strategy, which is rooted in a strong commitment to community participation and to developing strong community-government partnerships (Murray-Darling Basin Ministerial Council 2003). It also operates as one component of Australia's Pest Animal Control Cooperative Research Program.

To date, the daughterless carp program has engaged in a set of "community awareness and involvement activities" focusing on the problems that pest carp pose for native fish and the merits of conducting research to develop and test transgenic methods of control. These activities have included a regularly published brochure on non-native fish called *Aliens in the Basin*, a carp poster called *Villains or Victims*, a display for public forums, public presentations and workshops in many communities within the basin, various news articles and web-based information, and an educational video released in 2005 (Adrian Wells, Murray Darling Association, personal communication, June 2004; Wells 2004). Such activities should better prepare stakeholders for future participation in a deliberation process. They cannot take the place of a more substantive deliberation process needed to achieve broad and durable support of decisions regarding development and deployment of a genetic biocontrol method.

Recommendations

If a decision is made to further explore the use of genetic methods for biological control of non-native fish in the Gila River basin, the leaders of such an effort should develop a strategy for substantive multi-stakeholder deliberation. Deliberation should be staged to inform go/no-go steps (decision points) in the research, development, assessment and regulatory review of a genetic biocontrol method. We strongly recommend using an analytic-deliberative approach in order to maximize chances of gaining durable and widespread public trust in decisions. Problem Formulation and Options Assessment is a particularly promising approach. We therefore recommend monitoring the lessons learned as the PFOA process is further refined and implemented in policy and regulation of other kinds of genetically engineered organisms.

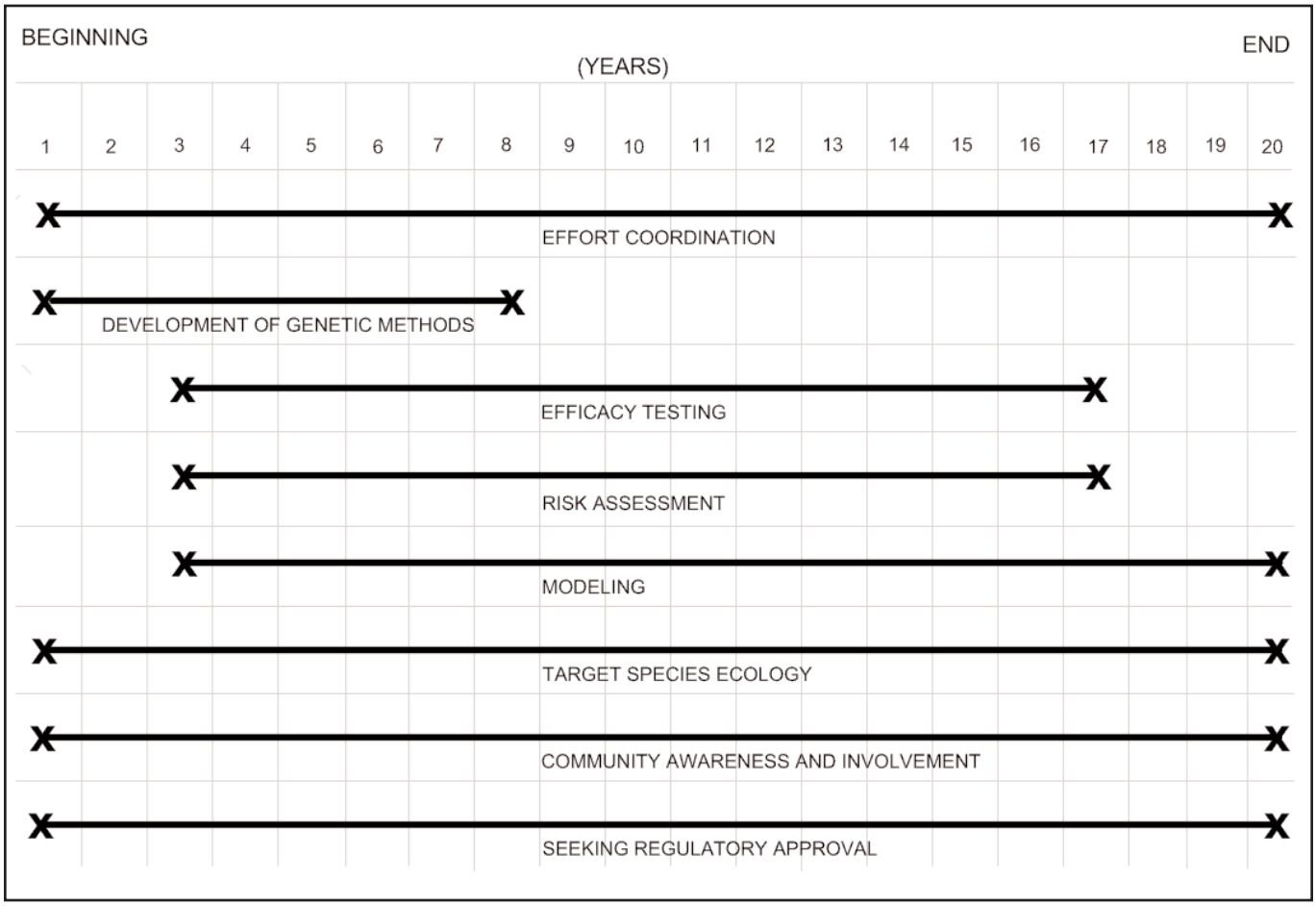
Chapter 6 presents potential points for deliberation to inform go/no-go steps of an entire genetic biocontrol program (Table 6.1, Figure 6.2). It will be extremely important to design the deliberations so that they fit the differing cultural norms and communication needs of stakeholders. Leaders of a biocontrol program could use our preliminary stakeholder list (Table 5.1) and additional organizations represented on the newly-formed Arizona Invasive Species Advisory Council (Napolitano 2005) as a starting point to assemble the proper scope of inclusion for deliberation. Each group should be allowed to decide who will represent it in order to assure the group's acceptance of conclusions and recommendations to which its representative contributed.

Chapter 6

Preliminary Roadmap of Programmatic Activities and General Cost Estimates

We propose seven important components for organizing a research and development program on genetic methods for biological control of non-native fish. We also suggest go/no-go steps to guide decisions on whether to proceed from simple contained laboratory tests, to more complex and confined tests, to the most complex tests in isolated stream reaches, to a final application for regulatory approval for field release. We also present estimates for the general categories of costs involved in developing either a triploid fish or a transgenic fish as a biological control agent. This preliminary roadmap of programmatic activities is heavily inspired by the ongoing work sponsored by the Murray-Darling Basin Commission in Australia to develop transgenic fish for biological control of non-native carp in the Murray-Darling River Basin. The CSIRO marine pest control group in Hobart is conducting laboratory research for this effort and the Pest Animal Cooperative Research Centre in Canberra is coordinating the overall effort. We met with individuals at these organizations in Australia (Appendix 1).

Figure 6.1. Multiple components for development and implementation of a transgenic fish biological control program.



Multiple Research Components

The development of transgenic fish for biological control is at very early stages of research. As of the writing of this report, no transgenic animal has been purposefully released into the environment in the United States. A future proposal to release a transgenic fish for biological control in the Gila River basin could end up being the first involving intentional environmental release of a transgenic animal in the United States. A research and development program in this area would be highly complex from both a scientific and a public policy perspective. We recommend a staged execution of multiple components of information gathering and testing as the best strategy for pro-actively addressing the scientific, social and regulatory needs and questions laid out in chapters 2-5 of this report. These components are presented in Figure 6.1. The discussion below briefly describes the important aspects of each component and how the various components complement each other. Two guiding principles for pursuing these components in parallel include: (1) to proceed from simpler to progressively more complex tests of efficacy and potential risks; and (2) to focus risk analysis, multi-stakeholder deliberations and seeking regulatory approval one step ahead of the current status of development and efficacy testing of the biological control methods.

This approach is a long-term and fairly expensive undertaking. Even the Australian group–probably the world's leader in genetic biological control of invasive fish–is still quite far away from demonstrating that a particular transgenic method will work well enough to warrant deployment. We estimate that it would take 15-20 years to develop transgenic methods from laboratory research to the point of field release for controlling one or perhaps 2-3 species in the Gila River. This assumes full-time dedication of an interdisciplinary team of senior scientists and support staff; parallel work on each component; focusing the first 10-15 years on figuring out if the control method works and what risks it entails; and focusing the last 5 years on obtaining regulatory approval and preparing for field release. Figure 6.1 highlights necessary components of developing a transgenic method over a 20-year period.

Although development of a triploid fish for biological control would require much less time and research effort than development of a transgenic fish, either approach requires the same multiple program components. We estimate that it would take approximately 5 years to develop a triploid fish for biological control. This is a much shorter time commitment than required for transgenic methods simply because the technical knowledge and research capabilities for reliably producing and verifying sterile triploid fish is well established. One option could be to develop and release triploid fish for biocontrol in the near term while pursing research and development of transgenic fish. A lack of sufficient understanding about the courtship behavior exhibited by triploid fish however, may demand more time for risk assessment and efficacy testing.

Development of genetic methods

We suggest taking an adaptive and bet-hedging approach to work on more than one method of developing transgenic fish because no one method is likely to be the silver bullet solution and because one should expect difficulties in getting particular genetic methods to work with different target species. Parallel pursuit of more than one method could also include different variants of any particular method such as different sequence-targets for interference RNA (see chapter 2).

Efficacy testing

Efficacy testing should be directed toward figuring out whether the method works. We agree with the staged approach planned by the Australians, i.e., to begin efficacy testing in simple lab environments and then progress to more complex ones, eventually to isolated stream reaches. Efforts should include testing for unintended trait changes that could reduce efficacy, such as changes in behavior that

would undermine the desired spread of the deleterious trait through the non-native fish population. It is important to define ahead of time what data will constitute sufficient evidence of efficacy at each stage of testing. Efficacy testing depends on the ease of captive propagation of the target non-native species. If there is a need to develop reliable culture methods for the target species, costs of doing so would need to be added to programmatic cost estimates presented below.

Risk assessment

Risk assessment should entail identifying potential ecological and human hazards and estimating the risk of potential harms posed by each hazard. We have identified a broad range of possible hazards and associated harms (see chapter 3). But a genetic biocontrol program should launch a thorough risk assessment process that draws on information that will come from progress in research and development of a genetic biocontrol agent (chapter 2) and multi-stakeholder deliberations (chapter 5). Risk assessment efforts also need to include an explicit analysis of uncertainty and must be linked to pro-active deliberation engaging affected and interested parties (chapter 5). It is important to carefully select endpoints in risk assessments because it is better to be less ecologically complete but have a more robust prediction with less uncertainty.

Modeling

Computer modeling is important to inform development of the genetic methods, efficacy testing, risk assessment, as well as post-release monitoring. Modeling efforts must be flexible and interact iteratively with the other program components. Early modeling can uncover inconsistencies and major information gaps. It is useful to start with a fairly simple conceptual model of the target fish population, its habitat and the management system. Modelers might then sparingly add complexity to the model based on results obtained from laboratory studies, more complex research on efficacy and potential risks, and field studies on target species ecology.

Target species ecology

Information about target species ecology should be gathered to inform efficacy testing, risk assessment and modeling efforts. Important information includes: population genetic structure, population dynamics (including density dependence), spatial distribution, migration patterns if applicable, inter-species interactions, and environmental variability. Distribution and abundance information of target species should be gathered throughout the duration of the program to inform the planning and possible deployment of genetic biological control agents.

Multi-stakeholder deliberation

Multi-stakeholder deliberations should be science driven, transparent, equitable and led by qualified experts trained in the social sciences (e.g., sociology, psychology, conflict management) and communication (see chapter 5). We recommend staging deliberations to occur at specific go/no-go steps in the program. This iterative approach progressively builds trust and a shared understanding among stakeholders. It also focuses deliberations on informing the decision on whether to proceed to the next phase in the program. Leaders of this deliberation component need to work closely with scientists developing the genetic biocontrol agent and researching its potential risks (chapters 2 and 3) and the regulatory analyst (chapter 4). A deliberation strategy should be developed very early in the research phase and revised as the program moves forward. This will require careful planning to coordinate the timing of this with points at which the technical work is far enough along to be able to share concrete information with some reasonable level of confidence.

Seeking regulatory approval

Early efforts should be made to understand requirements for regulatory approval via ongoing consultations and interagency coordination. Policy and regulation in this area is very much in flux and fraught with uncertainties, as stressed in chapter 4. Therefore, it will be important to keep abreast of evolving policy and regulation as the genetic

biocontrol program moves forward. Information required for submission of final applications for regulatory approval should be identified and built into the research program. It is critically important to coordinate the process of applying for regulatory approval with multi-stakeholder deliberation at this point.

Effort coordination

We also recommend an effort coordination component to knit together the work under the above seven components into an integrated program (Figure 6.1, 6.2). The objectives of effort coordinate are to recruit and retain essential staff, resources and capabilities; to establish a framework for inter-component coordination and decision making; to compile and review results; and to ensure proper program review via an independent scientific review panel.

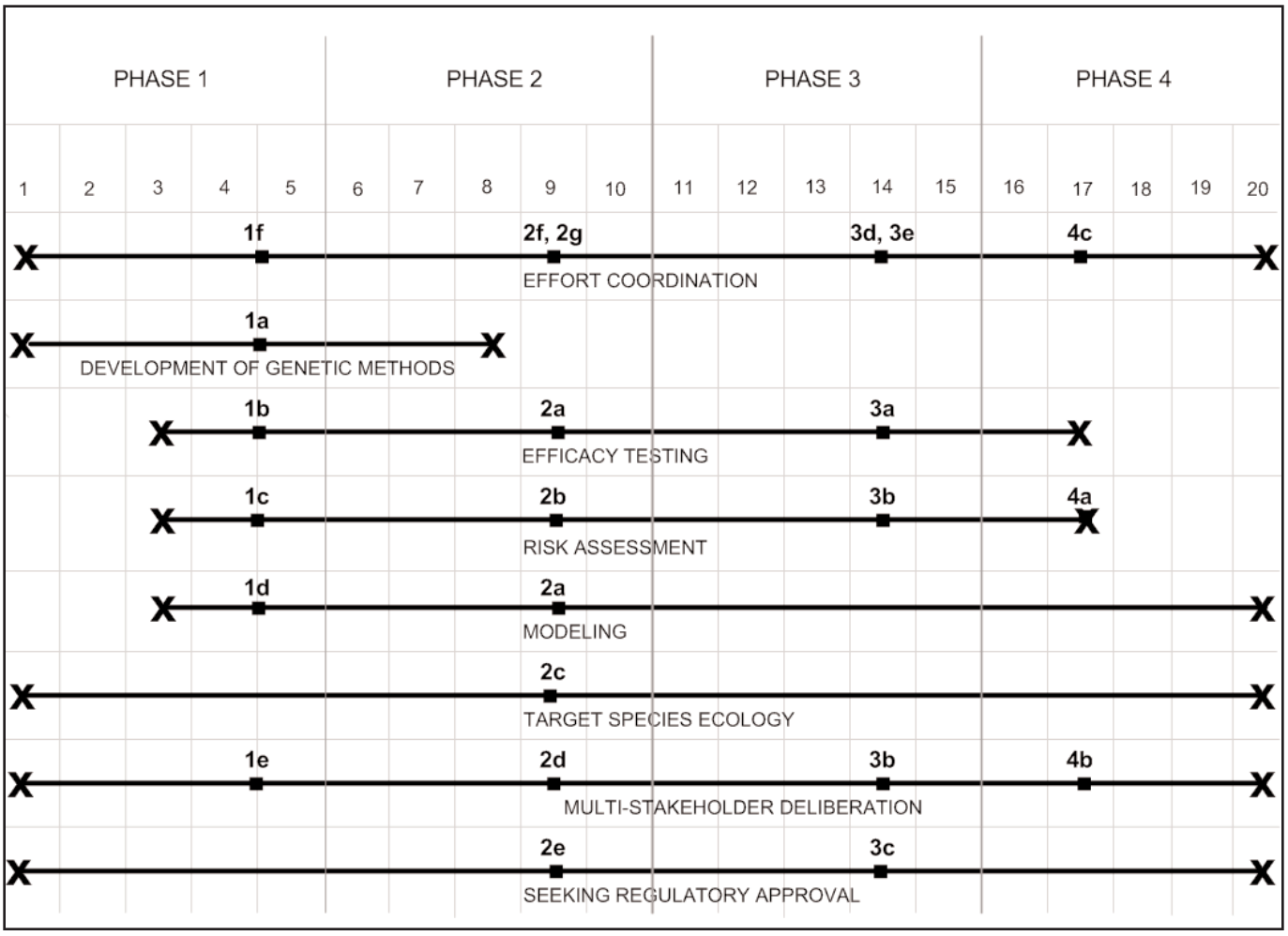
Suggested Go/No-Go Steps

We suggest four research program phases and specific go/no-go steps or decision points to guide transition of the program from one phase to another. These go/no-go steps should help program managers ensure that necessary support, information and resources have been obtained for each of the major components within each phase. Table 6.1 highlights these phases and suggested go/no-go steps. Figure 6.2 illustrates suggested timing of component-specific go/no-go steps during each phase. In presenting this phased approach, we recognize that any research program to develop genetic methods for biological control of non-native fish will have to be flexible enough to adapt to new information and scientific breakthroughs and change direction if needed. The details we have laid out may therefore change but we would still recommend the general approach.

Table 6.1. Preliminary outline of go/no-go steps to consider for development and implementation of a transgenic fish biocontrol program.

Phase 1- Contained Lab Tests	
1a. Was it possible to develop a stable line of transgenic fish and does it show the expected phenotype? If yes, then proceed.	1d. Do early population dynamics models of transgene spread, incorporating preliminary Gila River site-specific and population biology data, show desired trends? If yes, then proceed.
1b. Is the intended trait successfully passed on to wild relatives in the simplest laboratory efficacy tests? If yes, then proceed.	1e. Do results of multi-stakeholder deliberations indicate broad support for exploring the feasibility of genetic biocontrol through this research program? If yes, then proceed.
1c. Have appropriate ecological risk assessment studies been completed in the simplest environment? If yes, then proceed; if no, carry out such studies before proceeding, in order to inform next round of tests.	1f. Is necessary funding available to continue on all components? If yes, then proceed.
Phase 2- More Complex and Confined Tests	
2a. Are expected population declines observed in more complex efficacy experiments and simulation modeling? If yes, then proceed.	2d. Are efforts underway to conduct multi-stakeholder deliberations at this phase and do results indicate support for continued research? If yes, then proceed.
2b. Has the project now generated an adequate base of scientific information on benefits and risks to allow well-informed deliberations by stakeholder groups and decisions by the project-executing and regulatory agencies? If yes, then proceed.	2f. Has an appropriate independent scientific team reviewed progress to date and supported moving forward? If yes, then proceed.
2c. Is enough known about the biology and ecology of the target species within the Gila River to properly inform more complex efficacy tests, risk assessment and modeling? If yes, then proceed; if no, gather the key missing data.	2e. Can identified obstacles to regulatory approval be surmounted? If yes, then proceed.
	2g. Is necessary funding available to continue? If yes, then proceed.
Phase 3- Most Complex Tests in Isolated Stream Reaches	
3a. Are expected population responses observed in the most complex efficacy tests? If yes, then proceed.	3c. Have additional obstacles to regulatory approval been identified and does it look like they can be surmounted? If yes, then proceed.
3b. Do multi-stakeholder deliberations on needs, options and risk and informed by research results obtained by this point indicate continued strong support? Are identified hazards and risks within the ranges of risks acceptable to the multi-stakeholder group? If yes, then proceed.	3d. Has an appropriate independent scientific team reviewed progress to date and supported moving forward? If yes, then proceed.
	3e. Is necessary funding available to continue? If yes, then proceed.
Phase 4- Final Application for Regulatory Approval	
4a. Are there adequate plans in place for managing risks (including monitoring for desired effects and unintended problems and for taking corrective action if needed? If yes, then proceed	and risks/safety that will be presented in the application? If yes, then proceed.
4b. Are adequate plans in place for multi-stakeholder deliberation informed by the scientific results on efficacy	4c. Is necessary funding available to continue? If yes, then proceed.

Figure 6.2. Timeline of suggested go/no-go steps within multiple components of a program for developing and implementing transgenic fish for biocontrol. Numbers and letters directly above a line correspond to go/no-go steps in Table 6.1.



Cost Estimates for the General Categories of Costs

We estimate that development of a transgenic fish, taking into account all necessary components of a research program, will cost 15-20 million dollars over a 20 year period. We estimate the development of a triploid fish to cost 3-5 million dollars over a 5 year period (Table 6.2). Detailed cost estimates for each of the major research components described above appear in Tables 6.3 and 6.4 for development of transgenic and triploid fish, respectively. Table 6.3 presents phase-specific costs. Costs for food safety evaluation of transgenic fish included under the risk assessment component in Table 6.3 represent our best available estimates given the limited available information on this subject (see Appendix 6).

The nature of a complex, adaptive research program could warrant serious consideration of whether to suspend research and development during any phase for a variety of reasons. These include: insufficient funds, changes in multi-stakeholder support and general public opinion, insurmountable regulatory requirements, or results from efficacy testing and risk assessment research which do not support moving forward. Program coordinators should communicate this possibility to all stakeholders, policy makers and the general public before initial financial investments in the program. This is the best way to avoid unrealistic expectations of a result which cannot be assured at the outset.

Table 6.2. Summary of estimated costs for developing a transgenic and a triploid fish for biological control of non-native Fish in the Gila River. All costs are in 2004 dollars and based on detailed cost estimates in Tables 6.3 and 6.4.

General Cost Category	Development of Transgenic Fish- 20 year plan	Development of Triploid Fish- 5 year plan
Effort Coordination	$3,257,000	$400,900
Development of Genetic Methods	$2,500,000	$262,600
Efficacy Testing	$4,037,400	$966,200
Risk Assessment	$2,294,800	$203,600
Modeling	$545,800	$67,100
Target Species Ecology	$4,746,000	$1,779,500
Community Awareness and Involvement	$726,800	$121,700
Seeking Regulatory Approval	$539,300	$54,300
Total Costs	**$18,647,100**	**$3,855,900**

Table 6.3. General categories of costs and cost estimates to develop a transgenic fish for biological control of non-native fish in the Gila River Basin. Cost estimates in 2004 dollars and in thousands of dollars. NOTE: Estimates may be very conservative–agencies need to carefully reassess if they decide to begin a research effort.

Component	Phase 1				
	Year 1	Year 2	Year 3	Year 4	Year 5
EFFORT COORDINATION					
Project Manager Base Salary @ GS level 15 (% time varies during course of program)	97.0	99.4	101.9	104.4	107.0
Estimated Percent Time Required	0.40	0.50	0.40	0.30	0.25
Project Manager Salary (adjusted for % time)	**38.8**	**49.7**	**40.8**	**31.3**	**26.8**
Administrative Assistant @ GS level 7	33.1	33.9	34.7	35.6	36.5
Assistant Salary (adjusted for % time- same % as Project Manager)	**13.2**	**16.9**	**13.9**	**10.7**	**9.1**
Cross-Component Database Manager Salary- M.S. level @ GS level 11 (100% time)			**48.9**	**50.2**	**51.4**
Cross-Component Statistician- Ph.D. level @ GS level 13 (50% time)			69.8	71.5	73.3
Statistician Salary (adjusted for 50% time)			**34.9**	**35.8**	**36.6**
Independent Scientific Review Panel Input	5.0	5.0	5.0	5.0	5.0
Travel Related to Independent Scientific Review	5.0	5.0	5.0	5.0	5.0
Independent Scientific Review Total	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Annual Program-wide Planning, Review and Coordination Meetings	3.0	3.0	3.0	3.0	3.0
Travel for Recruitment Program Staff and Program-wide Meetings	5.0	5.0	5.0	5.0	5.0
Annual Planning, Review and Coordinating Expenses	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**
Total Effort Coordination	**70.0**	**84.6**	**156.5**	**145.9**	**142.0**
DEVELOPMENT OF GENETIC METHODS (CONTRACTED OUT)					
Operational, Equiptment and Salary Costs to Develop Transgenic Fish	**250.0**	**500.0**	**500.0**	**400.0**	**400.0**
Total Development of Genetic Methods	**250.0**	**500.0**	**500.0**	**400.0**	**400.0**
EFFICACY TESTING					
Efficacy Research Program Leader- Ph.D. Level Senior Scientist @ GS level 14 (100% time)			82.4	84.5	86.6
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Risk Assessment)			24.5	25.1	25.7
2 Half-time or 1 Full time B.S. Level Technician @ GS level 5 (overtime for weekend, etc. not included)			26.7	27.4	28.1
Efficacy Testing Staff Salary			**133.6**	**137.0**	**140.4**
Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same Equiptment					
Simplest Environment- Laboratory Tests:					
One-time Construction Costs for Retrofitting an Existing Laboratory Facility for Biocontainment and Security	400.0				
One-time Costs for Equiptment for Conducting Laboratory Experiments, Including Tanks, Circulation System, etc.	100.0				
Annual Lab Operating Costs (filters, chemicals, feed)			12.0	12.0	12.0
Miscellaneous Supplies			5.0	5.0	5.0
Total for Simplest Environment Laboratory Tests	**500.0**		**17.0**	**17.0**	**17.0**
More Complex Environment- Outdoor Artificial Streams:					
Design and Construction of Secure Outdoor Systems (~10 1/4 hectare ponds)				100.0	
Additional Staff Salary Required for Security of Outdoor Complex @ GS 5 level (.75 time)					
Estimated Percent Time Required					
Total for Additional Staff Salary for Security					
Annual Pond Operation Costs (filters, chemicals, feed)					
Miscellaneous Supplies					
Total for Artificial Outdoor Tests				**100.0**	
Most Complex- Isolated Reach of Actual Stream:					
One-time Design and Construction of Secure Research Site					
Travel Costs to Research Site					
Additional Staff Salary Required for Security of Research Site @ GS 5 level (100% time)					
Miscellaneous Supplies					
Total for Isolated Research of Actual Stream					
Total Efficacy Testing	**500.0**		**150.6**	**254.0**	**157.4**

Table 6.3. Phase 1 *Continued.*

Component	Phase 1				
	Year 1	Year 2	Year 3	Year 4	Year 5
RISK ASSESSMENT					
Ecological Risk Assessment					
Risk Assessment Specialist- Ph.D. level @ GS level 13 (100% time)			69.8	71.5	73.3
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Efficacy Testing)			24.5	25.1	25.7
Total Staff for Ecological Risk Assessment			**94.2**	**96.6**	**99.0**
Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same equiptment					
Additional Research Supplies			5.0	5.0	5.0
Travel for Program-wide Meetings			2.0	2.0	2.0
Total Ecological Risk Assessment Operating Expenses			**7.0**	**7.0**	**7.0**
Food Safety Risk Assessment (Assumed that Work is Contracted Out)					
Food Safety Research (Evaluating Potential of Harm for Human Consumption of Transgenic Fish)					
Total Food Safety Assessment Expenses					
Total Risk Assessment			**101.2**	**103.6**	**106.0**
MODELING					
Modeler- Ph.D level @ GS level 13 (% time varies during course of program)			69.8	71.5	73.3
Estimated Percent Time Required			0.25	0.25	0.40
Modeler Salary (Adjusted for % time)			**17.4**	**17.9**	**29.3**
Supplies			2.5	2.5	2.5
Travel for program-wide meetings			2.5	2.5	2.5
Total Operating Expenses			**5.0**	**5.0**	**5.0**
Total Modeling			**22.4**	**22.9**	**34.3**
TARGET SPECIES ECOLOGY					
Research on Non-native Species Distribution and Abundance (Agency Coordinated- Part of Existing Work):					
Project Leader- M.S. level biology @ GS 11 level (50% time)	48.9	50.2	51.4	52.7	54.0
Project Leader- Salary Adjusted at 50% time	24.5	25.1	25.7	26.4	27.0
Field Crew Leader- B.S./M.S. level @ GS 8 level (50% time)	36.6	37.5	38.5	39.4	40.4
Field Crew Leader- Salary Adjusted at 50% time	18.3	18.8	19.2	19.7	20.2
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	26.7	27.4	28.1	28.8	29.5
Field Crew member- Salary Adjusted at 50% time	13.3	13.7	14.0	14.4	14.7
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	26.7	27.4	28.1	28.8	29.5
Field Crew member- Salary Adjusted at 50% time	13.3	13.7	14.0	14.4	14.7
Total Salary for Target Species Ecology	**69.5**	**71.2**	**73.0**	**74.8**	**76.7**
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Travel to Reseach Sites	6.0	6.0	6.0	6.0	6.0
Survey Equiptment	5.0	1.0	1.0	1.0	1.0
Total for Reseach on Non-native Species Distribution and Abundance	**82.5**	**80.2**	**82.0**	**83.8**	**85.7**
Research on Biology of Target Organism(s) (Contracted out) (5 years of funding at 100K year):	100.0	100.0	100.0	100.0	100.0
Total for Research on Biology of Target Organisms	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Research on Ecology of Target Organism(s) (Contracted out) (5 years of funding at 100 K year):	100.0	100.0	100.0	100.0	100.0
Total for Research on Ecology of Target Organisms	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Total Target Species Ecology	**352.0**	**351.4**	**355.0**	**358.7**	**362.4**

Table 6.3. Phase 1 *Continued.*

Component	Phase 1				
	Year 1	Year 2	Year 3	Year 4	Year 5
MULTI-STAKEHOLDER DELIBERATION					
Multi-Stakeholder Deliberation Specialist- M.S. or Ph.D. level @ GS 12 level	58.7	60.1	61.6	63.2	64.8
Estimated Percent Time Required	0.20	0.20	0.25	0.25	0.25
Multi-Stakeholder Deliberation Specialist Salary (Adjusted for % time)	**11.7**	**12.0**	**15.4**	**15.8**	**16.2**
Travel for Program-wide Meetings	2.5	2.5	2.5	2.5	2.5
Expenses for Interactions with the Community	10.0	10.0	12.5	12.5	12.5
Total Operating Expenses	**12.5**	**12.5**	**15.0**	**15.0**	**15.0**
Total Multi-Stakeholder Deliberation	**24.2**	**24.5**	**30.4**	**30.8**	**31.2**
SEEKING REGULATORY APPROVAL					
Policy/Legal/Regulatory Specialist- M.S., J.D. or Ph.D. level @ GS 13 level	69.8	71.5	73.3	75.1	77.0
Estimated Percent Time Required	0.10	0.10	0.10	0.10	0.10
Policy/Legal/Regulatory Specialist Salary (Adjusted for % time)	**7.0**	**7.2**	**7.3**	**7.5**	**7.7**
Travel for Program-wide and Other Meetings	2.0	2.0	2.0	2.0	2.0
Total Operating Expenses	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**
Total Seeking Regulatory Approval	**9.0**	**9.2**	**9.3**	**9.5**	**9.7**
TOTAL- All Components Phase 1	**1205.2**	**969.8**	**1325.5**	**1325.3**	**1242.9**

Table 6.3. Phase 2

Component	Phase 2				
	Year 6	Year 7	Year 8	Year 9	Year 10
EFFORT COORDINATION					
Project Manager Base Salary @ GS level 15 (% time varies during course of program)	109.7	112.5	115.3	118.1	121.1
Estimated Percent Time Required	0.20	0.20	0.20	0.20	0.20
Project Manager Salary (adjusted for % time)	**21.9**	**22.5**	**23.1**	**23.6**	**24.2**
Administrative Assistant @ GS level 7	37.4	38.4	39.3	40.3	41.3
Assistant Salary (adjusted for % time- same % as Project Manager)	**7.5**	**7.7**	**7.9**	**8.1**	**8.3**
Cross-Component Database Manager Salary- M.S. level @ GS level 11 (100% time)	**52.7**	**54.0**	**55.4**	**56.8**	**58.2**
Cross-Component Statistician- Ph.D. level @ GS level 13 (50% time)	75.1	77.0	78.9	80.9	82.9
Statistician Salary (adjusted for 50% time)	**37.6**	**38.5**	**39.5**	**40.5**	**41.5**
Independent Scientific Review Panel Input	5.0	5.0	5.0	5.0	5.0
Travel Related to Independent Scientific Review	5.0	5.0	5.0	5.0	5.0
Independent Scientific Review Total	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Annual Program-wide Planning, Review and Coordination Meetings	3.0	3.0	3.0	3.0	3.0
Travel for Recruitment Program Staff and Program-wide Meetings	5.0	5.0	5.0	5.0	5.0
Annual Planning, Review and Coordinating Expenses	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**
Total Effort Coordination	**137.7**	**140.7**	**143.8**	**146.9**	**150.1**
DEVELOPMENT OF GENETIC METHODS (CONTRACTED OUT)					
Operational, Equiptment and Salary Costs to Develop Transgenic Fish	**250.0**	**100.0**	**100.0**		
Total Development of Genetic Methods	**250.0**	**100.0**	**100.0**		
EFFICACY TESTING					
Efficacy Research Program Leader- Ph.D. Level Senior Scientist @ GS level 14 (100% time)	88.8	91.0	93.3	95.6	98.0
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Risk Assessment)	26.4	27.0	27.7	28.4	29.1
2 Half-time or 1 Full time B.S. Level Technician @ GS level 5 (overtime for weekend, etc. not included)	28.8	29.5	30.2	31.0	31.7
Efficacy Testing Staff Salary	**143.9**	**147.5**	**151.2**	**154.9**	**158.8**
*****Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same Equiptment*****					
Simplest Environment- Laboratory Tests:					
One-time Construction Costs for Retrofitting an Existing Laboratory Facility for Biocontainment and Security					
One-time Costs for Equiptment for Conducting Laboratory Experiments, Including Tanks, Circulation System, etc.					
Annual Lab Operating Costs (filters, chemicals, feed)	12.0	12.0	12.0	12.0	12.0
Miscellaneous Supplies	5.0	5.0	5.0	5.0	5.0
Total for Simplest Environment Laboratory Tests	**17.0**	**17.0**	**17.0**	**17.0**	**17.0**
More Complex Environment- Outdoor Artificial Streams:					
Design and Construction of Secure Outdoor Systems (~10 1/4 hectare ponds)					
Additional Staff Salary Required for Security of Outdoor Complex @ GS 5 level (.75 time)	26.7	27.4	28.1	28.8	29.5
Estimated Percent Time Required	0.75	0.75	0.75	0.75	0.75
Total for Additional Staff Salary for Security	20.0	20.5	21.0	21.6	22.1
Annual Pond Operation Costs (filters, chemicals, feed)	12.0	12.0	12.0	12.0	12.0
Miscellaneous Supplies	8.0	8.0	8.0	8.0	8.0
Total for Artificial Outdoor Tests	**40.0**	**40.5**	**41.0**	**41.6**	**42.1**
Most Complex- Isolated Reach of Actual Stream:					
One-time Design and Construction of Secure Research Site			100.0		
Travel Costs to Research Site					6.0
Additional Staff Salary Required for Security of Research Site @ GS 5 level (100% time)					26.7
Miscellaneous Supplies					15.0
Total for Isolated Research of Actual Stream			**100.0**		**47.7**
Total Efficacy Testing	**200.9**	**205.0**	**309.2**	**213.5**	**265.6**

Table 6.3. Phase 2 *Continued.*

Component	Phase 2				
	Year 6	Year 7	Year 8	Year 9	Year 10
RISK ASSESSMENT					
Ecological Risk Assessment					
Risk Assessment Specialist- Ph.D. level @ GS level 13 (100% time)	75.1	77.0	78.9	80.9	82.9
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Efficacy Testing)	26.4	27.0	27.7	28.4	29.1
Total Staff for Ecological Risk Assessment	**101.5**	**104.0**	**106.6**	**109.3**	**112.0**
Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same equiptment					
Additional Research Supplies	5.0	5.0	5.0	5.0	5.0
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Total Ecological Risk Assessment Operating Expenses	**7.0**	**7.0**	**7.0**	**7.0**	**7.0**
Food Safety Risk Assessment (Assumed that Work is Contracted Out)					
Food Safety Research (Evaluating Potential of Harm for Human Consumption of Transgenic Fish)					**250.0**
Total Food Safety Assessment Expenses					
Total Risk Assessment	**108.5**	**111.0**	**113.6**	**116.3**	**369.0**
MODELING					
Modeler- Ph.D level @ GS level 13 (% time varies during course of program)	75.1	77.0	78.9	80.9	82.9
Estimated Percent Time Required	0.40	0.50	0.50	0.40	0.40
Modeler Salary (Adjusted for % time)	**30.1**	**38.5**	**39.5**	**32.4**	**33.2**
Supplies	2.5	2.5	2.5	2.5	2.5
Travel for program-wide meetings	2.5	2.5	2.5	2.5	2.5
Total Operating Expenses	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**
Total Modeling	**35.1**	**43.5**	**44.5**	**37.4**	**38.2**
TARGET SPECIES ECOLOGY					
Research on Non-native Species Distribution and Abundance (Agency Coordinated- Part of Existing Work):					
Project Leader- M.S. level biology @ GS 11 level (50% time)	55.4	56.8	58.2	59.6	61.1
Project Leader- Salary Adjusted at 50% time	27.7	28.4	29.1	29.8	30.6
Field Crew Leader- B.S./M.S. level @ GS 8 level (50% time)	41.4	42.5	43.5	44.6	45.7
Field Crew Leader- Salary Adjusted at 50% time	20.7	21.2	21.8	22.3	22.9
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	30.2	31.0	31.7	32.5	33.3
Field Crew member- Salary Adjusted at 50% time	15.1	15.5	15.9	16.3	16.7
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	30.2	31.0	31.7	32.5	33.3
Field Crew member- Salary Adjusted at 50% time	15.1	15.5	15.9	16.3	16.7
Total Salary for Target Species Ecology	**78.6**	**80.6**	**82.6**	**84.7**	**86.8**
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Travel to Reseach Sites	6.0	6.0	6.0	6.0	6.0
Survey Equiptment	5.0	1.0	1.0	1.0	1.0
Total for Reseach on Non-native Species Distribution and Abundance	**91.6**	**89.6**	**91.6**	**93.7**	**95.8**
Research on Biology of Target Organism(s) (Contracted out) (5 years of funding at 100K year):					
Total for Research on Biology of Target Organisms					
Research on Ecology of Target Organism(s) (Contracted out) (5 years of funding at 100 K year):					
Total for Research on Ecology of Target Organisms					
Total Target Species Ecology	**170.2**	**170.2**	**174.2**	**178.3**	**182.6**

Table 6.3. Phase 2 *Continued.*

Component	Phase 2				
	Year 6	Year 7	Year 8	Year 9	Year 10
MULTI-STAKEHOLDER DELIBERATION					
Multi-Stakeholder Deliberation Specialist- M.S. or Ph.D. level @ GS 12 level	66.4	68.0	69.7	71.5	73.3
Estimated Percent Time Required	0.25	0.40	0.40	0.40	0.40
Multi-Stakeholder Deliberation Specialist Salary (Adjusted for % time)	**16.6**	**27.2**	**27.9**	**28.6**	**29.3**
Travel for Program-wide Meetings	2.5	2.5	2.5	2.5	2.5
Expenses for Interactions with the Community	12.5	20.0	20.0	20.0	20.0
Total Operating Expenses	**15.0**	**22.5**	**22.5**	**22.5**	**22.5**
Total Multi-Stakeholder Deliberation	**31.6**	**49.7**	**50.4**	**51.1**	**51.8**
SEEKING REGULATORY APPROVAL					
Policy/Legal/Regulatory Specialist- M.S., J.D. or Ph.D. level @ GS 13 level	78.9	80.9	82.9	85.0	87.1
Estimated Percent Time Required	0.20	0.30	0.40	0.40	0.40
Policy/Legal/Regulatory Specialist Salary (Adjusted for % time)	**15.8**	**24.3**	**33.2**	**34.0**	**34.8**
Travel for Program-wide and Other Meetings	2.0	2.0	2.0	2.0	4.0
Total Operating Expenses	**2.0**	**2.0**	**2.0**	**2.0**	**4.0**
Total Seeking Regulatory Approval	**17.8**	**26.3**	**35.2**	**36.0**	**38.8**
TOTAL- All Components Phase 2	**951.8**	**846.4**	**970.8**	**779.5**	**1096.1**

Table 6.3. Phase 3

Component	Phase 3				
	Year 11	Year 12	Year 13	Year 14	Year 15
EFFORT COORDINATION					
Project Manager Base Salary @ GS level 15 (% time varies during course of program)	124.1	127.2	130.4	133.7	137.0
Estimated Percent Time Required	0.20	0.30	0.40	0.50	0.40
Project Manager Salary (adjusted for % time)	**24.8**	**38.2**	**52.2**	**66.8**	**54.8**
Administrative Assistant @ GS level 7	42.3	43.4	44.5	45.6	46.7
Assistant Salary (adjusted for % time- same % as Project Manager)	**8.5**	**13.0**	**17.8**	**22.8**	**18.7**
Cross-Component Database Manager Salary- M.S. level @ GS level 11 (100% time)	**59.6**	**61.1**	**62.7**	**64.2**	**65.8**
Cross-Component Statistician- Ph.D. level @ GS level 13 (50% time)	85.0	87.1	89.3	91.5	93.8
Statistician Salary (adjusted for 50% time)	**42.5**	**43.6**	**44.7**	**45.8**	**46.9**
Independent Scientific Review Panel Input	5.0	5.0	5.0	5.0	5.0
Travel Related to Independent Scientific Review	5.0	5.0	5.0	5.0	5.0
Independent Scientific Review Total	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Annual Program-wide Planning, Review and Coordination Meetings	3.0	3.0	3.0	3.0	3.0
Travel for Recruitment Program Staff and Program-wide Meetings	5.0	5.0	5.0	5.0	5.0
Annual Planning, Review and Coordinating Expenses	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**
Total Effort Coordination	**153.4**	**173.9**	**195.3**	**217.6**	**204.2**
DEVELOPMENT OF GENETIC METHODS (CONTRACTED OUT)					
Operational, Equiptment and Salary Costs to Develop Transgenic Fish					
Total Development of Genetic Methods					
EFFICACY TESTING					
Efficacy Research Program Leader- Ph.D. Level Senior Scientist @ GS level 14 (100% time)	100.4	103.0	105.5	108.2	110.9
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Risk Assessment)	29.8	30.6	31.3	32.1	32.9
2 Half-time or 1 Full time B.S. Level Technician @ GS level 5 (overtime for weekend, etc. not included)	32.5	33.3	34.2	35.0	35.9
Efficacy Testing Staff Salary	**162.8**	**166.9**	**171.0**	**175.3**	**179.7**
*****Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same Equiptment*****					
Simplest Environment- Laboratory Tests:					
One-time Construction Costs for Retrofitting an Existing Laboratory Facility for Biocontainment and Security					
One-time Costs for Equiptment for Conducting Laboratory Experiments, Including Tanks, Circulation System, etc.					
Annual Lab Operating Costs (filters, chemicals, feed)	12.0	12.0	12.0	12.0	12.0
Miscellaneous Supplies	5.0				
Total for Simplest Environment Laboratory Tests	**17.0**	**12.0**	**12.0**	**12.0**	**12.0**
More Complex Environment- Outdoor Artificial Streams:					
Design and Construction of Secure Outdoor Systems (~10 1/4 hectare ponds)					
Additional Staff Salary Required for Security of Outdoor Complex @ GS 5 level (.75 time)	30.2	31.0	31.7		
Estimated Percent Time Required	0.75	0.75	0.75		
Total for Additional Staff Salary for Security	22.7	23.2	23.8		
Annual Pond Operation Costs (filters, chemicals, feed)	12.0	12.0	12.0		
Miscellaneous Supplies	8.0	8.0	8.0		
Total for Artificial Outdoor Tests	**42.7**	**43.2**	**43.8**		
Most Complex- Isolated Reach of Actual Stream:					
One-time Design and Construction of Secure Research Site					
Travel Costs to Research Site	6.0	6.0	6.0	6.0	6.0
Additional Staff Salary Required for Security of Research Site @ GS 5 level (100% time)	26.7	26.7	26.7	26.7	26.7
Miscellaneous Supplies	15.0	15.0	15.0	15.0	15.0
Total for Isolated Research of Actual Stream	**47.7**	**47.7**	**47.7**	**47.7**	**47.7**
Total Efficacy Testing	**270.1**	**269.8**	**274.5**	**235.0**	**239.4**

Table 6.3. Phase 3 *Continued.*

Component	Phase 3				
	Year 11	Year 12	Year 13	Year 14	Year 15
RISK ASSESSMENT					
Ecological Risk Assessment					
Risk Assessment Specialist- Ph.D. level @ GS level 13 (100% time)	85.0	87.1	89.3	91.5	93.8
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Efficacy Testing)	29.8	30.6	31.3	32.1	32.9
Total Staff for Ecological Risk Assessment	**114.8**	**117.7**	**120.6**	**123.6**	**126.7**
Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same equiptment					
Additional Research Supplies	5.0	5.0	5.0	5.0	5.0
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Total Ecological Risk Assessment Operating Expenses	**7.0**	**7.0**	**7.0**	**7.0**	**7.0**
Food Safety Risk Assessment (Assumed that Work is Contracted Out)					
Food Safety Research (Evaluating Potential of Harm for Human Consumption of Transgenic Fish)	**250.0**				
Total Food Safety Assessment Expenses					
Total Risk Assessment	**371.8**	**124.7**	**127.6**	**130.6**	**133.7**
MODELING					
Modeler- Ph.D level @ GS level 13 (% time varies during course of program)	85.0	87.1	89.3	91.5	93.8
Estimated Percent Time Required	0.25	0.25	0.25	0.25	0.25
Modeler Salary (Adjusted for % time)	**21.2**	**21.8**	**22.3**	**22.9**	**23.5**
Supplies	2.5	2.5	2.5	2.5	2.5
Travel for program-wide meetings	2.5	2.5	2.5	2.5	2.5
Total Operating Expenses	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**
Total Modeling	**26.2**	**26.8**	**27.3**	**27.9**	**28.5**
TARGET SPECIES ECOLOGY					
Research on Non-native Species Distribution and Abundance (Agency Coordinated- Part of Existing Work):					
Project Leader- M.S. level biology @ GS 11 level (50% time)	62.7	64.2	65.8	67.5	69.2
Project Leader- Salary Adjusted at 50% time	31.3	32.1	32.9	33.7	34.6
Field Crew Leader- B.S./M.S. level @ GS 8 level (50% time)	46.9	48.1	49.3	50.5	51.8
Field Crew Leader- Salary Adjusted at 50% time	23.4	24.0	24.6	25.2	25.9
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	34.2	35.0	35.9	36.8	37.7
Field Crew member- Salary Adjusted at 50% time	17.1	17.5	18.0	18.4	18.9
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	34.2	35.0	35.9	36.8	37.7
Field Crew member- Salary Adjusted at 50% time	17.1	17.5	18.0	18.4	18.9
Total Salary for Target Species Ecology	**88.9**	**91.2**	**93.5**	**95.8**	**98.2**
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Travel to Reseach Sites	6.0	6.0	6.0	6.0	6.0
Survey Equiptment	5.0	1.0	1.0	1.0	1.0
Total for Reseach on Non-native Species Distribution and Abundance	**101.9**	**100.2**	**102.5**	**104.8**	**107.2**
Research on Biology of Target Organism(s) (Contracted out) (5 years of funding at 100K year):					
Total for Research on Biology of Target Organisms					
Research on Ecology of Target Organism(s) (Contracted out) (5 years of funding at 100 K year):					
Total for Research on Ecology of Target Organisms					
Total Target Species Ecology	**190.9**	**191.3**	**195.9**	**200.6**	**205.4**

Table 6.3. Phase 3 *Continued.*

Component	Phase 3				
	Year 11	Year 12	Year 13	Year 14	Year 15
MULTI-STAKEHOLDER DELIBERATION					
Multi-Stakeholder Deliberation Specialist- M.S. or Ph.D. level @ GS 12 level	75.1	77.0	78.9	80.9	82.9
Estimated Percent Time Required	0.40	0.25	0.25	0.25	0.25
Multi-Stakeholder Deliberation Specialist Salary (Adjusted for % time)	**30.0**	**19.2**	**19.7**	**20.2**	**20.7**
Travel for Program-wide Meetings	2.5	2.5	2.5	2.5	2.5
Expenses for Interactions with the Community	20.0	12.5	12.5	12.5	12.5
Total Operating Expenses	**22.5**	**15.0**	**15.0**	**15.0**	**15.0**
Total Multi-Stakeholder Deliberation	**52.5**	**34.2**	**34.7**	**35.2**	**35.7**
SEEKING REGULATORY APPROVAL					
Policy/Legal/Regulatory Specialist- M.S., J.D. or Ph.D. level @ GS 13 level	89.3	91.5	93.8	96.2	98.6
Estimated Percent Time Required	0.50	0.50	0.50	0.50	0.40
Policy/Legal/Regulatory Specialist Salary (Adjusted for % time)	**44.7**	**45.8**	**46.9**	**48.1**	**39.4**
Travel for Program-wide and Other Meetings	4.0	4.0	4.0	4.0	4.0
Total Operating Expenses	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**
Total Seeking Regulatory Approval	**48.7**	**49.8**	**50.9**	**52.1**	**43.4**
TOTAL- All Components Phase 3	**1113.7**	**870.5**	**906.3**	**899.0**	**890.3**

Table 6.3. Phase 4

Component	Phase 4				
	Year 16	Year 17	Year 18	Year 19	Year 20
EFFORT COORDINATION					
Project Manager Base Salary @ GS level 15 (% time varies during course of program)	140.4	144.0	147.6	151.2	155.0
Estimated Percent Time Required	0.30	0.30	0.30	0.30	0.30
Project Manager Salary (adjusted for % time)	**42.1**	**43.2**	**44.3**	**45.4**	**46.5**
Administrative Assistant @ GS level 7	47.9	49.1	50.3	51.6	52.9
Assistant Salary (adjusted for % time- same % as Project Manager)	**14.4**	**14.7**	**15.1**	**15.5**	**15.9**
Cross-Component Database Manager Salary- M.S. level @ GS level 11 (100% time)	**67.5**	**69.2**	**70.9**	**72.7**	**74.5**
Cross-Component Statistician- Ph.D. level @ GS level 13 (50% time)	96.2	98.6	101.0	103.6	106.2
Statistician Salary (adjusted for 50% time)	**48.1**	**49.3**	**50.5**	**51.8**	**53.1**
Independent Scientific Review Panel Input	5.0	5.0	5.0	5.0	5.0
Travel Related to Independent Scientific Review	5.0	5.0	5.0	5.0	5.0
Independent Scientific Review Total	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**
Annual Program-wide Planning, Review and Coordination Meetings	3.0	3.0	3.0	3.0	3.0
Travel for Recruitment Program Staff and Program-wide Meetings	5.0	5.0	5.0	5.0	5.0
Annual Planning, Review and Coordinating Expenses	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**
Total Effort Coordination	**190.1**	**194.4**	**198.8**	**203.3**	**207.9**
DEVELOPMENT OF GENETIC METHODS (CONTRACTED OUT)					
Operational, Equiptment and Salary Costs to Develop Transgenic Fish					
Total Development of Genetic Methods					
EFFICACY TESTING					
Efficacy Research Program Leader- Ph.D. Level Senior Scientist @ GS level 14 (100% time)	113.6	116.5			
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Risk Assessment)	33.7	34.6			
2 Half-time or 1 Full time B.S. Level Technician @ GS level 5 (overtime for weekend, etc. not included)	36.8	37.7			
Efficacy Testing Staff Salary	**184.2**	**188.8**			
*****Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same Equiptment*****					
Simplest Environment- Laboratory Tests:					
One-time Construction Costs for Retrofitting an Existing Laboratory Facility for Biocontainment and Security					
One-time Costs for Equiptment for Conducting Laboratory Experiments, Including Tanks, Circulation System, etc.					
Annual Lab Operating Costs (filters, chemicals, feed)	12.0	12.0			
Miscellaneous Supplies					
Total for Simplest Environment Laboratory Tests	**12.0**	**12.0**			
More Complex Environment- Outdoor Artificial Streams:					
Design and Construction of Secure Outdoor Systems (~10 1/4 hectare ponds)					
Additional Staff Salary Required for Security of Outdoor Complex @ GS 5 level (.75 time)					
Estimated Percent Time Required					
Total for Additional Staff Salary for Security					
Annual Pond Operation Costs (filters, chemicals, feed)					
Miscellaneous Supplies					
Total for Artificial Outdoor Tests					
Most Complex- Isolated Reach of Actual Stream:					
One-time Design and Construction of Secure Research Site					
Travel Costs to Research Site	6.0	6.0			
Additional Staff Salary Required for Security of Research Site @ GS 5 level (100% time)	26.7	26.7			
Miscellaneous Supplies	15.0	15.0			
Total for Isolated Research of Actual Stream	**47.7**	**47.7**			
Total Efficacy Testing	**243.9**	**248.5**			

Table 6.3. Phase 4 *Continued.*

Component	Phase 4				
	Year 16	Year 17	Year 18	Year 19	Year 20
RISK ASSESSMENT					
Ecological Risk Assessment					
Risk Assessment Specialist- Ph.D. level @ GS level 13 (100% time)	96.2	98.6			
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Efficacy Testing)	33.7	34.6			
Total Staff for Ecological Risk Assessment	**129.9**	**133.2**			
Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same equiptment					
Additional Research Supplies	5.0	5.0			
Travel for Program-wide Meetings	2.0	2.0			
Total Ecological Risk Assessment Operating Expenses	**7.0**	**7.0**			
Food Safety Risk Assessment (Assumed that Work is Contracted Out)					
Food Safety Research (Evaluating Potential of Harm for Human Consumption of Transgenic Fish)					
Total Food Safety Assessment Expenses					
Total Risk Assessment	**136.9**	**140.2**			
MODELING					
Modeler- Ph.D level @ GS level 13 (% time varies during course of program)	96.2	98.6	101.0	103.6	106.2
Estimated Percent Time Required	0.25	0.20	0.20	0.20	0.20
Modeler Salary (Adjusted for % time)	**24.0**	**19.7**	**20.2**	**20.7**	**21.2**
Supplies	2.5	2.5	2.5	2.5	2.5
Travel for program-wide meetings	2.5	2.5	2.5	2.5	2.5
Total Operating Expenses	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**
Total Modeling	**29.0**	**24.7**	**25.2**	**25.7**	**26.2**
TARGET SPECIES ECOLOGY					
Research on Non-native Species Distribution and Abundance (Agency Coordinated- Part of Existing Work):					
Project Leader- M.S. level biology @ GS 11 level (50% time)	70.9	72.7	74.5	76.3	78.2
Project Leader- Salary Adjusted at 50% time	35.4	36.3	37.2	38.2	39.1
Field Crew Leader- B.S./M.S. level @ GS 8 level (50% time)	53.0	54.4	55.7	57.1	58.6
Field Crew Leader- Salary Adjusted at 50% time	26.5	27.2	27.9	28.6	29.3
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	38.7	39.6	40.6	41.6	42.7
Field Crew member- Salary Adjusted at 50% time	19.3	19.8	20.3	20.8	21.3
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	38.7	39.6	40.6	41.6	42.7
Field Crew member- Salary Adjusted at 50% time	19.3	19.8	20.3	20.8	21.3
Total Salary for Target Species Ecology	**100.6**	**103.2**	**105.7**	**108.4**	**111.1**
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Travel to Reseach Sites	6.0	6.0	6.0	6.0	6.0
Survey Equiptment	5.0	1.0	1.0	1.0	1.0
Total for Reseach on Non-native Species Distribution and Abundance	**113.6**	**112.2**	**114.7**	**117.4**	**120.1**
Research on Biology of Target Organism(s) (Contracted out) (5 years of funding at 100K year):					
Total for Research on Biology of Target Organisms					
Research on Ecology of Target Organism(s) (Contracted out) (5 years of funding at 100 K year):					
Total for Research on Ecology of Target Organisms					
Total Target Species Ecology	**214.3**	**215.3**	**220.5**	**225.7**	**231.2**

Table 6.3. Phase 4 *Continued.*

Component	Phase 4				
	Year 16	Year 17	Year 18	Year 19	Year 20
MULTI-STAKEHOLDER DELIBERATION					
Multi-Stakeholder Deliberation Specialist- M.S. or Ph.D. level @ GS 12 level	85.0	87.1	89.3	91.5	93.8
Estimated Percent Time Required	0.25	0.20	0.20	0.20	0.20
Multi-Stakeholder Deliberation Specialist Salary (Adjusted for % time)	**21.2**	**17.4**	**17.9**	**18.3**	**18.8**
Travel for Program-wide Meetings	2.5	2.5	2.5	2.5	2.5
Expenses for Interactions with the Community	12.5	10.0	10.0	10.0	10.0
Total Operating Expenses	**15.0**	**12.5**	**12.5**	**12.5**	**12.5**
Total Multi-Stakeholder Deliberation	**36.2**	**29.9**	**30.4**	**30.8**	**31.3**
SEEKING REGULATORY APPROVAL					
Policy/Legal/Regulatory Specialist- M.S., J.D. or Ph.D. level @ GS 13 level	101.0	103.6	106.2	108.8	111.5
Estimated Percent Time Required	0.30	0.20	0.10	0.10	0.10
Policy/Legal/Regulatory Specialist Salary (Adjusted for % time)	**30.3**	**20.7**	**10.6**	**10.9**	**11.2**
Travel for Program-wide and Other Meetings	2.0	2.0	2.0	2.0	2.0
Total Operating Expenses	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**
Total Seeking Regulatory Approval	**32.3**	**22.7**	**12.6**	**12.9**	**13.2**
TOTAL- All Components Phase 4	**882.7**	**875.6**	**487.4**	**498.4**	**509.7**

Table 6.4. General categories of costs and cost estimates to develop a triploid fish for biological control of non-native fish in the Gila River Basin. Cost estimates in 2004 dollars and in thousands of dollars. NOTE: Estimates may be very conservative; agencies need to carefully reassess if they decide to begin a research effort.

Component	Year 1	Year 2	Year 3	Year 4	Year 5
EFFORT COORDINATION					
Project Manager Base Salary @ GS level 15 (25% time)	97.0	99.4	101.9	104.4	107.0
Estimated Percent Time Required	0.20	0.20	0.20	0.20	0.20
Project Manager Salary (adjusted for % time)	**19.4**	**19.9**	**20.4**	**20.9**	**21.4**
Administrative Assistant @ GS level 7	33.1	33.9	34.7	35.6	36.5
Assistant Salary (adjusted for % time- same % as Project Manager)	**6.6**	**6.8**	**6.9**	**7.1**	**7.3**
Cross-Component Database Manager Salary- M.S. level @ GS level 11 (25% time)			48.9	50.2	51.4
Estimated Percent Time Required			0.25	0.25	0.25
Database Manager Salary Adjusted for % time			**12.2**	**12.5**	**12.9**
Cross-Component Statistician- Ph.D. level @ GS level 13 (25% time)			69.8	71.5	73.3
Statistician Salary (adjusted for 50% time)			**17.4**	**17.9**	**18.3**
Independent Scientific Review Panel Input	3.00	3.00	3.00	3.00	3.00
Travel Related to Independent Scientific Review	3.00	3.00	3.00	3.00	3.00
Independent Scientific Review Total	**6.00**	**6.00**	**6.00**	**6.00**	**6.00**
Annual Program-wide Planning, Review and Coordination Meetings	3.00	3.00	3.00	3.00	3.00
Travel for Recruitment Program Staff and Program-wide Meetings	3.00	3.00	3.00	3.00	3.00
Annual Planning, Review and Coordinating Expenses	**6.00**	**6.00**	**6.00**	**6.00**	**6.00**
Total Effort Coordination	**38.0**	**38.7**	**105.7**	**108.1**	**110.5**
DEVELOPMENT OF GENETIC METHODS					
Lead Laboratory Biologist or Fish Geneticist- Ph.D. level @ GS level 13 (50% time)	69.8	71.5	73.3		
Estimated Percent Time Required	0.50	0.50	0.50		
Lead Laboratory Biologist Salary (Adjusted for % time)	**34.9**	**35.8**	**36.6**		
Research Assistant- M.S. level @ GS level 11 (50% time)	48.9	50.2	51.5		
Estimated Percent Time Required	0.50	0.50	0.50		
Research Assistant Salary (Adjusted for % time)	**24.5**	**25.1**	**25.7**		
Equiptment and Supply Costs to Refine Methods and Produce Triploid Fish	**60.0**	**5.0**	**5.0**	**5.0**	**5.0**
Total Development of Genetic Methods	**119.4**	**65.8**	**67.4**	**5.0**	**5.0**
EFFICACY TESTING					
Efficacy Research Program Leader- Ph.D. level senior scientist @ GS level 14 (50% time)			82.4	84.5	86.6
Estimated Percent Time Required			0.50	0.50	0.50
Efficacy Research Program Leader Salary (Adjusted for % time)			**41.2**	**42.2**	**43.3**
Research Assistant- M.S. level @ GS level 11 (50% time- other 50% shared with Risk Assessment)			**24.5**	**25.1**	**25.7**
2 Half-time or 1 Full-time B.S. level technician @ GS level 5 (overtime for weekend, etc. not included)			**26.7**	**27.4**	**28.1**
Travel for Program-wide Meetings			**2.0**	**2.0**	**2.0**
*****Assume that Efficacy Testing and Risk Assessment Will Use Portions of the Same Equiptment*****					
Simplest Environment- Laboratory Tests:					
One-time Construction Costs for Retrofitting an Existing Laboratory Facility for Biocontainment and Security	400.0				
One-time Costs for Equiptment for Conducting Laboratory Experiments, Including Tanks, Circulation System, etc.	100.0				
Annual Lab Operating Costs (filters, chemicals, feed)			12.0	12.0	
Miscellaneous Supplies			5.0	5.0	
Total for Simplest Environment Laboratory Tests	**500.0**		**17.0**	**17.0**	
Most Complex- Isolated Reach of Actual Stream:					
One Time Design and Construction of a Secure Research Site			100.0		
Travel Costs to Research Site				6.0	6.0
Miscellaneous Supplies				15.0	15.0
Total for Isolated Research of Actual Stream			**100.0**	**21.0**	**21.0**
Total Efficacy Testing	**500.0**		**211.4**	**134.7**	**120.1**

Table 6.4. *Continued.*

Component	Year 1	Year 2	Year 3	Year 4	Year 5
TARGET SPECIES ECOLOGY					
Research on Non-native Species Distribution and Abundance (Agency coordinated- part of existing work):					
Project Leader- M.S. level biology @ GS 11 level (50% time)	48.9	50.2	51.4	52.7	54.0
Project Leader- Salary Adjusted at 50% time	24.5	25.1	25.7	26.4	27.0
Field Crew Leader- B.S./M.S. level @ GS 8 level (50% time)	36.6	37.5	38.5	39.4	40.4
Field Crew Leader- Salary Adjusted at 50% time	18.3	18.8	19.2	19.7	20.2
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	26.7	27.4	28.1	28.8	29.5
Field Crew member- Salary Adjusted at 50% time	13.3	13.7	14.0	14.4	14.7
Field Crew member- B.S./M.S. level @ GS 6 level (50% time)	26.7	27.4	28.1	28.8	29.5
Field Crew member- Salary Adjusted at 50% time	13.3	13.7	14.0	14.4	14.7
Total Salary for Target Species Ecology	**69.5**	**71.2**	**73.0**	**74.8**	**76.7**
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Travel to Reseach Sites	6.0	6.0	6.0	6.0	6.0
Survey Equiptment	5.0	1.0	1.0	1.0	1.0
Total for Reseach on Non-native Species Distribution and Abundance	**82.5**	**80.2**	**82.0**	**83.8**	**85.7**
Research on Biology of Target Organism(s) (Contracted out) (5 years of funding at 100K year):	100.0	100.0	100.0	100.0	100.0
Total for Research on Biology of Target Organisms	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Research on Ecology of Target Organism(s) (Contracted out) (5 years of funding at 100 K year):	100.0	100.0	100.0	100.0	100.0
Total for Research on Ecology of Target Organisms	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Total Target Species Ecology	**352.0**	**351.4**	**355.0**	**358.7**	**362.4**
MULTI-STAKEHOLDER DELIBERATION					
Multi-Stakeholder Deliberation Specialist- M.S. or Ph.D. level @ GS 12 level	58.7	60.1	61.6	63.2	64.8
Estimated Percent Time Required	0.20	0.20	0.20	0.20	0.20
Multi-Stakeholder Deliberation Specialist Salary (Adjusted for % time)	**11.7**	**12.0**	**12.3**	**12.6**	**13.0**
Travel for Program-wide Meetings	2.0	2.0	2.0	2.0	2.0
Expenses for Interactions with the Community	10.0	10.0	10.0	10.0	10.0
Total Operating Expenses	**12.0**	**12.0**	**12.0**	**12.0**	**12.0**
Total Multi-Stakeholder Deliberation	**23.7**	**24.0**	**24.3**	**24.6**	**25.0**
SEEKING REGULATORY APPROVAL					
Policy/Legal/Regulatory Specialist- M.S., J.D. or Ph.D. level @ GS 13 level	69.8	71.5	73.3	75.1	77.0
Estimated Percent Time Required	0.10	0.10	0.10	0.15	0.15
Policy/Legal/Regulatory Specialist Salary (Adjusted for % time)	**7.0**	**7.2**	**7.3**	**11.3**	**11.6**
Travel for Program-wide and Other Meetings	2.0	2.0	2.0	2.0	2.0
Total Operating Expenses	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**
Total Seeking Regulatory Approval	**9.0**	**9.2**	**9.3**	**13.3**	**13.6**
TOTAL 5-YEAR PROJECT COSTS	**1042.1**	**489.1**	**861.5**	**734.6**	**728.7**

References

ABRAC (Agricultural Biotechnology Research Advisory Committee). 1995. Performance standards for safely conducting research with genetically modified fish and shellfish. Parts I and II. USDA (United States Department of Agriculture). Available: *http://www.isb.vt.edu/perfstands/psmain.cfm*. (May 2005).

AFS (American Fisheries Society). 2004. Guidelines for the use of fishes in research. American Fisheries Society. Available: *http://www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml*. (June 2004).

Aldrich, M. 1997. Safety first: Technology, labor and business in the building of American worker safety 1870-1939. The John Hopkins University Press, Baltimore, MD.

Amendola, A. 2001. Recent paradigms for risk informed decision making. Safety Science 40:17-30.

Animal Welfare Information Center. 2003. Information resources on fish welfare, 1970-2003. United States Department of Agriculture, Agricultural Research Service, National Agricultural Library, and Animal Welfare Information Center. Available: *http://www.nal.usda.gov/awic/pubs/Fishwelfare/fishwelfare.htm*. (May 2005).

Anonymous. 2002. Carp management in the Murray-Darling Basin: Daughterless carp technology. Murray-Darling Basin Commission, and Commonwealth Scientific and Industrial Research Organization. Available: *http://www.marine.csiro.au/LeafletsFolder/pdfsheets/Daughterless_carp_13may02.pdf*. (May 2005).

Aquatic Nuisance Species Task Force. 1994. Report to Congress: Findings, conclusions, and recommendations of the intentional introductions policy review. Available: *http://nas.nfrcg/gov/iirpt.htm*. (May 2005).

Arizona Commission of Indian Affairs. 2003. Game and fish. Arizona Commission of Indian Affairs. Available: *http://www.indianaffairs.state.az.us/agreements/gamefish.html*. (May 2005).

Benfey, T. 1999. The physiology and behavior of triploid fishes. Reviews in Fisheries Science 7:39-67.

Braig, H. R., and G. Yan. 2002. The spread of genetic constructs in natural insect populations. Pages 251-314 in Letourneau, D. K., and B. E. Burrows, editors. Genetically engineered organisms: Assessing the environmental and human effects. CRC Press, Boca Raton, FL.

Brummelkamp, T. R., R. Bernards, and R. Agami. 2002. A system for stable expression of short interfering RNAs in mammalian cells. Science 296:550-553.

Burgman, M. 2005. Risks and decisions for conservation and environmental management. Cambridge University Press, Cambridge, UK.

Burkhead, N. M., and D. H. Huge. 2002. The case of the Red Shiner: What happens when a fish goes bad? United States Geological Survey. Available: *http://cars.er.usgs.gov/Southeastern_Aquatic_Fauna/Freshwater_Fishes/Shiner_Research/shiner_research.html.* (May 2005).

Burt, A. 2003. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. Proceedings of the Royal Society of London, B 270:921-928.

Capalbo, D. M. F., M. F. Simon, R. O. Notary, S. Valle, R. Ferreira dos Santos, M. J. A. Sampaio, L. Cora din, J. de O. Duarte, J. E. Miranda, A. L. Assay, E. P. F. Dias, L. Q. Queen, E. Underwood, and K. C. Nelson. 2005. Problem formulation and option assessment (PFOA) in Brazil: Bt cotton case study. Pages 00-00 in Andow, D. A., A. Hilbeck, and E. M. G. Fontes, editors. Kapuscinski, A. R., and P. J. Schei, series editors. Environmental risk assessment of genetically modified organisms, Volume 2 - Methodologies for assessing Bt cotton in Brazil. 2, CABI Publishing, Wallingford, UK. In press.

CEQ (Council on Environmental Quality). 1997. Memorandum to the heads of agencies on the application of the National Environmental Policy Act to proposed Federal action in the United States with transboundary effects. Council on Environmental Quality. Available: *http://ceq.eh.doe.gov/nepa/regs/transguide.html.* (May 2005).

CEQ-OSTP (Council on Environmental Quality - Office of Science and Technology Policy). 2001. Case study no. 1: Growth-enhanced salmon. Office of Science and Technology Policy. Available: *http://www.ostp.gov/html/ceq_ostp_study2.pdf.* (May 2005).

Childers, W. F. 1967. Hybridization of four species of sunfishes (Centrarchidae). Illinois Natural History Survey Bulletin 29:159-214.

Codex Alimentarius Commission. 2003. Principles for the risk analysis of foods derived from modern biotechnology. International Portal on Food Safety, Animal and Plant Health. Available: *www.ipfsaph.org/En/default.jsp*. (May 2005).

Codex Alimentarius Commission. 2003a. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. International Portal on Food Safety, Animal and Plant Health. Available: *www.ipfsaph.org/En/default.jsp*. (May 2005).

Codex Alimentarius Commission. 2003b. Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms. International Portal on Food Safety, Animal and Plant Health. Available: *www.ipfsaph.org/En/default.jsp*. (May 2005).

Collodi, P. 2003. Targeted inactivation of the fish aromatase gene. Illinois-Indiana Sea Grant. Available: *http://www.iisgcp.org/research/projects/aqua/ra0301.htm*. (May 2005).

Commission for Environmental Cooperation. 1999. Ribbon of life: An agenda for preserving transboundary migratory bird habitat on the Upper San Pedro River. Commission for Environmental Cooperation. Available: *http://www.cec.org/files/pdf/SP-ENGL_EN.PDF*. (May 2005).

Cotter, D., V. O. Donovan, N. O'Maioleidigh, G. Rogan, N. Roche, and N. P. Wilkins. 2000. An evaluation of the use of triploid Atlantic salmon (Salmo salar) in minimizing the impact of escaped farmed salmon on wild populations. Aquaculture 186:61-75.

CVM (Center for Veterinary Medicine, United States Food and Drug Administration). 2003. Recent incident at the University of Illinois. Letter from Dr. Stephen Sundlof, Director of the CVM, to various scientists. USDA (United States Food and Drug Administration). Available: *http://www.fda.gov/cvm/Documents/Mar-Apr03.pdf*. (May 2005).

Davis, S. A. , E. A. Catchpole, and R. P. Pech. 1999. Models for introgression of a transgene into a wild population within a stochastic environment, with applications to pest control. Ecological Modeling 119(3):267-275.

Davis, S., N. Bax, and P. Grewe. 2001. Engineered underdominance allows efficient and economical introgression of traits into pest populations. Journal of Theoretical Biology 212:83-98.

Dawson, V. K., and C. S. Kolar, editors. 2003. Integrated management techniques to control non-native fishes. U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, December 2003. 146 pp. Appendices A-F.

Dayton, P. K. 1998. Reversal of the burden of proof in fisheries management. Science 279:821-822.

Desert Fishes Team. 2003. Status of federal and state listed warm water fishes of the Gila River basin, with recommendations for management. Desert Fishes Team Report 1:1-20.

Desert Fishes Team. 2004. Status of unlisted native fishes of the Gila River basin, with recommendations for management. Desert Fishes Team Report 2:1-25.

Donaldson, E. M., and R. H. Devlin. 1996. Uses of biotechnology to enhance production. Pages 969-1020 in Pennell, W., and B. A. Barton, editors. Principles of salmon culture, developments in aquaculture and fisheries science, No. 29. Elsevier Publishers, Amsterdam.

Donaldson, E. M., R. H. Devlin, I. I. Solar, and F. Piferrer. 1993. The reproductive containment of genetically altered salmonids. Pages 113-129 in Cloud, J. G., and G. H. Thorgaard, editors. Principles of salmonid culture. Plenum Press, New York.

Dunham, R. A. 2004. Aquaculture and fisheries biotechnology: genetic approaches. CABI Publishing, Wallingford, UK.

Ehler, L. E. 2000. Critical issues related to non-target effects in classical biological control of insects. Pages 1-13 in Follett, P. A., and J. Duan, editors. Non-target effects of biological control. Kluwer Academic Publishers, Boston, MA.

Etnier, D. A. 1968. Reproductive success of natural populations of hybrid sunfish in three Minnesota lakes. Transactions of the American Fisheries Society 97:466-471.

FAO (Food and Agriculture Organization) and WHO (World Health Organization). 2004. Safety assessment of foods derived from genetically modified animals, including fish. Report of the joint FAO/WHO expert consultation, Rome, 17-21 November 2003. Food and Agriculture Organization and World Health Organization. Available: *www.who.int/foodsafety/biotech/meetings/ec_nov2003/en*. (June 2005).

Fiorino, D. J. 1990. Citizen participation and environmental risk: A survey of institutional mechanisms. Science, Technology, and Human Values 15(2):226-243.

Fire, A., S. Xu, M. Montgomery, S. Kostas, S. Driver, and C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA Caenorhabditis elegans. Nature 391(6669):806-811.

Fitzsimmons, K. 1998. Review of rules and regulations affecting aquaculture in Arizona. University of Arizona. Available: *http://ag.arizona.edu/azaqua/extension/rules.html*. (May 2005).

Fitzsimmons, K. 2002. Arizona state aquatic nuisance species management plan. University of Arizona. Available: *http://Ag.Arizona.Edu/azaqua/extension/ANS/ArizonaPlan.htm*. (May 2005).

Getches, D., C. Wilkinson, and R. Williamson. 1998. Cases and materials on Federal Indian Law, Volume 4. West Group, St. Paul, MN.

Gibbons, M. 1999. Science's new social contract with society. Nature 402 (Supp. 29): C81-C84.

Gould, F., and P. Schliekelman. 2004. Population genetics of autocidal control and strain replacement. Annual Review of Entomology 49:193-217.

Grewe, P. 1996. Review and evaluation of the potential of molecular approaches for the environmentally benign management of common carp (Cyprinus carpio) in Australian waters. Commonwealth Scientific and Industrial Research Organization. Available: *http://www.marine.csiro.au/crimp/reports/CRIMPTechReport10.pdf*. (May 2005).

Hackett, P. B. 1993. The molecular biology of transgenic fish. Pages 207-240 in Hochachka, P. W., And T. P. Mommsen, editors. Biochemistry and molecular biology of fishes, Vol II: Molecular biology frontiers. Elsevier Publishers, Amsterdam.

Hallerman, E. M., and A. R. Kapuscinski. 1993. Potential impacts of transgenic and genetically manipulated fish on natural populations: Addressing uncertainties through field testing. Pages 93-112 in Cloud, J. G., and G. H. Thorgaard, editors. Genetic conservation of salmonid fishes. Plenum Press, New York.

Hayes, K. R. 2002. Identifying hazards in complex ecological systems. Part 1: Fault tree analysis for biological invasions. Biological Invasions 4:235-249.

Hayes, K. R. 2002a. Identifying hazards in complex ecological systems. Part 2: Infection modes and effects analysis for biological invasions. Biological Invasions 4:251-261.

Hayes, K. R., P. Gregg, V. Gupta, R. Jessop, W. Lonsdale, B. Sindel, J. Stanley, and C. Williams. 2004. Identifying hazards in complex ecological systems. Part 3: Hierarchical holographic model for herbicide tolerant oilseed rape. Environmental Biosafety Research 3:109-128.

Hew, C. L., and G. L. Fletcher. 1992. Transgenic fish. World Scientific Publishing Company, Singapore.

Houdebine, L. M. 1996. Transgenic Animals: Generation and Use, Hardwood Academic, Amsterdam.

Howard, R. D., J. A. DeWoody, and W. M. Muir. 2004. Transgenic male mating advantage provides opportunity for Trojan gene effect in a fish. Proceedings of the National Academy of Sciences 101(9):2934-2938.

Hurst, G. D. D., and J. Werren. 2001. The role of selfish genetic elements in eukaryotic evolution. Nature Reviews 2(8):597-606.

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
.

Indiana Department of Natural Resources. 2003. Indiana aquatic nuisance species management plan. Indiana Department of Natural Resources. Available: *http://www.in.gov/dnr/invasivespecies/inansmanagementplan.html*. (May 2005).

Inoue, K., and S. Yamashita. 1996. The techniques using electroporation to generate transgenic fish. Pages 129-136 in L. M. Houdebine, editors. Transgenic animals: generation and use. Hardwood Academic, Amsterdam.

Kapuscinski, A. R. 2002. Controversies in designing useful ecological assessments of genetically engineered organisms. Pages 385-415 in Letourneau, D. and B. Burrows, editors. Genetically engineered organisms: Assessing environmental and human health effects. CRC Press, Boca Raton, FL.

Kapuscinski, A. R. 2003. Marine GEO's: Products in pipeline. Marine biotechnology briefs 1(1):1-5, Table 1. University of Minnesota. Available: *http://www.fw.umn.edu/isees/MarineBrief/gmmobrf.htm*. (May 2005).

Kapuscinski, A. R. 2005. Current scientific understanding of the environmental biosafety of transgenic fish and shellfish. Scientific and Technical Review Office International des Epizooties 24(1): 309-322. Available: *http://www.oie.int/eng/publicat/RT/2401A_R240127.htm* (July 2005).

Kapuscinski, A. R. 2005a. Status of environmental biosafety science on genetically engineered fish and policy implications. Canadian Technical Reports on Fisheries and Aquatic Science: in press.

Kapuscinski, A. R., and E. Hallerman. 1991. Implications of introduction of transgenic fish into natural ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 48:99-107.

Kapuscinski, A. R., R. M. Goodman, S. D. Hann, L. R. Jacobs, E. Pullins, C. S. Johnson, J. D. Kinsey, R. L. Krall, A. G. M. La Vina, M. G. Mellon, and V. W. Ruttan. 2003. Making safety first a reality for biotechnology products. Nature Biotechnology 21(6):599-601.

Kelso, D. T. 2000. Aquarian transitions: Technology change, environmental uncertainty, and salmon production on North America's Pacific coast. Dissertation. University of California, Berkley, CA.

Kelso, D. T. 2004. Genetically engineered salmon, ecological risk, and environmental policy. Bulletin of Marine Science 74(3):508-528.

Kitamura, S., H. Ogata, and H. Onozato. 1991. Triploid male masu salmon Oncorhynchus masu shows normal courtship behavior. Bulletin of the Japanese Society of Scientific Fisheries 57:2157.

Koufopanou, V., M. Goddard, and A. Burt. 2002. Adaptation for horizontal transfer in homing endonuclease. Molecular Biology and Evolution 19:239-246.

Kurita, K., S. M. Burgess, and N. Sakai. 2004. Transgenic zebrafish produced by retroviral infection of in vitro cultured sperm. Proceedings of the National Academy of Science U.S.A. 101(5):1263-1267.

Lapidge, K. L. 2003. Proceedings of the national carp control workshop. Cooperative Research Centre for Pest Animal Control. Available: *http://www.pestanimal.crc.org.au/info/CarpProc.pdf*. (May 2005).

Lee, D., C. Gilbert, C. Hocutt, R. Jenkins, D. McAllister, and J. Stauffer, Jr. 1980. Atlas of North American Fishes, North Carolina State Museum of Natural History, 867 pp.

Lewis, G. W. 1999. Use of sterile grass carp to control aquatic weeds. Leaflet No. 418. University of Georgia College of Agriculture and Environmental Sciences, Cooperative Extension Service. Available: *http://www.ces.uga.edu/pubcd/L418-w.htm#Permit*. (June 2005).

Louet, S. 2001. EC study reveals an informed public. Nature Biotechnology 19(1):15-16.

Mackenzie, R., F. Burhenne-Guilmin, A. G. M. La Vina, and J. D. Werksman in cooperation with A. Ascencio, J. Kinderlerer, K. Kummer, and R. Tapper. 2003. An explanatory guide to the Cartagena Protocol on biosafety. IUCN, Gland, Switzerland and Cambridge, UK.

Malakoff, D. 1999. Bayes offers a 'new' way to make sense of numbers. Science 286:1460-1464.

Marris, C., B. Wynne, P. Simmons, and S. Weldon. 2001. Public perceptions of agricultural biotechnology in Europe. Final report of the PABE research project. Lancaster University. Available: *http://www.lancs.ac.uk/depts/ieppp/pabe/docs/pabe_finalreport.pdf*. (May 2005).

Marsh, P., and C. Pacey. 2005. Immiscibility of native and non-native fishes. Pages 59-63 in M.J. Brouder, C.L. Springer, and S.C. Leon, editors. Proceedings of two symposia: Restoring native fish to the lower Colorado River: interactions of native and non-native fish. July 13-14, 1999, Las Vegas, Nevada, and Restoring natural function within a modified riverine environment: the lower Colorado River. July 8-9, 1998. U.S. Fish and Wildlife Service, Southwest Region, Albuquerque, New Mexico.

Martin, A. November 12, 2003. One fish, two fish, genetically new fish. Section A1. Chicago Tribune.

Mazurek, J. P. 1998. American Indian law deskbook: Conference of western Attorneys General. Volume 2. University Press of Colorado, Niwot, CO.

McIntyre, G. R. 2000. Patterns of safety thinking. Ashgate Publishing, UK.

McMillan, M., and D. Wilcove. 1994. Gone but not forgotten: Why have species protected by the Endangered Species Act become extinct? Endangered Species Update 11:5-6.

Miller, L. M., A. R. Kapuscinski, and W. Senanan. 2004. A biosafety approach to assessing risks posed by aquaculture escapees. Pages 56-65 in Gupta, M. V., D. M. Bartley, and B. O. Acosta, editors. Use of genetically improved and alien species for aquaculture and conservation of aquatic biodiversity in Africa, World Fish Center conference proceedings. World Fish Center, Penang, Malaysia.

Muir, W. M., and R. D. Howard. 2002. Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. Transgenic Research 11(2):101-104.

Muir, W., and R. D. Howard. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis. Proceedings of the National Academy of Science 96:13853-13856.

Murray-Darling Association. 2003. Let's just get rid of 'em! A communication strategy for the daughterless carp project. Report of Murray-Darling Association to Daughterless Carp Reference Group, Australia.

Murray-Darling Basin Ministerial Council. 2003. Native fish strategy for the Murray-Darling basin 2003-2013. Murray-Darling Ministerial Council. Available: *http://www.mdbc.gov.au/naturalresources/fish/pdf/Fish-Strat_ful.pdf*. (May 2005).

Napolitano, J. 2005. Invasive Species Advisory Council. Executive Order 2005-09. April 1, 2005, Phoenix, AZ.

NIH (National Institutes of Health). 1986. Guidelines for research involving recombinant DNA molecules. Federal Register 51: 16957-16985 (and as subsequently amended).

National Invasive Species Council. 2001. Meeting the invasive species challenge: National invasive species management plan. National Agricultural Library. Available: *http://www.invasivespecies.gov/council/nmp.shtml*. (May 2005).

Nelson, K. C., G. Kibata, M. Lutta, J. O. Okuro, F. Muyekho, M. Odinho, A. Ely, and J. M. Waquil. 2004. Problem formulation and option assessment (PFOA) for genetically modified organisms: The Kenya case study. Pages 57-82 in Hilbeck, A. and D. A. Andow, editors. Kapuscinski, A. R., and P. J. Schei, series editors. Environmental risk assessment of genetically modified organisms Vol 1: A case study of Bt maze in Kenya. CABI Publishing, Oxfordshire, UK.

NRC (National Research Council). 1996. Understanding risk: Informing decisions in a democratic society. National Academy Press, Washington, D. C.

NRC (National Research Council). 1996a. Guide for the care and use of laboratory animals. National Academy Press, Washington, D. C.

NRC (National Research Council). 2002. Environmental effects of transgenic plants: The scope and adequacy of regulation. National Academy Press, Washington, D. C.

NRC (National Research Council). 2002a. Animal biotechnology: Science-based concerns, National Academy Press, Washington, D.C.

NRC (National Research Council). 2004. Biological confinement of genetically engineered organisms. National Academy Press, Washington, D. C.

NRC (National Research Council). 2004a. Safety of genetically engineered foods: Approaches to assessing unintended health effects. The National Academy Press. Available: *http://www.nap.edu/catalog/10977.html*. (May 2005).

OIE (Office International des Epizooties - World Organization for Animal Health). 2005. Biotechnology and animal health. Office International des Epizooties Scientific and Technical Review 24(1):in press.

OIE (Office of International des Epizooties). 2004. International aquatic animal health code. Office of International des Epizooties. Available: *http://www.oie.int/eng/normes/fcode/A_summry.htm*. (May 2005).

OSTP (Office of Science and Technology Policy). 1985. U.S. Government principles for the utilization and care of vertebrate animals used in testing, research, and training. OSTP (Office of Science and Technology Policy). Available: *http://www.absc.usgs.gov/research/vet/policies/IRACPRIN.htm*. (May 2005).

OTA (Office of Technology Assessment). 1993. Harmful non-indigenous species in the United States. United States Government Printing Office, Washington, D.C.

Pew Initiative on Food and Biotechnology. 2003. Future fish: Issues in science and regulation of transgenic fish. Pew Initiative on Food and Biotechnology, Washington, D.C. Available: *http://pewagbiotech.org/research/fish/* (March 2005).

Pew Initiative on Food and Biotechnology. 2004. Issues in the regulation of genetically engineered plants and animals. Pew Initiative on Food and Biotechnology, Washington, D.C. Available: *http://pewagbiotech.org/research/RegulationExecSum.pdf*. (May 2005).

Pollack, A. January 25, 2004. So, the fish glow. But will they sell? Page 5. The New York Times.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Review of Ecology and Systematics 27:83-109.

Rinne, J. N., and W. L. Minckley. 1991. Native fishes of arid lands: a dwindling resource of the desert southwest. General Technical Report RM-206. U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO.

Sagar, A., A. Daemmrich, and M. Ashiya. 2000. The tragedy of the commoners: Biotechnology and its publics. Nature Biotechnology 18(1):2-4.

Sawyer, A. J. 1980. Prospects for integrated pest management of the sea lamprey (Petromyzon marinus). Canadian Journal of Fisheries and Aquatic Sciences 37: 2081-2092.

Schliekelman, P., and F. Gould. 2000. Pest control by introduction of a conditional lethal trait on multiple loci: Potential limitations and optimal strategies. Journal of Economic Entomology 93(6):1543-1565.

Scientists' Working Group on Biosafety. 1998. Manual for assessing ecological and human health effects of genetically engineered organisms. Part One: Introductory text and supporting text for flowcharts. Part Two: Flowcharts and worksheets. The Edmunds Institute. Available: *www.edmonds-institute.org/manual.html.* (May 2005).

Sorensen P. W., and N. E. Stacey. 2004. Brief review of pheromones and discussion of their possible uses in the control of non-indigenous teleost fishes. New Zealand Journal of Marine and Freshwater Research 38: 399-417.

Stanford Environmental Law Society. 2001. The Endangered Species Act, Stanford University Press, Stanford, CA.

Sui, G., C. Soohoo, E. B. Affar, F. Gray, Y. Shi, W. Forrester, and Y. Shi. 2002. A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proceedings of the National Academy of Science 99:5515-5520.

Susskind, L., Levy, P. F. and J. Thomas-Larmer. 2000. Negotiating environmental agreements: How to avoid escalating confrontation, needless costs, and unnecessary litigation. Island Press, Washington, D.C.

Syvanen, M. 1994. Horizontal gene transfer: Evidence and possible consequences. Annual Review of Genetics 28:237-261.

Takeuchi, Y., G. Yoshizaki, and T. Takeuchi. 2003. Generation of live fry from intraperitoneally transplanted primordial germ cells in rainbow trout. Biology of Reproduction 69:1142-1149.

Thompson, D. Q., R. L. Stuckey, and E. B. Thompson. 1987. Spread, impact and control of purple loosestrife (Lythrum salicaria) in North American wetlands. U. S. Fish and Wildlife Service, Washington, D.C. Fish and Wildlife Research Report No. 2.

Thorgaard, G. H. 1983. Chromosome set manipulation and sex control in fish. Pages 405-434 in Hoar, W. S., D. J. Randall and E. M. Donaldson, editors. Fish physiology. Academic Press, New York.

Thorpe, J. E., R. S. Wright, C. Talbot, and M. S. Miles. 1987. Secondary sexual characteristics developed by 60-Co sterilized Atlantic salmon. Pages 139 in Idler, D. R., L. W. Crim and J. M. Walsh, editors. Reproductive physiology of fish. Memorial University of Newfoundland, St. John's, NL.

Thresher, R. E., L. Hinds, P. Grewe, and J. Patil. 2002. Genetic control of sex ratio in animal populations. International Publication number WO 02/30183 A1. World International Property Organization.

Thresher, R. E., L. Hinds, P. Grewe, C. Hardy, S. Whyard, J. Patil, D. McGoldrick, and S. Vignarajan. 1999. Reversible sterility in animals. Australian Patent Number PG4884. Australia.

Twohey, M. B., J. Heinrich, J. Seelye, R. Bergstedt, K. Fredricks, C. Kaye, R. Scholefield, R. McDonald, and G. Christie. 2003. The sterile-male-release technique in Great Lakes sea lamprey management. Journal of Great Lakes Research 29(Supp. 1):410-423.

Tyus, H. M., and J. F. Saunders, III. 2000. Non-native fish control and endangered fish recovery: lessons from the Colorado River. Fisheries (Bethesda) 25(9):17-24.

USDOI (United States Department of Interior). 2001. National Environmental Policy Act of 1979. Volume Departmental Manual, Part 516. United States Department of Interior, Washington, D. C.

USFDA (United States Food and Drug Administration). 2003. FDA statement regarding Glofish. United States Food and Drug Administration. Available: *www.fda.gov/bbs/topics/NEWS/2003/NEW00994.html*. (December 2003).

USFWS (United States Fish and Wildlife Service). 1998. Final intra-service consultation handbook. United States Fish and Wildlife Service, Washington, D. C.

USFWS (United States Fish and Wildlife Service). 2001. Background information in the Central Arizona Project and non-native aquatic species in the Gila River basin (excluding Santa Cruz River sub-basin). United States Fish and Wildlife Service, Phoenix, AZ.

USFWS (United States Fish and Wildlife Service). 2004. Grass carp inspection and certification program. United States Fish and Wildlife Service. Available: *http://policy.fws.gov/fh_handbook/Volume_9/Volume9.htm*. (May 2005).

Warren, M. L., Jr. and B. M. Burr. 1994. Status of freshwater fishes of the United States: overview of an imperiled fauna. Fisheries (Bethesda) 19(1):6-18.

Weiss, K. December 10, 2003. In reversal, FDA says it will not regulate bioengineered fish. Page 31. The Los Angeles Times.

Wells, A. 2004. Daughterless carp community engagement activities report. Murray-Darling Association, Albury, NSW, Australia.

Whitten, M. J. 1992. Pest management in 2000: What we might learn from the twentieth century. Pages 9-44 in Kadir, A., and H. Barlow, editors. Pest management and the environment in 2000. CAB International and Agricultural Institute of Malaysia, Wallingford, UK.

Wilkinson, C. 1998. The role of bilaterlism in fulfilling the Federal-Tribal relationship: The Tribal Rights-Endangered Species Secretarial Order. Washington Law Review 72:1063-1107.

Wimmer, E. A. 2003. Genetically engineered, embryo-specific lethality for insect pest management. Information Systems for Biotechnology. Available: *http://www.isb.vt.edu/news/2003/artspdf/mar0301.pdf*. (May 2005).

Wondolleck, J. M. and S. L. Yaffee. 2000. Making collaboration work: Lessons from innovation in natural resource management. Island Press, Washington, D.C.

Wood, M. C. 1994. Indian land and the promise of native sovereignty: The trust doctrine revisited. Utah Law Review 4:1471-1569.

Yamazaki, F. 1976. Application of hormones in fish culture. Journal of the Fisheries Research Board Canada 33:948-958.

Appendix 1

List of Information-Gathering Meetings in Australia and Arizona during 2004

Australia

Ron Thresher, Commonwealth Scientific and Industrial Research Organization (CSIRO) Marine Pest Control Group, 5-24-04 through 5-28-04

Nic Bax, CSIRO Marine Pest Control Group, 5-24-04 through 5-28-04

Keith Hayes, CSIRO Marine Pest Control Group, 5-24-04 through 5-28-04

Peter Grewe, CSIRO Marine Pest Control Group, 5-24-04 through 5-28-04

Jawahar Patil, CSIRO Marine Pest Control Group, 5-24-04 through 5-28-04

GM Fish Hazard Identification Workshop, CSIRO Marine Laboratories, Hobart 5-25-04

Adrian Wells, Murray Darling Association Inc., 6-11-04

Brad Tucker, Pest Animal Control Cooperative Research Centre (CRC), 6-15-04

Tony Peacock, Pest Animal Control CRC, 6-15-04

Jim Barrett, Murray-Darling Basin Commission, 6-15-04

Arizona

Rob Clarkson, U.S. Bureau of Reclamation, 6-28-04

Larry Riley, Arizona Game and Fish Department, 6-28-04

Bob Miles, Arizona Game and Fish Department, 6-28-04

Jeff Humphrey, U.S. Fish and Wildlife Service, 6-28-04

William Matter, University of Arizona, 6-29-04

Paul Barrett, U.S. Fish and Wildlife Service, 6-29-04

Doug Duncan, U.S. Fish and Wildlife Service, 6-29-04

Marty Tuegel, U.S. Fish and Wildlife Service, 6-29-04

Anne Browning-Aiken, University of Arizona, 6-29-04

Briefing Concerning Transgenic Fish for Biological control at Meeting of the Central Arizona Project Fund Transfer Program Policy Committee, Arizona Game and Fish Department, Phoenix 7-1-04

Appendix 2

Gila River Basin-Specific Ecological Risk Considerations Related to Transgene Spread to Non-Target Populations

Transgene Spread to Native Range of Species

Forty non-native fish species are known to be established and reproducing in the Gila River basin (USFWS 2001; Table A2.1). An additional 24 species have been reported from the basin but are not established or their status is unknown (USFWS 2001). Of those established and reproducing (species listed in bold-font type in Table A2.1), 20 have native distributions in states adjacent to Arizona and New Mexico, 13 have native distributions in other areas of North America not adjacent to Arizona and New Mexico, and 7 have native distributions on other continents, but not in North America.

The probability of a transgenic fish released for biocontrol escaping to areas of the species' native distribution is greatest for fish with native distributions close to the Gila River system, such as adjacent states and Mexico. Transgenic fish for which the species' native distributions cover other areas of North America, not adjacent to Arizona or New Mexico, have a lower but still high probability of escape, and fish with native distributions on other continents have a low probability of escape. Although the hazard of transgenic fish escape to areas of its native distribution has a high potential severity of harm, the hazard of escape to areas with imperiled native populations possesses an even higher potential severity of harm.

Most of the basin's established non-native species with native ranges on other continents are either economically important in another region of the U.S.A., eaten as human food, or fit both descriptors. Any future effort to release transgenic lines for biocontrol of these species, therefore, would likely raise substantial socio-economic and regulatory concerns. We further explore these issues in chapter 4 on Legal, Policy and Regulatory Framework and chapter 5 on Community Awareness and Involvement.

Transgene Spread to Closely Related Species by Hybridization

Hybridization and introgression have played a major role in the extinction of many species across a wide range of taxa (Rhymer and Simberloff 1996). This hazard presents the potential for serious harm because of the high level of imperilment native fish in the Gila River basin. To further understand the potential harms that could arise from this hazard, a full risk assessment would need to identify the proximity of closely related species (both within and outside of the basin) and the hybridization potential of the transgenic fish with those species.

Consider, for example, three potential targets for biological control in the Gila basin: green sunfish (*Lepomis cyanellus*), red shiner (*Cyprinella lutrensis*) and mosquitofish (*Gambusia affinis*). Although green sunfish don't share Family Centrarchidae with a native Gila River species, they do frequently hybridize with at least seven other Lepomis species (Childers 1967), raising the concern that they could potentially hybridize with closely related species outside of the basin. The resulting hybrids tend to grow faster and to larger size than their parents and are usually males, but are thought to seldom reproduce in natural environments (Etnier 1968). Red shiners, which share the Family Cyprinidae with nine Gila River native fish (but not the same genus), also hybridize frequently and have been

known to displace native members of the same genus via competition and dilution of native gene pools (Burkhead and Huge 2002). Mosquitofish share Family Poeciliidae with one Gila River native fish. Although little is known about their hybridization potential, at least one instance of mosquitofish hybridization with a native species has been implicated as a major factor in the extinction of the Amistad gambusia (*Gambusia amistadensis*) from Texas (McMillan and Wilcove 1994).

Table A2.1. Non-native aquatic fish reported from the Gila River basin (Adapted from Table 5, USFWS 2001) Bold font entries denotes established and reproducing in the basin.

Species Name	Established? Y=yes, N=no, U=unknown	ASU GIS Database of Fish	AGFD Database	Minckley 1973	Native Distribution Source: www.natureserve.org accessed 3-17-04 and Lee et. al 1980.
Threadfin shad	**Y**	**X**	**X**	**X**	**North America**
Cutthroat trout	U	X	X	X	Northern Arizona, Adjacent States
Rainbow trout	**Y**	**X**	**X**	**X**	**Adjacent States**
Brook trout	**Y**	**X**	**X**	**X**	**North America**
Brown trout	**Y**	**X**	**X**	**X**	**Europe**
Lake trout	N	X			North America
Golden trout	N			X	Adjacent States
Kokanee	U		X	X	North America
Arctic grayling	**Y**	**X**	**X**	**X**	**North America**
Northern pike	**Y**	**X**	**X**	**X**	**North America**
Common carp	**Y**	**X**	**X**	**X**	**Europe and Asia**
Goldfish	**Y**	**X**	**X**	**X**	**Europe and Asia**
Grass carp	N	X	X	X	Asia
Silver carp	N	X			Asia
Golden shiner	**Y**	**X**	**X**	**X**	**Adjacent States**
Red shiner	**Y**	**X**	**X**	**X**	**Adjacent States**
Beautiful shiner	N			X	Native to Arizona, New Mexico
Central stoneroller	N	X			Adjacent States
Fathead minnow	**Y**	**X**	**X**	**X**	**Adjacent States**
Pacu	U				South America
Bigmouth buffalo	**Y**	**X**	**X**	**X**	**Adjacent States**
Black buffalo	**Y**	**X**		**X**	**North America**
Smallmouth buffalo	**Y**	**X**	**X**	**X**	**Adjacent States**
Rio Grande sucker	**Y**	**X**		**X**	**Native to New Mexico**
White sucker	U		X		Adjacent States
Flathead catfish	**Y**	**X**	**X**	**X**	**Adjacent States**
Channel catfish	**Y**	**X**	**X**	**X**	**Adjacent States**
Yaqui catfish	N	X			Native to Arizona and Mexico
Black bullhead	**Y**	**X**	**X**	**X**	**Adjacent States**
Yellow bullhead	**Y**	**X**	**X**	**X**	**Adjacent States**
Brown bullhead	**Y**	**X**	**X**		**Adjacent States**
Suckermouth catfish	N	X			Central and South America
Mosquitofish	**Y**	**X**	**X**	**X**	**Adjacent States**
Variable platyfish	N			X	Mexico

Table A2.1. Non-native aquatic fish reported from the Gila River basin (Adapted from Table 5, USFWS 2001) Bold font entries denotes established and reproducing in the basin.

Species Name	Established? Y=yes, N=no, U=unknown	ASU GIS Database of Fish	AGFD Database	Minckley 1973	Native Distribution Source: www.natureserve.org accessed 3-17-04 and Lee et. al 1980.
Green swordtail	N			X	Mexico and Central America
Sailfin molly	**Y**	**X**	**X**	**X**	**Adjacent States**
Mexican (shortfin) molly	N	X		X	Mexico and Central America
Guppy	**Y**	**X**	**X**	**X**	**South America and Caribbean**
Striped bass	**Y**	**X**	**X**		**Eastern North America**
White bass	**Y**	**X**	**X**	**X**	**Adjacent States**
Yellow bass	**Y**	**X**	**X**	**X**	**North America**
Smallmouth bass	**Y**	**X**	**X**	**X**	**North America**
Largemouth bass	**Y**	**X**	**X**	**X**	**North America**
Spotted bass	**Y**	**X**	**X**	**X**	**Adjacent States**
Warmouth	**Y**	**X**	**X**	**X**	**Adjacent States**
Green sunfish	**Y**	**X**	**X**	**X**	**Adjacent States**
Bluegill	**Y**	**X**	**X**	**X**	**Adjacent States**
Redear sunfish	**Y**	**X**	**X**	**X**	**Adjacent States**
Pumpkinseed	N	X		X	North America
Rock bass	**Y**	**X**	**X**	**X**	**North America**
White crappie	**Y**	**X**	**X**	**X**	**North America**
Black crappie	**Y**	**X**	**X**	**X**	**North America**
Sacramento perch	N			X	Adjacent States
Walleye	**Y**	**X**	**X**	**X**	**Adjacent States**
Yellow perch	N	X		X	North America
Oscar	N				South America
Convict cichlid	N			X	Central America
Firemouth cichlid	N				Mexico and Central America
Rio Grande cichlid	N				Adjacent States and Mexico
Mozambique tilapia	**Y**	**X**		**X**	**Africa**
Nile tilapia	N			X	Africa and Middle East
Red-belly tilapia (*T. zillii*)	**Y**	**X**	**X**	**X**	**Africa**
Blue tilapia	**Y**	**X**			**Africa**
Longjaw mudsucker	N	X		X	Adjacent States and Mexico

Appendix 3

FDA Regulation of Transgenic Fish Under the New Animal Drug Provisions of the Federal Food, Drug, and Cosmetic Act

The Federal Food, Drug, and Cosmetic Act (FFDCA) defines a "drug" broadly to include any "articles . . . intended to affect the structure, or any function of the body of man or other animals." 21 U.S.C § 321(g). By interpreting the genetic construct as well as its protein product—a growth hormone—to be drugs under this definition, the FDA has informally indicated it would regulate transgenic fish under procedures based on the law's new animal drug provisions (CEQ-OSTP 2001).

Secrecy of Regulatory Process Under the FFDCA

The Trade Secrets Act, 18 U.S.C. § 1905 and section 301 (j) of the act, requires FDA to keep investigations, review and approval of commercial applications and pre-market notifications for new animal drugs secret, including the mere existence of an application, unless the applicant chooses to disclose the information. The FFDCA also protects these actions from disclosure (via various sections listed in 21 C.F.R 25.50). The FDA would publicly disclose its final approval of an application for marketing of a transgenic fish and a summary of its decision, including a summary of the Environmental Assessment or Environmental Impact Statement. In the event of disapproval, however, the agency would not publish summaries of the decision and the EA or EIS.

Publicly Known Exercise of Authority Over Research Involving Transgenic Fish

Public knowledge of the FDA regulating transgenic fish so far involves two situations. First, the agency issued an Investigative New Animal Drug Permit (INAD) to AquaBounty Technologies, Inc. (Martin 2003). This allows the company to test transgenic Atlantic salmon under contained conditions in order to generate some of the data the company would eventually include in an application to FDA for commercial approval. The public is presently aware of this situation only because the company disclosed this fact; the company has not disclosed any contents of its applications or permits. Additionally, the agency's Center for Veterinary Medicine alerted Land Grant Universities that they must obtain an INAD permit for any research project involving transgenic animals (CVM 2003).

A new animal drug is considered to be "unsafe" until approved for its intended use. 21 U.S.C. § 360b(a)(1). Given its mandate to protect human health, the FDA could perhaps assert jurisdiction over a transgenic fish intended for use as a biocontrol agent in two situations: (1) if the transgenic fish could potentially enter the human food supply (e.g. green sunfish, an invasive species in the Gila basin); or (2) if the transgenic fish could directly or indirectly, affect the "health of man or animal". This may be too narrow an authority to give the FDA the power to disapprove releases of transgenic animals with potential adverse environmental effects that do not affect the health of man or animals (CEQ-OSTP 2001), such as the

ecological hazards and harms posed by transgenic fish for biocontrol (see preliminary list of potential hazards and harms in table 3.2, chapter 3). Although the FDA has said that it intends to publish a guidance document on how the new animal drug provisions apply to transgenic animals and to hold workshops to clarify the scientific issues posed by various uses of transgenic animals (CEQ-OSTP 2001), it had not yet done so by May 2005.

FDA Good Laboratory Practices Regulations

If the FDA decided to assert regulatory jurisdiction over transgenic fish developed for biocontrol uses, the agency would have specific authority over laboratory research with such transgenic fish. The FDA may withdraw approval of a new animal drug application if any non-clinical study in support of the application was not conducted in compliance with the agency's good laboratory practices regulations. 21 C.F.R. 514.111(b)(4). The appropriate regulations include requirements for animal care facilities (21 C.F.R. 58.43), standard operating procedures for the housing, feeding, handling, and care of animals. 21 C.F.R. 58.90. Data from studies conducted at testing facilities that failed to comply with the good laboratory practices regulations could be disqualified in any subsequent application for release of the transgenic fish until the FDA determined that non-compliance did not occur during or did not affect the validity of the data generated by the study. 21 C.F.R. 58.200(a)(1). No further studies from a disqualified facility may be submitted until the facility satisfies the Commissioner of the FDA that it will comply with the regulations.

Appendix 4

Triploid Grass Carp Oversight: State Regulation and Federal Inspection Program

Arizona

The Arizona Game and Fish Department requires parties to obtain a white amur (grass carp) stocking license for importation, transportation, stocking, and possession of triploid grass carp. R12-4-424. The fish can only be stocked in closed aquatic systems for the purpose of control of aquatic weeds that interfere with recreational, domestic, municipal, agricultural, or industrial use of water. R12-4-424. All shipments of white amur must be accompanied by certification from the U.S. Fish and Wildlife Service verifying triploidy. R12-4-412. If the stocking will be in watersheds containing threatened native fish, the applicant must submit a written proposal addressing the biological ramifications of the introduction, and the application may be denied if the Department determines that a negative impact may result from the stocking. R12-4-424. All fish must be of a certain size before they can be stocked (Fitzsimmons, 1998).

California

The California legislature set rather strict guidelines on the state's triploid grass carp stocking program. The California Game and Fish Code allows the Department of Fish and Game to issue regulations providing for control of aquatic plant pests using triploid grass carp introduced under a permit from the Department. California Game and Fish Code, 6450. The Code specifies eight conditions on the regulations, such as requiring that individual fish be checked for triploidy; employing only documented, certified fish; requiring the identification by tagging of individual fish as the property of each owner; requiring posting of notices at stocked bodies of water declaring penalties for removing triploid grass carp. California Game and Fish Code, 6450. Prior to receiving the permit, the applicant must provide the Department with certain information, including all sensitive plant or animal species within the waterway to be stocked and connecting waterways. California Game and Fish Code, 6452(c)(4). The Fish and Game Department is required to impose conditions on the permit that it finds necessary to prevent escape of the triploid grass carp from the targeted area. In addition, the legislature provided for a stiff penalty of a fine up to five thousand dollars and imprisonment in the county jail for up to one year for unauthorized introduction or transfer of triploid grass carp.

New Mexico

The New Mexico Game and Fish Department allows importation of fish from a limited number of families, which does not include Family Cyprinidae, to which grass carp belong. 19.35.7(C) NMAC. However, the Department may grant permission to import prohibited species if the applicant can demonstrate that there will be no conflict with native animals, human health, or livestock, and if the applicant can demonstrate the importation is for a good cause. 19.35.7.8(E) NMAC. Triploid grass carp may be imported into New Mexico for the purpose of aquatic weed control.

FWS Triploid Grass Carp Inspection and Certification Program

The U.S. Fish and Wildlife Service Grass Carp Inspection and Certification (Program) is a service to state natural resource agencies that require a reliable inspection procedure to protect their aquatic

habitats. As of 1995, the Inspection Program conducted more than 550 inspections annually (USFWS 2004). In January 1995, Congress authorized the Secretary of the Interior to charge private producers of triploid fish reasonable fees to sustain continuing operation of the Program, with the current charge being about thirty cents per fish plus travel expenses for the inspectors (USFWS 2004).

The basic elements of the Program consist of standards for inspectors and for producers of triploid grass carp. Inspectors must verify the triploidy in 120 randomly-selected fish from groups ready to be shipped. Inspectors must verify that the group of fish to be certified is isolated from those in production ponds to avoid inadvertent mixing of triploids and diploids. Any non-triploid fish in the inspected sample disqualifies the entire group from certification. Producers participate in the program on a voluntary basis and must verify that they have individually tested each fish in the group to be shipped prior to re-testing of the statistical sample by the Inspector. Producers agree to sell or ship the fish within four working days of certification or re-certification is required. Once inspected, no additional fish may be added to the certified group. Producers must keep records of the certification and provide copies to truck drivers and others delivering the fish. And producers must agree to abide by the laws, regulations, and guidelines of the states where the fish are delivered.

Appendix 5

Possible State Regulation of Transgenic Fish Released for Biocontrol in the Gila Basin: Arizona, California and New Mexico

Arizona Laws

Although Arizona does not explicitly regulate transgenic fish, a number of aquatic wildlife regulations could be applied to regulate potential use of transgenic fish for biocontrol.

Definition of "aquatic wildlife"

The Arizona Revised Statutes (A.R.S.) contains two different definitions of "aquatic wildlife". The chapter on aquaculture narrowly defines "aquatic wildlife" to mean "amphibians, fish, mollusks, crustaceans and soft shelled turtles *found in a state of nature* (emphasis added)." A.R.S. 3-2901. Title 17 (Game and Fish) more broadly defines "aquatic wildlife" as referring to "all fish, amphibians, mollusks, crustaceans and soft-shelled turtles (emphasis added)". A.R.S. 17-101. The latter definition is further extended by the accompanying definition of "wildlife" which includes the eggs or spawn of fish. A.R.S. 17-101. And the definition of "wild" states that, in reference to mammals and birds (but presumably not fish), "wild" describes species normally found in nature. A.R.S. 17-101.

The applicability of these two definitions of "aquatic wildlife" to transgenic fish for biocontrol purposes may need to be addressed before undertaking such a biocontrol project. Under the narrow definition of "aquatic wildlife," the supervisor of aquaculture may lack statutory authority to regulate the production and purposeful release of transgenic fish for biocontrol purposes. In contrast, the broader definition in Title 17 appears to give sufficient regulatory powers of the Game and Fish Commission over release of transgenic fish.

Permit requirements

Operation of a transgenic fish biocontrol project in Arizona would require obtaining a permit from the Game and Fish Commission, whose authority is based on A.R. S. 17-306 and the corresponding Arizona Administrative Code (A.A.C. R12-4-402). Regulated activities include import, export, possession, sale, trade, propagation and release within the state including stocking, any of which could be involved in operating a transgenic fish biocontrol project.

A special license would be required for activities involving species listed as "restricted live wildlife." A.A.C. R12-4-406. Restricted species are those that the Commission has determined to be an actual or potentially-significant threat to indigenous wildlife or to public safety. A.A.C. R12-4-401. Some of the restricted non-native fish in the Gila River system include: northern pike, various bass species, walleye, and yellow perch. A.A.C. R12-4-406. This list also includes the green sunfish, one of the potential targets of a transgenic fish biocontrol project.

An aquatic wildlife stocking permit would be required to release a transgenic fish for biocontrol purposes. The application for the permit must adequately address the biological and socioeconomic ramifications of the introduction. A.A.C. R12-4-410. One important item on the list of biological concerns which is particularly relevant for stocking transgenic species is anticipated hybridization. Hybridization presents a potential pathway for spread of a transgene to non-target species, as discussed in chapter 3.

California Laws

Compared to Arizona, California presents a considerably more restrictive regulatory environment and explicitly regulates transgenic fish. Several aspects of California regulations on transgenic organisms may be relevant to a biocontrol project involving transgenic fish in the Gila River system.

Transgenic aquatic animal regulations

The California Code of Regulations includes "transgenic aquatic animals" on the state's list of restricted species. C.C.R. § 671(c)(9). Transgenic aquatic animals (including freshwater and marine fishes, invertebrates, crustaceans, mollusks, amphibians and reptiles) are classified as "detrimental animals" because they pose a threat to native wildlife. Consequently, under the Fish and Game Code, institutions engaging in scientific research on transgenic aquatic animals are not eligible for an exemption from permit requirements. Fish & Game 2150(e). Without a permit it is unlawful for any person to import, export, transport, maintain, dispose of, or use for any purpose a transgenic aquatic animal. C.C.R. § 671.1. Release of transgenic animals or their progeny from permitted facilities into the waters of the state is prohibited. C.C.R. § 671.1(a)(9)(D).

In addition, regulations prohibit release into the wild without written permission from the California Fish and Game Commission of any wild animal. This includes domestically reared stocks of such animals, which may be genetically detrimental to native wildlife. C.C.R. § 671.6(a)(3). It is possible that transboundary movement of a transgenic fish from the Gila River system into California could be considered an un-permitted release of a genetically detrimental wild animal within California. Proponents of a transgenic fish biocontrol project in a neighboring state could perhaps seek written permission to indemnify such a release from regulatory penalties in advance. However securing such "insurance" may involve public notification.

Public notification

In February 2003, the California Fish and Game Commission adopted regulations providing for public participation in the process for permitting facilities containing transgenic organisms. C.C.R. § 671.1(a)(9)(H). Consequently, any discussions of transboundary issues between the proponents of a transgenic fish biocontrol project in Arizona and the California Fish and Game Department would likely have to be conducted through a relatively transparent process, with some opportunity for input from a public concerned about the potential environmental risks of transgenic aquatic organisms.

California's regulations specify that the Department of Fish and Game shall provide written notice of all permit applications to any interested party submitting a request to be notified. And the Department shall consider all written comments regarding a permit application received prior to approval of the application. Approved applications are reviewed by the Fish and Game Commission during a regularly scheduled public meeting, and, following public comment, the Commission may deny the issuance of a permit if the applicant can not meet regulatory requirements.

New Mexico Laws

Like Arizona, New Mexico does not specifically regulate transgenic aquatic wildlife. Within the New Mexico Department of Game and Fish, the Conservation Services Division is charged with consulting other agencies and organizations on conservation-related issues. The Conservation Services Division administers the main biodiversity protection statute, the Wildlife Conservation Act. NMSA 1978 §§ 17-2-37 et seq. The statute

contains a citizen suit provision as an additional check to ensure that state agencies carry out their conservation responsibilities. NMSA § 17-2-43.1.

New Mexico has several programs targeted at non-native invasive species. In order to protect game animals, birds, and fish against importation of undesirable species and introduction of infectious or contagious diseases, it is a misdemeanor to import any live animals, birds, or fish without a permit from the Department of Game and Fish. NMSA § 17-3-32.

Appendix 6

Estimation of Costs for Food Safety Evaluation

Estimation of Costs of Food Safety Testing of Transgenic Fish for Biocontrol

In Table 6.3, we estimate that conducting the proper food safety testing will cost approximately $500,000. This estimate is based on personal communication with one FDA scientist and represents the low end of what costs actually could be. Costs could range from $500,000 to as high as $2 million.

A major present obstacle to formulating a more reliable estimate is that regulatory policy for determining food safety of all GM animals—including fish –is at a very early stage of development and is very much in flux. In the U.S.A., the FDA has not yet issued official guidance on the kinds of tests that they will routinely expect for determining food safety of GM animals, including fish (see chapter 4 for background on role of FDA here). FDA might require the typical set of tests used to determine human health safety of traditional drugs, which usually include ex-situ biochemical testing as well as in-situ live animal food safety testing (e.g., feeding a 'drug' or in this case, transgenic fish tissues, to rodents). Alternatively, the FDA might require a simpler approach, consisting solely of ex-situ biochemical and nutritional comparisons between the tissues of transgenic fish and their unmodified counterpart. Internationally, the U.S.A. has a history of following food safety standards set by the Codex Alimentarius Commission, the body that sets consensus standards for establishing safety of different foods involved in global trade (see policy section). However, the Commission has taken only one early step in its typical process for developing standards: it convened an Expert Consultation that issued scientific recommendations on methods for testing food safety of GM animals including fish (FAO and WHO 2004). The Commission has not yet formally taken up consideration of these recommendations and it is unclear when it will do so.

TITLES OF RELATED INTEREST:

Kapuscinski, A. R. 2005. Current scientific understanding of the environmental biosafety of transgenic fish and shellfish. OIE Scientific and Technical Review Office International des Épizooties 24(1): 309-322. www.oie.int/eng/publicat/RT/2401/A_R240127.htm

Marine GEOs: Products in the Pipeline. Marine Biotechnology Briefs 1 (February 2003): 1-5 plus tables and hotlinks. www.fw.umn.edu/isees/MarineBrief/gmmobrf.htm

Kapuscinski, A. R., R. M. Goodman, S. D. Hann, L. R. Jacobs, E. E. Pullins, C. S. Johnson, J. D. Kinsey, R. L. Krall, A. G. M. La Viña, M. G. Mellon, and V. W. Ruttan. 2003. Making Safety First a Reality for Biotechnology Products. Nature Biotechnology 21(6): 599-601.

The Minnesota Sea Grant College Program supported the printing of this report.

For additional copies, contact:

Minnesota Sea Grant
2305 East 5th Street
Duluth, MN 55812-1445
Phone: (218) 726-8106
Fax: (218) 726-6556
seagr@d.umn.edu
www.seagrant.umn.edu

This report is a publication of the Institute for Social, Economic and Ecological Sustainability of the University of Minnesota. Further information about the Institute, including its Biotechnology Governance Program is available at: www.fw.umn.edu/isees/