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Genetic Risks Associated with Marine Aquaculture

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Genetic Risks Associated with Marine Aquaculture

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Foreword

The National Marine Fisheries Service (NMFS) Office of Aquaculture and scientists at NMFS fisheries science centers are leading the agency's efforts to increase scientific knowledge in support of its regulatory and management missions pertaining to marine aquaculture. Critical scientific uncertainties need to be addressed to guide research, provide and communicate knowledge, and inform policy and regulatory decisions. There is a range of genetic issues in marine aquaculture, including genetic stock improvement for commercial culture, genetic risks to natural populations, management strategies for mitigating genetic risks, and development of tools to assess relative and acceptable risk. NMFS is sponsoring a series of white papers to address these genetic issues. This technical memorandum, Genetic Risks Associated with Marine Aquaculture, one such paper, is intended to provide an overview of potential genetic risks from a conservation perspective. It is a state-of-the-art summary, identifying areas where additional studies are needed, providing a framework for balancing risks, and supplying examples of how adequate monitoring and evaluation might be done. Additional papers will address genetic improvement, risk mitigation, and a risk assessment modeling effort.

This report is intended for a broad audience, including researchers, natural resource managers, policy makers, the aquaculture and fishing industries, the recreational fishing community, and the public. Accordingly, it is written in a style that should be readable by a broad but informed audience. Genetic terms are defined, complex genetic principles are described, and scientific jargon is avoided or moved to appendices.

The authors are experts in finfish genetics, particularly in conservation of genetic resources of salmonid populations and the interaction of natural and hatchery-reared populations. They have a global perspective and have drawn from the experience of a similar effort in Europe with the development of best management practices for genetic guidelines in aquaculture.

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Executive Summary

Introduction

This technical memorandum is intended to provide managers with a better understanding of the genetic effects of marine aquaculture on natural populations, so that these factors can more effectively be incorporated into informed decisions pertaining to federal marine aquaculture policy and regulatory decisions. We do not attempt an exhaustive literature review of scientific information regarding this complex topic. Instead, in the main body of this report we attempt to synthesize relevant information in an accessible way, providing key references; interested readers can find more detailed treatments in Appendix A. Furthermore, the document does not provide a detailed treatment of ecological factors such as waste discharge/assimilation, competition, and disease, as those factors are considered elsewhere. We focus on commercial aquaculture of marine finfish, but also consider information for salmon hatcheries and marine stock enhancement.

Aquaculture is used to produce aquatic species for food and other human uses. Hatchery-produced stocks are also used to replenish or supplement wild stocks of importance to recreational and commercial fishing, restore threatened or endangered species, or rebuild habitat such as oyster or coral reefs (referred to as enhancement or restoration aquaculture undertaken by public agencies). The global harvest of wild fish and shellfish has leveled off, but demand for protein from aquatic species continues to grow. As a consequence, aquaculture has been a fast-growing system of food production worldwide during the past two decades (although not for the past 5 years in the United States). The last two to three decades have also seen a growing recognition of the importance of conserving natural populations and the profound ways in which anthropogenic changes to natural ecosystems can affect biodiversity. The paradigm of sustainable development emerged and is now widely applied to natural resources management. The Brundtland Commission report, Our Common Future (1987), defined sustainable development as “development that meets the needs of the present without compromising the ability of future generations to meet their own needs.” Applied to aquaculture, the concept of sustainable development raises an important question: How can global aquaculture production continue to increase without compromising long-term viability and sustainability of natural populations and the ecosystems on which they depend?

In the United States, the National Marine Fisheries Service (NMFS) has diverse and potentially competing responsibilities with respect to marine aquaculture:

1. Enable and support the growth of sustainable seafood production in the United States by aquaculture, including stock enhancement activities and commercial production.
2. Ensure that such growth is compatible with NMFS’ stewardship responsibility for living marine resources.

3. Encourage marine aquaculture activities that complement and support sustainable marine fisheries.
4. Protect and restore threatened or endangered species and habitats.
5. Develop a scientific framework to assist in permitting and regulating aquaculture and in making public policy decisions about commercial and replenishment aquaculture.
6. Develop a research program that takes advantage of opportunities provided by aquaculture operations to address critical uncertainties and to educate the public about the benefits and risks of aquaculture.

This technical memorandum is intended to assist the agency in meeting some of these responsibilities. Numerous marine species are already the subjects of commercial and enhancement aquaculture in the United States, and NMFS must make regulatory and policy decisions about aquaculture that are consistent with the agency's stewardship responsibility for living marine resources. These decisions must consider a complex range of factors from the natural and the social sciences.

We begin by summarizing what has been learned over the past several decades from experience in three major arenas: salmon (Salmonidae) aquaculture and hatcheries, propagation of marine species, and agriculture. Important points include the following:

1. The most reliable way to ensure long-term sustainability is to conserve a diverse array of natural populations.
2. Some genetic change associated with artificial propagation is inevitable and can be desirable from a production standpoint; however, it is not inevitable that changes to cultured populations will have substantial genetic effects on natural populations.
3. A certain level of escapes probably cannot be avoided, particularly when production is scaled to increase profitability; therefore, risk assessments should accept this reality and account for potential effects on natural populations.
4. However, to make a genetic impact, escapes must survive and reproduce successfully in the wild. The capability of escaped fish to do this can vary widely, depending on a variety of factors. In general, fitness in the wild of captively reared individuals decreases with number of generations in captivity.
5. Some genetic risks are inversely correlated, such that reducing one risk simultaneously increases another. For example, creating a genetically divergent aquaculture population might reduce the chances that escapes can survive and reproduce, but those that do can pass on maladapted genes to the natural population.
6. An effective monitoring component is important but cannot compensate for failure to implement risk-averse strategies. Even ambitious monitoring programs might have low power to detect adverse effects before serious harm is caused.

Characterizing Risks and Benefits

Informed decisions about appropriate use of marine aquaculture are best made following a comprehensive assessment of potential risks and benefits. The major genetic risks of aquaculture include loss of genetic diversity within populations, loss of genetic diversity among populations, and loss of fitness. These risks have been known and characterized for several decades and their potential effects on natural populations are increasingly well documented, if not always easy to predict. The genetic risks discussed in this document should be considered in conjunction with ecological risks discussed elsewhere.

Potential benefits are of two types: benefits to society and benefits to natural populations. Evaluating societal benefits (economic, social, political) involves a host of normative considerations and is not attempted here, but this is a crucial component of any comprehensive risk-benefit analysis. Of the potential benefits of artificial propagation for natural populations, reducing short-term extinction risk by maintaining a gene pool in captivity is best supported by empirical evidence. Artificial propagation also has been used successfully for some terrestrial or freshwater species to facilitate reintroduction into formerly occupied habitat. Neither of these activities is typically a goal of commercial marine aquaculture. However, two types of indirect benefits of aquaculture for natural populations might be applicable in some situations: 1) abundant aquaculture products can sometimes help alleviate fishing pressure on wild populations, and 2) biological information obtained through aquaculture operations can potentially aid species conservation.

Loss of Diversity within Populations

Genetic variability within populations provides the raw material for evolution and populations with low levels of genetic variability have less capacity to respond to changes in their environments. Levels of genetic variability within populations are determined largely by effective population size (N_e), which can be influenced by many factors, including aquaculture. Effective population size strongly influences levels of genetic diversity and the capacity to adapt to environmental change; N_e is typically lower—and in marine species, often substantially lower—than the population's census size. Marine species are particularly susceptible to loss of within-population variability, because high fecundity and high survival during early life stages in captivity make it theoretically possible for just a few adults to contribute a large fraction of the genes to the natural population. In general, risks increase when N_e in the captive population is much lower than in the wild and when the fraction of natural spawners that are of captive origin is relatively high.

Loss of Diversity among Populations

Genetic diversity among populations confers resilience to natural systems on a variety of temporal and spatial scales. On an equivalent spatial scale, marine species generally show lower levels of molecular genetic differentiation among populations than do anadromous or freshwater species. However, several recent studies show surprisingly strong evidence for local adaptations among populations of marine species, including mobile and highly fecund fishes such as Atlantic cod (*Gadus morhua*). The major concern regarding loss of among-population diversity is that widespread escapes from one or a few aquaculture broodstocks will lead to replacement of existing patterns of locally adapted populations with a smaller number of relatively homogeneous ones. In many marine species, diversity among populations has evolved over many hundreds to thousands of years and cannot easily be regenerated once lost.

Loss of Fitness

Domestication is a process by which populations become better adapted to artificial environments. Because of the profound differences in selective regimes and mortality patterns between wild and aquaculture environments, domestication will occur to some extent when animals are reared under controlled conditions over several generations. Note that domestication does not require (nor generally involve) genetic change to or modification of individual fish; rather, it occurs across generations as certain individuals and their associated genotypes survive and reproduce at different rates than they would in the wild.

Although some level of domestication is probably essential in a profitable aquaculture operation, domesticated individuals are less fit in the wild, and if they reproduce successfully their progeny can depress fitness and compromise viability of the wild population. A related phenomenon, outbreeding depression, is the loss of fitness that occurs when individuals from two genetically divergent populations interbreed. Outbreeding depression can be facilitated by aquaculture operations that import nonlocal stocks that escape and interbreed with local populations. Use of local broodstocks will not avoid domestication, but it will avoid losses of fitness arising from translocation and subsequent interbreeding of genetically divergent populations. Some aquaculture operations will involve both factors (broodstock derived from a nonlocal source and strong domestication for market-related reasons).

Managing Risk

We discuss several tools that might be used to help manage genetic risks associated with marine aquaculture. We developed a framework for an Aquaculture Genetics Management Plan; this is based on a tool developed for hatchery salmon programs in the U.S. Pacific Northwest by the Hatchery Science Review Group, but modified here to better reflect particular issues associated with marine aquaculture programs. We also review Florida’s Genetic Policy for the Release of Finfishes in Florida, recent developments involving aquaculture and endangered Atlantic salmon (*Salmo salar*) in Maine, and Genimpact, a project supported by the European Union to evaluate the genetic impact of aquaculture activities on wild fish populations. An example using information for a permit application for a hypothetical marine aquaculture operation is used to illustrate some of the issues involved with assessing and managing genetic risks. A section on frequently asked questions addresses some specific topics that arise repeatedly in considering genetic consequences of artificial propagation. A number of risk-averse strategies can be employed to manage risk, including the use of triploid or sterile fish, shore-based systems, site selection, and improved containment technology, among others.

Discussion

Although marine aquaculture is a relatively new enterprise, artificial propagation of fish populations has been conducted for centuries, and the major genetic risks associated with salmon hatcheries and marine stock enhancement (loss of fitness, loss of diversity within and among natural populations) have been characterized for several decades. A key feature of these genetic risks is that they are proportional to the fraction of successful spawners in nature that were produced in captivity. Each of the major genetic concerns associated with these hatchery/enhancement programs is also a potential risk of marine aquaculture. However, important differences also exist between marine aquaculture and hatchery/enhancement programs that can affect the likelihood that genetic effects will materialize. For this to occur, three conditions must be met: 1) captively reared individuals must enter the marine environment; 2) some of these individuals must survive to sexual maturity, and 3) some of the mature adults must successfully reproduce, including some level of interbreeding with wild fish. For salmon hatcheries and marine stock enhancement programs, all three conditions are commonly met: captive individuals are intentionally released into the wild in the hope that at least some will survive long enough to be harvested at a substantially larger size, and those that aren't harvested often reproduce in natural habitat (sometimes by design). The goals of marine aquaculture are quite different, as each of the three conditions leads to undesirable outcomes: escapes are economically costly and therefore unintentional, and survival and reproduction of escapes increases regulatory scrutiny while providing no economic benefits.

These considerations indicate that successful containment of genetic risks associated with marine aquaculture should focus on two general strategies: 1) prevent escapes and 2) ensure that individuals that do escape have a low probability of surviving to reproduce in the wild. The magnitude of genetic effects of marine aquaculture is determined primarily by how effective programs are at these two control points. Regarding the first point, a variety of methods can be used to reduce the probability of escapes, but it is generally recognized that no marine containment system will be 100% effective. The realized genetic effects of marine aquaculture on natural populations, therefore, will depend heavily on the subsequent fate of individuals that escape into natural habitats. According to one view, significant genetic introgression due to escapes from aquaculture is unlikely, because stocks used for aquaculture will either be sterile or so highly domesticated that survival and reproduction in nature is greatly compromised. The extent to which (and time frame over which) this actually occurs, however, is likely to vary considerably among species.

Currently, marine aquaculture is experimenting (or contemplating experimenting) with a wide variety of slightly domesticated and not-yet-domesticated species. Eventually, attention might focus on a relatively few, highly domesticated species, as occurs with terrestrial grain production and animal husbandry. If this happens, it might be the case that escaped individuals would have minimal success in the wild. In the interim, however, it seems likely that at least some marine aquaculture operations will use stocks that have an appreciable probability of surviving and reproducing in the wild, and in these cases the effectiveness of the first control point (preventing escapes in the first place) becomes increasingly crucial. Because no aquaculture system in the marine environment is perfect, it is important to consider program scale and duration in developing strategies to provide adequate safeguards for natural populations.

An alternative strategy for dealing with the consequences of escapes is to minimize domestication in the aquaculture broodstock, with the goal of minimizing the deleterious genetic effects of captive-wild interbreeding events that do occur. This strategy is widely used, for example, in hatchery supplementation programs for salmon that have a goal of improving the conservation status of a natural population. However, the differences between the captive and wild environments are so substantial that some erosion of fitness is impossible to avoid, even in the most enlightened programs; furthermore, this strategy will generally enhance survival and reproduction of aquaculture escapes and lead to elevated rates of introgression. Therefore, although development of locally derived broodstocks might have a role to play in the early stages of development of marine aquaculture, it is not a panacea.

Although the genetic risks posed by marine aquaculture are well understood in a general sense, considerable uncertainty is associated with predicting the exact consequences of any particular program. Furthermore, risks will vary according to biological attributes of the target species, but relatively little is known about the basic biology of many marine species that might be used in aquaculture. As a consequence, it is important for scientists, managers, and policy makers to discuss how to deal with uncertainty and what is an appropriate way to assign burden of proof. Should proposed operations be allowed to go forward unless it can be convincingly demonstrated that they will cause serious harm? Or should proposed operations not be allowed to proceed unless it can be demonstrated with a high degree of certainty that adverse effects will not occur?

These questions will repeatedly arise in considering the most appropriate ways to implement marine aquaculture. They cannot be answered by science alone, but how they are answered can have a profound effect on the nature and magnitude of aquaculture programs that are considered permissible. The same general questions, it should be noted, are relevant to assessing the consequences of other anthropogenic actions that affect marine populations (such as fishing or habitat modification), as well as the consequences of propagation programs that release large numbers of individuals of other taxa into the wild (e.g., birds, insects, trees).

Two critical uncertainties merit particular attention for focused research efforts. First, the factors that determine how likely escaped fish are to spawn naturally are diverse and poorly understood, but have a strong influence on actual genetic risks posed by marine aquaculture. Therefore, achieving a better understanding of how escapes function in the wild should be an important research priority. Second, the relative genetic consequences for natural populations of using local, lightly domesticated versus nonlocal, strongly domesticated broodstocks are complicated to assess. The first strategy will reduce divergence between captive and wild populations and hence the severity of interbreeding events that do occur, but will also generally lead to more captive-wild genetic interactions. The second strategy should reduce survival or reproductive capacity of escapes, but each interbreeding event that does occur is more likely to be deleterious. More research is needed to determine the conditions under which each strategy is more consistent with sustainability of natural populations.

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Introduction

Background

Aquaculture (farming of aquatic organisms) has a long history in freshwater and marine systems. In the United States, scientists from the National Marine Fisheries Service (NMFS) or its precursor agencies have played a role in development and evaluation of marine aquaculture since the late 1800s (e.g., Earll 1880). But two recent developments have generated a new wave of interest in aquaculture. First, continued rapid growth of the global human population generates increasing demand for high-protein food sources, a trend expected to continue into the foreseeable future. However, for the last two decades, worldwide harvest of wild fish and shellfish has leveled off at around 80–90 million metric tons annually. In contrast, over this period aquaculture has been a fast-growing system of food production, increasing at a rate of nearly 9% per year (Figure 1). Aquaculture is widely viewed as becoming increasingly important in the future in helping to lessen the gap between worldwide demand for and production of protein, at least if it can do so without compromising the sustainable production of protein from fisheries. Nearly half of the fish consumed worldwide is now farmed, compared to 9% in 1980, with most of the current farmed production involving freshwater species. Estimated aquaculture production would have to nearly double by 2050 to keep up with current per-capita consumption levels (FAO 2006).

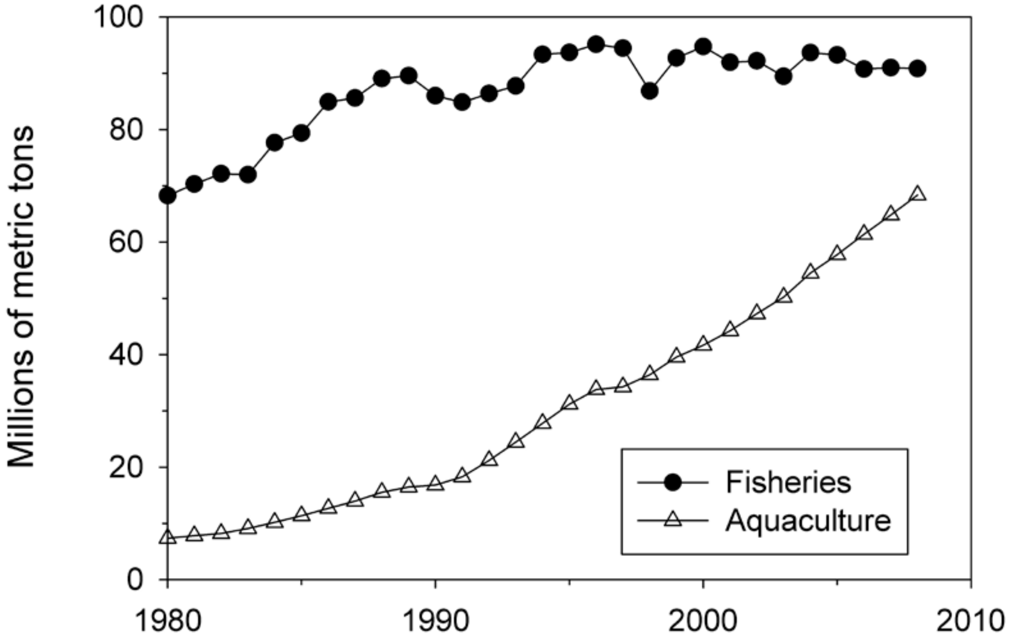

Figure 1. Worldwide production from wild-capture fisheries and aquaculture. Data from FAO 2010.

A second major development over the last 2–3 decades has been growing recognition of the importance of biodiversity conservation and the profound ways in which anthropogenic changes to natural ecosystems can affect biodiversity (e.g., the Convention on Biological Diversity that emerged from the 1992 U.N. Conference on Environment and Development). On a global scale, the most pervasive anthropogenic changes to natural ecosystems involve loss and degradation of habitat, overharvest, and species introductions (Diamond 1989, Hilton-Taylor 2000, Mace and Reynolds 2001). Potential effects of agriculture (including aquaculture) on biodiversity are also substantial, even when they are not dependent on introduced species. Aquaculture and stock enhancement generally involve direct intervention in growth and reproduction of target species; in many cases, intentional selection for production-related traits also occurs. These evolutionary changes in cultured species can have profound consequences for natural populations when cultured and wild individuals interact (Costa-Pierce 2002, Bert 2007). Wild progenitors of most domesticated animals have long since disappeared from the natural landscape, but that is not the case with the vast majority of species subject to aquaculture.

Not surprisingly, these two developments have generated a certain amount of tension, and as a result, a new and challenging problem has been identified: How can global aquaculture production continue to increase at a rapid pace without compromising long-term viability and sustainability of natural populations and the ecosystems on which they depend? In the United States, legislative and policy events over the past decade have focused attention on the promise and risks of aquaculture and broadened NOAA's involvement in marine aquaculture (commercial and enhancement). Some of the key events include:

1. The Department of Commerce Aquaculture Policy (1999) set the following goals for 2025:
    - Increase the value of domestic aquaculture production (freshwater and marine) from $900 million annually to $5 billion.
    - Develop aquaculture technologies and methods not only to improve production but also to safeguard the environment.
    - Enhance depleted wild fish stocks through aquaculture.
2. The Final Report of the U.S. Commission on Ocean Policy (2004) called on the Secretary of Commerce to:
    - Design and implement national policies for environmentally and economically sustainable marine aquaculture.
    - Develop a comprehensive, environmentally sound permitting, leasing, and regulatory program for marine aquaculture.
    - Expand marine aquaculture research.
3. In June 2005, the Marine Fisheries Advisory Committee asked NOAA to prepare a plan to support development of marine aquaculture in the United States. In response to this request, NOAA (2007) produced a "10-year Plan for Marine Aquaculture" that identifies four goals:
    - A comprehensive regulatory program for marine aquaculture.

- Development of commercial marine aquaculture and replenishment of wild stocks.
- Public understanding of marine aquaculture.
- Increased collaboration and cooperation with international partners.

4. The Aquaculture Act of 1980 was reauthorized in the 2008 Farm Bill. The act calls on the departments of Agriculture, Commerce, and Interior to develop U.S. aquaculture.
5. The Magnuson-Stevens Reauthorization Act of 2006 calls for the end of overfishing, rebuilding of wild stocks managed by the federal government, and attention to the effects of fisheries regulations on coastal communities (e.g., alternative or supplemental employment).
6. NOAA (with other agencies) continues to administer the U.S. Endangered Species Act (ESA), the Marine Mammal Protection Act, the National Environmental Policy Act, and other laws aimed at the conservation and protection of marine species and the marine environment.
7. In June 2011, following public comment, the U.S. Department of Commerce Aquaculture Policy and the NOAA Marine Aquaculture Policy were released. Potential competitive and genetic effects of aquaculture on wild species were among the identified environmental challenges to be addressed.

Several marine species are already subjects of commercial aquaculture in the United States, including Atlantic cod (*Gadus morhua*), cobia (*Rachycentron canadum*), striped bass (*Morone saxatilis*), pompano (*Trachinotus* spp.), yellowtail jack (*Seriola lalandi*), moi (Pacific threadfin, *Polydactylus sexfilis*), kahala (greater amberjack, *Seriola dumerili*), American lobster (*Homarus americanus*), Pacific oyster (*Crassostrea gigas*), hard clam (*Meretrix lusoria*), goeduck (*Panopea abrupta*), abalone (*Haliotis* spp.), and others. On a regular basis, NMFS must make decisions about the nature of aquaculture programs that are consistent with the agency's stewardship responsibility for living marine resources. These decisions must consider a complex range of factors from the natural and the social sciences. This technical memorandum is intended to provide managers with a better understanding of the potential genetic effects of marine aquaculture for natural populations, so that these factors can be incorporated more effectively into informed decisions about the appropriate role of marine aquaculture.

Objectives of the NOAA Aquaculture Program

It is clear from the material reviewed above that NOAA has several responsibilities with respect to marine aquaculture:

1. Facilitate and support the growth of sustainable seafood production in the United States by aquaculture, including stock enhancement activities and commercial production.
2. Ensure that such marine aquaculture that comes under NOAA's legal and regulatory mandates is compatible with NOAA's stewardship responsibility for living marine resources.
3. Develop a scientific framework to assist in permitting and regulating aquaculture.

4. Develop a research program that takes advantage of opportunities provided by aquaculture operations to address critical uncertainties and educate the public about the benefits and risks of aquaculture.

Objectives of this Document

This report is intended to assist the agency in meeting some of the above responsibilities. We do not attempt an exhaustive literature review of scientific information regarding this complex topic; rather, we synthesize and summarize the relevant information and identify references that treat these topics in more detail for interested readers. Furthermore, the document will not consider ecological factors (such as waste discharge/assimilation, competition, and disease), except insofar as they might have evolutionary consequences for natural populations; ecological factors are treated elsewhere (e.g., Naylor et al. 2005, Ford and Myers 2008, Diana 2009, Duarte et al. 2009). We will focus on commercial aquaculture of marine finfish (which is typically designed to rear individuals in net pen cages that are intended to be self-contained) but will also consider information for marine stock enhancement (in which individuals are intentionally released into the wild for growth and maturity). Although the focus will be marine finfish, we also draw on the extensive literature for freshwater and anadromous species, as well as examples from aquaculture of marine invertebrates.

We begin with a summary of lessons that have been learned over the past several decades of experience with artificial propagation (including aquaculture) of anadromous and marine species. The next section, Characterizing Risks and Benefits, explores in detail the potential benefits and risks posed by commercial aquaculture and outlines the factors that should be included in a comprehensive risk-benefit assessment of a proposed aquaculture operation. In Managing Risk, we outline general and specific tools that can be used to help assess and manage risks. This section also includes discussion of methods for monitoring genetic consequences of marine aquaculture and identifies some critical uncertainties that merit additional research. We follow this with a section that includes answers to frequently asked questions about artificial propagation and its effects on natural populations. Finally, we include a worked example to show how the principles discussed here can be used to make decisions about appropriate use of marine aquaculture.

It is important to clarify our use of the word risk. Some authors (e.g., Currens and Busack 1995) have used the term to apply only to the probability that an event will occur. We use the term risk in the broader sense of “a factor, thing, element, or course involving uncertain danger”[1] because this usage facilitates the discussion of risks and benefits. In the Glossary, we provide definitions of other key terms used in this document, particularly those that might not be familiar to nongeneticists. To allow a briefer and (hopefully) more readable treatment in the main text, detailed supporting material and many additional references have been moved to Appendix A. A reader looking for more information on a particular topic can look at the material under the same heading in this appendix.

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[1] American Heritage Dictionary, third edition.

Lessons Learned

In this section, we synthesize and summarize what has been learned over the past several decades from experience in three major arenas: salmon aquaculture and hatcheries, propagation of marine species, and agriculture. More details and references on these topics can be found in Appendix A.

General Lessons

Expect the Unexpected

Unanticipated events inevitably occur during the course of a program or operation; this is particularly true for those working with new species or populations recently brought into culture. In most cases, these unexpected events lead to higher rather than lower risks to natural populations. This means that risk analyses that do not explicitly account for such events probably will underestimate risks and the potential for adverse effects on natural populations.

Program Goals

Risks and benefits are best evaluated in the context of program goals. The goals of stock enhancement and captive aquaculture are substantially different in terms of intended interactions with natural populations; this will influence how risks and benefits are monitored and managed (Table 1). Stock enhancement programs generally incorporate wild individuals into the broodstock to minimize divergence from the wild population and intentionally release individuals into the wild, with the goal of enhancing harvest, the naturally spawning population, or both. In contrast, any interactions of aquaculture and wild fish are incidental and intended to be minimized. See the Characterizing Risks and Benefits section for more on this topic.

Adaptive Management

Lessons about effects on natural populations are often slowly learned from experience. The major types of genetic effects of cultured fish on natural populations have been known since

Table 1. Contrasting goals of stock enhancement and aquaculture.

	Stock enhancement	**Aquaculture**
Incorporation of wild individuals into broodstock	Yes	Sometimes
Release of cultured individuals into the wild	Intentional	Accidental
Reproduction of cultured individuals in the wild	Intentional	To be minimized

the early 1980s and are, for example, outlined by Allendorf and Ryman (1987), Hindar et al. (1991), and Waples (1991). In subsequent years, these effects on natural populations have been increasingly well documented (Araki and Schmid 2010). However, it was a long time before attempts to address these concerns were made in many fishery management arenas, and even today these issues are commonly neglected in the fields of forestry and game management. Laikre et al. (2010) offer some possible explanations for this neglect; perhaps the most important is that potential benefits of stock enhancement are easy to visualize, while appreciation of long-term effects on natural populations requires a more nuanced understanding of biological systems. With respect to the current interest in taking advantage of potential benefits of marine aquaculture while providing adequate safeguards for natural biodiversity, the most effective approach will be to accept the reality of genetic risks and focus attention on understanding how the risks interact with key features of the marine environment, life histories of the species involved, and particular aspects of individual aquaculture programs.

Diversity of Natural Populations

Conservation of a diverse array of natural populations or native land races is the most reliable way to ensure long-term sustainability. This point has been made repeatedly by a long series of science panels that have reviewed this topic in recent decades (e.g., NRC 1996, NOU 1999, Myers et al. 2004, NASCO at http://www.nasco.int).

Monitoring

An effective monitoring component is important but cannot compensate for failure to implement risk-averse strategies. Monitoring is necessary to estimate the magnitude of effects of a particular aquaculture or enhancement program on natural populations, as well as to provide adaptive management information that can be applied more broadly to other programs. However, even ambitious monitoring programs often have low power to detect adverse effects before they have long-lasting consequences (Hard 1995b).

Lessons from Salmon Aquaculture

Artificial propagation involves direct intervention in the reproduction and survival of the target species. Because some leakage from the cultured to the wild population is almost inevitable, propagation programs also involve, either directly or indirectly, intervention in survival and reproduction of associated natural populations. Here are a few of the practical realities that can be expected as part of an aquaculture operation, starting with those that apply equally to captive commercial aquaculture and enhancement of natural populations.

Escapes

A certain level of escapes from marine cages (net pens) is almost inevitable, except perhaps for secure land-based operations. Key questions arise. How many individuals will escape and how often? What life stages will escape? How will they interact with wild populations?

Recapture of Escaped Fish

Efforts to recapture fish that have escaped from culture have had limited success. This means that managers cannot rely on mop-up operations and containment efforts are more effective when they focus on prevention of escapes in the first place.

Postescape Survival

Although many escaped individuals do not survive, because they are eaten by predators or are not conditioned to foraging for food, some do. Captively reared fish that do survive can disperse over large areas. As a consequence, wild populations at some distance from culture sites can be affected by escaped captively reared individuals.

Marking

Although efficient aquaculture operations keep close track of inventory and will generally notice any sizeable number of escapes, fish that do escape can be hard to detect and distinguish in the wild unless they are marked and monitoring is sufficiently frequent and adequate in spatial scale. This reality complicates efforts to control the effects of escaped fish (by targeting collection of marked fish), as well as efforts to monitor the consequences of escapes for natural populations.

Reproduction of Escaped Individuals

Reproductive capabilities of captively reared fish in the wild vary across species, life stages of release/escape, geographic locations, and other factors. If survival and reproduction in the wild are poor, the genetic consequences of escapes could be much less than might be suggested by their absolute numbers. Generally, individuals escaping at an early life stage have a lower probability of surviving to reproduce than do escapes that are nearly mature, and a higher reproductive success if they reach maturity after having spent most of their life in the wild. However, escapes at any life stage can lead to successful reproduction in the wild, and if large numbers escape at early life stages, the probability that at least some will survive to reproduce could be high.

Containment Effectiveness

Indices of containment effectiveness (e.g., the fraction of cultured individuals that escape) must be evaluated in the context of program scale. Although escapes cannot be eliminated, they can be controlled to some extent with sufficient experience, efficiency, and financial motivation. However, even if the fraction of escaping fish declines over time, the net effects of escapes on wild populations can continue to increase if overall production is also increasing. This pattern has been observed for Atlantic salmon (*Salmo salar*) in Norway.

Joint Lessons from Salmon Aquaculture and Hatcheries

Long-term Sustainability

Empirical results show that, in many cases, artificial propagation can maintain populations for several decades or fish generations. However, little evidence exists to show that artificial propagation can improve the long-term sustainability of natural populations. These results suggest that potential conservation benefits of artificial propagation might be largely limited to short-term efforts to prevent extinction. Long-term effects of artificial propagation are essentially unknown; even the longest-running programs have lasted only a tiny fraction of the time over which most aquatic species have persisted in the wild.

Genetic changes associated with artificial propagation are inevitable. Selective regimes and mortality profiles differ so dramatically between natural and cultured populations that some genetic change cannot be avoided—and from the aquaculture perspective, such changes might be desirable if they increase profitability. For the purposes of this paper, the key questions are: How much genetic change will occur and what will be the consequences for natural populations with which they might interact?

Fitness

Fitness of captively reared fish in the wild decreases with the number of generations in captivity. A large body of empirical information now documents reduced fitness of cultured fish in nature compared to their wild counterparts, and the fitness reductions increase as a function of the number of generations in captivity. Reduced fitness has also been documented among the wild-born descendants of captively reared fish (Araki et al. 2009). These fitness reductions become a concern when escaped fish interbreed with natural populations.

Genetic Risks

Genetic risks associated with fish culture can be reduced but not eliminated entirely except in a completely closed system. This is true even for state-of-the-art programs. Many risks are negatively correlated, such that reducing one inevitably increases another (see Waples and Drake 2004). For example, creating a genetically divergent aquaculture population might reduce the chances that escapes can survive and reproduce, but those that do can pass on very maladapted genes to the natural population.

Direct Effects

The consequences for natural populations of interbreeding with cultured fish depend in a complex way on a variety of factors. The factors that determine whether invasion and introgression are initially successful are difficult to predict (Drake and Lodge 2006, Hayes and Barry 2008). The factors that subsequently influence the consequences of introgression include the rate and duration of interbreeding and the magnitude of genetic differences between cultured and wild populations. Some conclusions are straightforward: 1) effects are cumulative across generations, so all else being equal, interbreeding is more detrimental the longer it occurs; 2) for a given level of population divergence, interbreeding is more harmful if it occurs at a higher frequency; and 3) for a given rate of interbreeding, the consequences are worse if the cultured population is strongly diverged from the wild population. However, in other cases firm predictions are difficult because of inherent trade-offs (see Frequently Asked Questions section below).

Indirect Effects

Reduced population size and other indirect genetic effects have been associated with fish culture. Effects on survival or abundance of wild fish populations have been linked to aquaculture production of the same or a closely related species. Moreover, stock recruitment analyses have shown reduced recruitment for the same stock size when part of the spawning stock has a cultured background.

Genetic Effects

Genetic effects have occurred in native populations following invasion of intentionally or accidentally released cultured fish. The genetic effects that have been found in wild populations receiving cultured fish include changes in molecular genetic diversity and changes in fitness traits that have a genetic component. When genetic effects on fitness traits have been detected, they are generally negative in comparison with unaffected native populations (Hindar et al. 1991).

Substantial genetic effects are not inevitable, even when large numbers of captively reared fish are released into the wild. For example, some enhancement programs for salmon have operated for many years without detectable genetic effects on natural populations (reviewed by Hindar et al. 1991). This does not prove that these programs have had no genetic consequences, since the power to detect effects might have been limited by sampling constraints and fitness has seldom been evaluated directly. However, this result does illustrate the difficulty in making quantitative predictions about consequences of any particular program.

Genetic Technologies

Breeding programs and application of genetic technologies have been developed for relatively few aquaculture species, and the potential consequences of escaped cultured fish for wild populations have not yet been carefully evaluated. Until recently, breeding programs in aquaculture have been limited to a few species such as salmon and trout (Salmonidae spp.), tilapia (e.g., *Tilapia*, *Oreochromis*, *Sarotherodon* spp.), seabass (e.g., *Morone* spp.), sea bream (e.g., *Pagrus major*, *Acanthopagrus schlegeli schlegeli*), catfish (e.g., *Ictalurus punctatus*), oysters, shrimp, and other invertebrates. In comparison to programs for terrestrial agricultural species, breeding programs for most aquaculture species have been ongoing for only a few years or decades. Consequently, knowledge of the phenotypic and genetic parameters of many economically important traits of interest to marine aquaculturists is limited. This is an important gap in knowledge, because this information might also be helpful in determining how interbreeding between escaped cultured and wild individuals might affect natural population structure and viability in the marine environment.

Lessons from Marine Stock Enhancement

A large number of marine species have been targets for marine stock enhancement or restoration (sometimes also called sea ranching, which entails intentional release of juveniles into the wild), but only a small fraction have been studied in detail. Lorenzen et al. (2010) provide an updated discussion of responsible approaches to marine stock enhancement programs. It is an open question whether the strong empirical record for salmonids provides a good indication of the likely effects in marine systems that can be affected by cultured populations. On the one hand, all the genetic and ecological concerns identified for salmonids also apply in theory to marine species. On the other hand, some (e.g., Bonhomme 2007) argue that most marine species with high fecundity and large population sizes should be less susceptible to at least some genetic risks.

Here are some factors associated with marine species that should be kept in mind in extrapolating from the lessons learned from anadromous species.

Biology of Marine Species

Much less is known about the basic biology of most marine species compared to salmon. As a consequence, except for a relatively few well-studied species, more uncertainty will be associated with all aspects of marine aquaculture programs. However, collectively marine species have a greater diversity of life history strategies and fill a greater range of ecological roles than do salmonids. This observation suggests that novel cultured/wild interactions are likely to occur in some marine species and that the lessons learned from salmonid systems will need to be supplemented based on experience with marine systems.

Population Differentiation

In general, on an equivalent spatial scale, marine species show lower levels of molecular genetic differentiation among populations than do anadromous or freshwater species. Presumably this arises from larger population sizes (for which random genetic changes are considerably reduced) and reduced barriers to dispersal.

Local Adaptations in Marine Species

Despite the previous point, several recent studies show surprisingly strong evidence for local adaptations in populations of marine species. This result emphasizes the empirical observation that molecular (and presumably selectively neutral) genetic variation is generally a poor predictor of the degree of adaptive genetic differentiation (Hard 1995a, Lynch 1996, Naish and Hard 2008).

Spawning in Net Pens or Cages

At least some marine species can successfully spawn in net pens or cages. This reality presents special challenges for managing aquaculture programs for marine species: even if managers are 100% successful in controlling escapes of cultured individuals, gametes or fertilized eggs produced by cultured adults can leave net pen cages and might lead to introgression with wild populations.

The high fecundity of many marine species means that relatively few individual breeders can have a large genetic impact on natural populations. This can potentially lead to an extreme example of the Ryman-Laikre effect, where the wild population is swamped by genes derived from a few captive individuals. This in turn can reduce levels of genetic variability and increase levels of inbreeding in natural populations.

Evaluation

Detecting and evaluating the genetic effects of cultured marine species on natural populations is extremely challenging and involves a number of linked steps. A fault tree of the chain of events necessary for introgression of genes from escaped cultured fish into the wild (Figure 2) describes the key events and what to look for.

Despite some obvious differences between marine species and salmonids, many important features of the marine species considered by the European Union project Genimpact are similar to those of Atlantic salmon, as illustrated in Table 2.

Lessons from Agriculture

In the previous two subsections, we focused on lessons that are relevant to consideration of potential effects on natural populations. These factors have not been primary considerations during the development of the agriculture and aquaculture industries. Agriculture has long relied on the incredible genetic and phenotypic diversity available in natural populations to develop cultured stocks that can be altered through control of breeding and environmental variation. The primary considerations in these industries are the cost-effective production of safe and high-quality products (see Gjedrem 2005). Breeding programs in agriculture have applied a combination of inbreeding, selection, and crossbreeding to develop strains with desirable characteristics that improve the efficiency of agricultural production. Chief among these characteristics are stage-specific survival, rapid growth, high tissue quality for consumption, ease of culture, rapid maturity, and improved resistance to pathogens. Here we briefly outline how these considerations help to shape the nature and types of agriculture programs that are implemented.

Selective Breeding

Agriculture can efficiently produce plants and animals with desirable marketable phenotypes through careful broodstock selection, intensive selective breeding, and judicious use of arable land. Agricultural operations have grown in efficiency with technological advances and more efficient use of land, but major advances have also been made by genetically improving stocks through the concerted application of stock selection, inbreeding, and crossbreeding. The combination of selective breeding, calculation of breeding values based on an individual’s performance and that of its relatives, and breeding to maximize genetic gain can rapidly increase the frequency of desirable phenotypes for market.

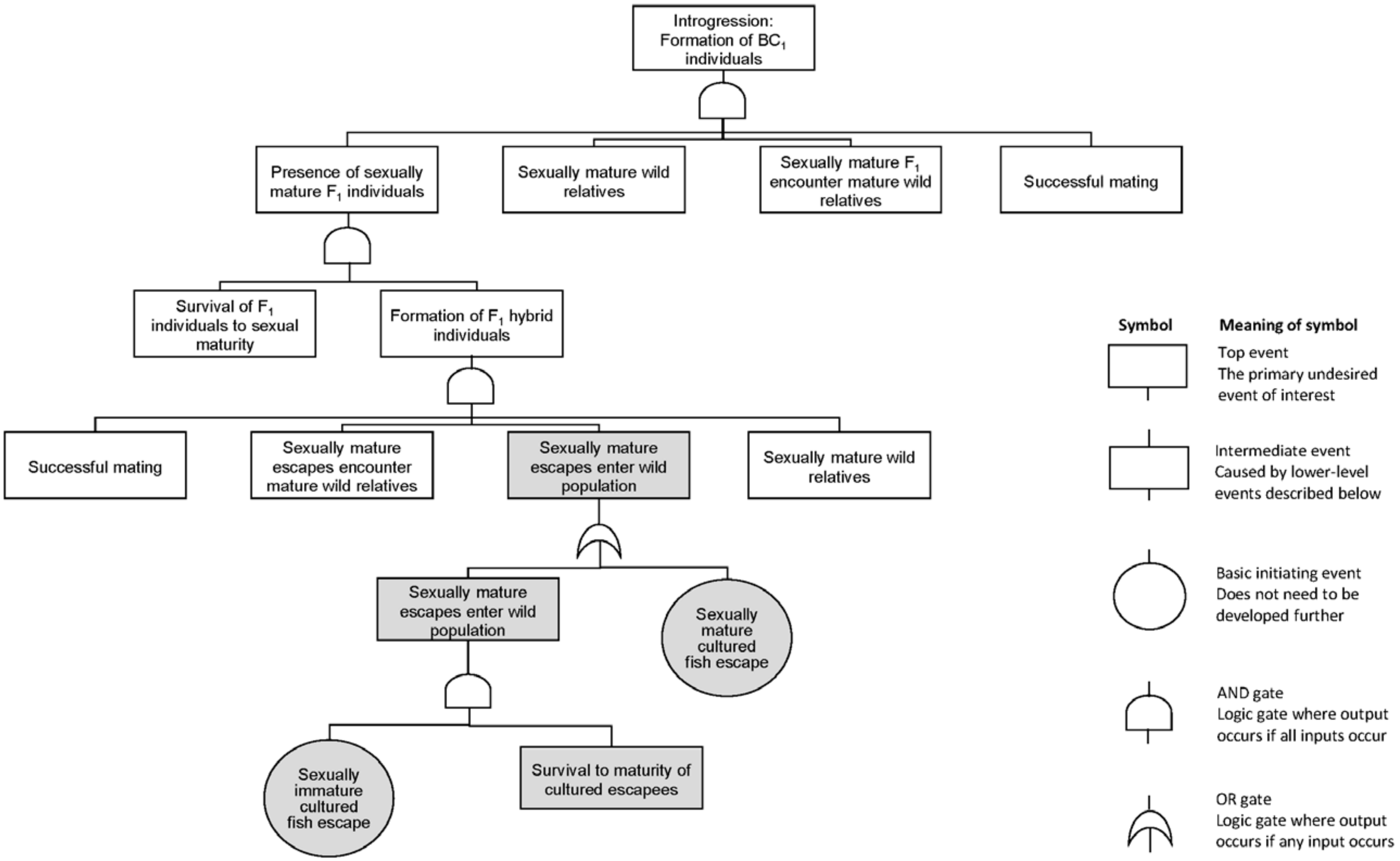

Figure 2. Fault tree of events leading to introgression of genes from escaping cultured fish into the gene pool of wild relatives. The fault tree should be read from the bottom to the top. Gray background = event leading to entry of sexually mature cultured fish into a breeding wild population. White background = postentry event leading to introgression. F_1 = first-generation hybrids from cultured and wild fish matings. BC_1 = first-generation backcrosses between F_1 hybrids and wild relatives. Additional lower level events could contribute to events in rectangular boxes but they are not shown for clarity. (Adapted with permission from Kapuscinski et al. 2007, copyright Centre for Agricultural Bioscience International.)

Table 2. Comparison of biological and aquaculture-relevant characteristics of Atlantic salmon and marine fish species considered for genetic impact studies in Europe by the European Union Genimpact project (Svåsand et al. 2007) and in Asia (i.e., large yellow croaker, Liu et al. 2008). FW = freshwater, LC = least concern, LR = low risk, V = vulnerable, E = endangered, and NE = not evaluated.

Species	Range	Ecology	Population genetics	Wild harvest	Breeding and culture	Interaction studies	IUCN status	Reference
Atlantic salmon	North Atlantic Ocean and freshwater; 72°N to 37°N, 77°W to 37°E	Anadromous, some FW; river spawning; long migration with homing; feeds on invertebrates and fish; spends 1–6 yr FW; 1–4 yr sea; sexually mature M 10–140 cm and 0.01–35 kg, F 40–120 cm and 1–20 kg; annual to biannual spawning; eggs 2,000–20,000, 6 mm diameter, benthic; max 150 cm, 45 kg, 13 yr	Moderate to strong genetic structure; global F_{ST} = 0.366 allozymes; E. Atlantic, W. Atlantic subspecies; empirical evidence for local adaptations	1,700 t (2008), declining 80% from 1970–1980s; extinct from many rivers in North America and Europe	1.3 M t; 300-fold increase since 1980; hatching and juvenile rearing in FW tanks 0.5–1.5 yr; growth in seawater cages 1–2 yr; large-scale selective breeding programs (since 1970s) for growth, age at maturity, disease resistance, flesh quality; thousands of single nucleotide polymorphism (SNP) markers	This report	LR	Genimpact[a] FishBase[b] FishStat Plus[c] Ward et al. 1994 Verspoor et al. 2007
Atlantic cod	North Atlantic Ocean; 35–80°N, 95°W–61°E; cont. shelves and banks	Marine; epibenthic pelagic; long migration, localized spawning sites; feeds on invertebrates and fish; sexually mature 30–100 cm, 3–15 yr; annual spawning; eggs 1,000s to 30 M, 1 mm diameter, pelagic; max 200 cm, 96 kg, 25 yr	Low to moderate structure; global F_{ST} = 0.076 allozymes; stationary and migratory population segments; empirical evidence for local adaptations	1M t (2004); declining 70% since 1960s; major stock collapses	10,000 t; 5-fold increase since 2000; larvae production difficult and partly relying on live feed; mature 2 yr, some growth in sea cages, partly from wild-caught juveniles; selective breeding programs (since 1990s) for growth, delayed maturity, disease resistance; genome sequenced 2009	Genetic marking in sea ranching experiments; spawning in sea cages	V	Genimpact FishBase Ward et al. 1994

Table 2 continued. Comparison of biological and aquaculture-relevant characteristics of Atlantic salmon and marine fish species considered for genetic impact studies in Europe by the European Union Genimpact project (Svåsand et al. 2007) and in Asia (i.e., large yellow croaker, Liu et al. 2008). FW = freshwater, LC = least concern, LR = low risk, V = vulnerable, E = endangered, and NE = not evaluated.

Species	**Range**	**Ecology**	**Population genetics**	**Wild harvest**	**Breeding and culture**	**Interaction studies**	**IUCN status**	**Reference**
Atlantic halibut (*Hippoglossus hippoglossus*)	North Atlantic Ocean; 79°N–36°N, 77°W–55°E	Marine; demersal, occasionally pelagic; migratory; depth range 50-2,000 m; feeds on invertebrates and fish; batch spawner; sexually mature M 55 cm, 4.5 yr, F 110 cm, 7 yr; eggs 1 M or more, small, deep, pelagic; larval metamorphosis; max M 470 cm, 320 kg, F 300 cm, 50 yr	Low genetic structure; F_{ST} = 0.012 allozymes; unclear if local adaptations occur	European catches 10-15K t (1950–1965), declined to 2,000 t (2004)	500 t; eggs stripped from wild-caught individuals; life-cycle closed in captivity in the 1990s but still limited success with egg and start-feeding survival; selective breeding programs planned	No data	E	Genimpact FishBase Ward et al. 1994
European seabass (*Dicentrarchus labrax*)	Atlantic Ocean; 72°N–11°N, 19°W–42°E	Brackish and marine; demersal, migratory; depth 10–100 m; feeds on invertebrates and fish; sexually mature 17–46 cm; eggs small 200,000/kg, batch spawner with pelagic eggs; max 103 cm, 12 kg, 15 yr	Atlantic, W. Med. and E. Med. groups, only the latter showing sub-divided populations; possible selection in some allozymes	11,000 t (2004), slightly increasing since 1980s	80,000 t (2005), 20-fold increase since 1990; eggs throughout the year, on-growth in sea cages, raceways and ponds; hundreds of SNP markers	No. of escapes unknown; no interaction studies, but genetic studies indicate some gene flow from hatchery stocks (W. Med. origin) to E. Med. natural populations	LC	Genimpact FishBase Lemaire et al. 2000

Table 2 continued. Comparison of biological and aquaculture-relevant characteristics of Atlantic salmon and marine fish species considered for genetic impact studies in Europe by the European Union Genimpact project (Svåsand et al. 2007) and in Asia (i.e., large yellow croaker, Liu et al. 2008). FW = freshwater, LC = least concern, LR = low risk, V = vulnerable, E = endangered, and NE = not evaluated.

Species	**Range**	**Ecology**	**Population genetics**	**Wild harvest**	**Breeding and culture**	**Interaction studies**	**IUCN status**	**Reference**
Gilthead seabream (*Sparus auratus*)	Atlantic Ocean; 62°N–15°N, 17°W–43°E	Marine and brackish; demersal; 1–30 (150) m depth; carnivorous, accessorily herbivorous; sexual maturity 20–40 cm; sex reversal at 2 yr (male to female); batch spawner with up to 1 M eggs, 1 mm diameter; max 70 cm, 17 kg, 11 yr	Moderate genetic structure; Atlantic and Med. F_{ST} = 0.031 allozymes, higher among some Tunisian samples	9,000 t (2004), slightly increasing since the 1980s	91,000 t (2004); tank and cage rearing, and restocking in lagoons; family based selection program since 2002; hundreds of SNP markers	Little knowledge about interactions, some Atlantic origin among Med. broodstock; possible spawning in cages	NE	Genimpact FishBase Alarcón et al. 2004 de Innocentiis et al. 2005
Turbot (*Psetta maxima*) (syn., *Scophthalmus maximus*)	Atlantic Ocean; 30–70°N, 23°W–42°E	Marine and brackish; demersal, 20–70 m, migratory; sexual maturity 41–54 cm; batch spawner with up to 5–10 M eggs, max 100 cm, 25 kg, 25 yr	Little or no structure based on allozymes Atlantic coast of Europe; hybrid zone between North Sea and Baltic Sea; two Med. lineages	7,000 t, fluctuating since 1980s	5,000 t (2002); reared in concrete tanks; high stocking density; selection programs since mid-1990s; chromosome manipulation	No data; information from restocking programs suggest rapid adoption of natural diet	NE	Genimpact FishBase Blanquer et al. 1992 Nielsen et al. 2004
Large yellow croaker (*Larimichthys crocea*)	Coastal waters of China and SW South Korea; 20–36°N, 110–127°E	Marine; benthopelagic soft bottom; migratory; feeds on crustaceans and fishes; spring and autumn spawning near river mouths; sexually mature M 17 cm+ and 2–4 yr; millions of eggs; max 80 cm, 29 yr with variation between stocks	Three putative stocks; loss of genetic variation in cultured stocks	Peak 240,000 t mid 1970s; collapse late 1980s; currently c. 70,000 t	Hatchery and release programs since 1980s; released at 6–10 cm after 6 months; cage rearing 70,000 t 2005; 2 billion juveniles produced 2005	Reduced genetic variation in captivity; suggestion of genetic problems following releases	NE	FishBase Liu et al. 2008

[a] Genimpact online at http://genimpact.imr.no

[b] FishBase online at http://www.fishbase.org

[c] FishStat Plus (FAO 2010) online at http://www.fao.org/fishery/statistics/software/en

Careful application of inbreeding and selection in agricultural settings can dramatically improve production gains, but the application of inbreeding and selection requires considerable care to minimize genetic problems, not only within the program but also when cultured and wild individuals interbreed (Lind et al. 2012). Inbreeding is used in culture programs to reduce undesirable variation and improve efficiency of selection and, so long as the rate of inbreeding is not too high, most cultured populations can thrive in protective environments. Empirical data suggest that cultured populations can withstand sustained inbreeding levels of about 1–2% per generation. However, levels of inbreeding that can be tolerated in a benign culture environment can be much higher than is typically experienced in the wild. The fitness consequences of inbreeding are very context specific and the largest decreases in fitness typically occur in the most stressful environments. Indeed, estimates of inbreeding depression in captive populations can substantially underestimate the loss of fitness that can occur in natural environments (Wang et al. 2002). Inbreeding is a particular risk in culturing species with high fecundity—the case with many marine species—because of the ability to found and maintain culture programs from only a few individuals (Gjedrem 2005).

Divergence in Captivity

Agricultural strains and varieties often diverge rapidly from wild progenitors. The agricultural environment and the improved well-being of cultured organisms typically lead to rapid phenotypic divergence of cultured individuals from wild relatives. This can involve several factors, including active selection for agriculturally important traits, adaptation to the cultured environment, and relaxation of natural selection that occurs in the wild.

Synthesis

The genetic objectives of fish culture and the potential genetic consequences of aquaculture for wild populations pose a management and regulatory challenge for resource management agencies like NOAA. In general, the factors that are typically associated with successful agriculture/aquaculture programs and which lead to what is considered “genetic improvement” from an agronomic perspective (rapid divergence from wild populations; strong artificial selection for traits that are desirable in the marketplace; heavy reliance on a few, high-performing stocks) are also factors that might pose the greatest potential risks to wild populations. Differences between cultured and wild populations arise from selection and inbreeding, which tend to reduce variation and lead to divergence between cultured and wild individuals.

Widespread monocultures of highly productive stocks can greatly enhance agriculture or livestock production, but these practices also leave the system as a whole more vulnerable to boom-bust cycles and attack by pests or diseases (Mooney et al. 1995). Agronomists increasingly pay careful attention to the rich store of genetic variation contained in native land races of species used for food production and ensuring that this diversity is maintained in situ and in seed banks (e.g., The Millennium Seed-Bank Partnership, http://www.kew.org/science-conservation/conservation-climate-change/millennium-seed-bank/index.htm). This approach is logistically difficult, if not completely infeasible, with most natural populations of marine species that might be affected by aquaculture—a reality that argues persuasively for careful attention to ensuring persistence of diverse wild populations in situ.

Although agriculture and aquaculture share many goals, the capacity to rely on genetically improved aquaculture stocks as a foundation for production efficiency is relatively undeveloped, especially for marine fishes. Aquaculture has a rich history but not nearly as long as agriculture. Carp have been domesticated the longest among fishes, beginning perhaps 4,000-5,000 years ago in what is now China. Agriculture has relied extensively on development and maintenance of domesticated stocks with desirable, marketable qualities. In aquaculture, such stocks are now widely used for some species (e.g., salmonids), but for most marine species such stocks do not yet exist. Nevertheless, the potential for selective breeding to rapidly change cultured stocks of marine species is considerable, as selection response is often higher in fish and shellfish than in terrestrial farm animals.

The scale of commercial and enhancement aquaculture production underway and under contemplation in some parts of the world has potentially large consequences for natural population structure and productivity. The actual consequences of conflicts between cultured and wild populations depend heavily on best management practices; the effectiveness of efforts to reduce risk, size, location, and distribution of production facilities in relation to natural populations; and a variety of other factors. The potentially competing goals of aquaculture and natural resource conservation are likely to become more problematic as the industry grows and strives to be more efficient in response to market forces. This reality emphasizes the need for a systematic and comprehensive framework for considering risks and benefits of marine aquaculture, which is the topic of the next section.

Characterizing Risks and Benefits

Informed decisions about appropriate use of marine aquaculture are best made following a comprehensive risk-benefit analysis. Such analyses might be conducted in a number of ways; below we modify the framework developed by Waples and Drake (2004) for marine stock enhancement, based on experience with Pacific salmon.

Potential benefits are of two kinds: benefits to society and benefits to natural populations. This document will not consider economic, social, or political benefits to society in any detail, because this requires consideration of a wide range of normative issues. Furthermore, benefits to society are measured in a fundamentally different currency than are risks to natural populations, and this reality greatly complicates the ability to incorporate both into an overall quantitative analysis. Nevertheless, it is important to recognize that these normative issues will be implicitly (if not explicitly) involved in most decisions about appropriate use of marine aquaculture. Potential societal benefits of commercial and stock enhancement aquaculture (e.g., jobs, recreational opportunities, species or habitat restoration) are outlined elsewhere in the literature.

Potential Benefits of Artificial Propagation for Natural Populations

Potential benefits include those that directly result from culture activities (1–4), as well as those that are indirect consequences of artificial production (5).

1. Reduce short-term extinction risks for endangered populations. Successful hatchery programs that provide a benign environment to enhance survival during a life-history stage with high natural mortality can help to reduce short-term risks faced by natural populations.
2. Help maintain a population at a safe level until factors for decline can be addressed. By helping to forestall extinction of a population that is not viable in its current environment, this strategy at least allows for the possibility that a self-sustaining natural population eventually can be achieved.
3. Speed recovery by providing a demographic boost to an existing population. Rapid increases in population size help to minimize the time a population spends at low abundance, when it is at highest risk. In theory, a successful short-term supplementation program might help speed sustainable natural recovery by temporarily increasing abundance of a depressed natural population, which then remains stable at the higher population size after the supplementation program terminates. However, few empirical examples exist to show that this has actually occurred.
4. Reseed vacant habitat. Translocations have been widely used with terrestrial and freshwater species and could potentially have a role for marine species.
5. Reduce harvest pressure on natural populations. Overfishing is a serious problem for many marine species and existence of a consistent supply of high quality cultured product

at a reasonable price could substantially reduce demand for (and profit from) wild harvests.

Artificial propagation has already provided benefit 1 for some critically endangered aquatic species, including Redfish Lake sockeye salmon (*Oncorhynchus nerka*) and white abalone (*Haliotis sorenseni*). Hatchery-based enhancement or replenishment programs undertaken by public agencies might or might not have tangible benefits for natural populations. In reality, however, the vast majority of commercial aquaculture operations will neither seek nor have any tangible benefits for natural populations. Therefore, most risk-benefit considerations for commercial aquaculture will have to focus on evaluating trade-offs between potential benefits to society and risks to natural populations.

Genetic Risks to Natural Populations from Aquaculture

Risks to natural populations depend on program goals and the methods used to achieve them; as a result, the risks will differ between commercial aquaculture and marine stock enhancement (Table 2). In stock enhancement, releasing juveniles (generally in large numbers) into the marine environment is integral to the program, and this creates widespread opportunities for introgression to occur, even when captive-wild interbreeding is not a specific objective. In contrast, aquaculture operations seek closed production systems; any leakage from these systems represents a cost that must be minimized to ensure profitability, and this creates an incentive to reduce events that could lead to interbreeding of captive and wild individuals. In this case, leakage is incidental and unintentional, although it can be nearly impossible to eliminate entirely, particularly in the marine environment. Furthermore, although it might be cost-effective to reduce escapes to a small fraction of production, the marginal costs for reducing escapes to near zero might increase exponentially.

A convenient metric for evaluating genetic risks from aquaculture is the fraction of cultured individuals that escape into the wild. However, a more informative measure for assessing potential impacts on natural populations is the fraction of natural spawners that originated from aquaculture, and assessing this requires more information than can be obtained by simply monitoring a culture facility.

Before discussing the risks from escapes, we briefly review circumstances that can create opportunities for escape and interbreeding with wild individuals. Escape events can range from relatively constant leakage of small numbers of individuals to large catastrophic events involving tens of thousands of individuals, millions of gametes or larvae, or more. Escapes from most shore-based, closed recirculating culture systems probably pose a negligible risk; in contrast, the probability of escapes is highest from ponds and net pen cages in near-shore or open-ocean sites. The life stages involved also affect the likely genetic consequences of escapes. A large event involving thousands of escaped juveniles would not be expected to lead to the same level of introgression as the same number of escaped adults, because many juveniles would be expected to die before maturity. Furthermore, the realized level of introgression will depend on the relative reproductive success of cultured and wild individuals. A variety of factors, including source of broodstock, husbandry practices, duration of the program, and proximity to suitable natural spawning habitat, can affect not only the reproductive success in the wild of escaped individuals, but also the genetic consequences of interbreeding events that do occur. Finally, genes from captive individuals are most likely to spread to the wild population when the probability of hatchery individuals encountering the natural spawning population is high, which generally occurs when culture programs contain reproductively mature individuals and culture sites that are close to natural spawning populations.

Strategies to Minimize Genetic Risks

Genetic risks associated with fish culture have been described in several key papers, including Allendorf and Ryman 1987, Hindar et al. 1991, Waples 1991, Busack and Currens 1995, Campton 1995, Waples 1999, Brannon et al. 2004, Waples and Drake 2004, Fraser 2008, Naish et al. 2008, and Araki and Schmid 2010. Here we briefly consider two general approaches for minimizing these risks: preventing escapes and reducing the genetic consequences of escapes that do occur. In the following subsections, we discuss strategies that can help alleviate specific genetic risks.

General approach 1: Reduce opportunities for escapes

The most reliable way to limit genetic interactions between cultured and wild individuals is to ensure that few or no captive individuals escape. Closed, land-based systems can be very effective in this regard, but such operations are not feasible for many marine species. A variety of methods can be used to reduce the number of escapes from marine enclosures. Escapes most often result from cage or net pen failures resulting from storm events or damage caused by potential predators. Appropriate siting and construction of aquaculture enclosures can limit exposure to strong wave or wind action, and the use of guard nets or other devices to discourage damage or intrusion by predators can be effective in limiting escapes from marine enclosures. In Norway, methods include mandatory reporting of all escape incidents, establishment of a special commission to learn from past escape events and disseminate knowledge to both fish farmers and equipment suppliers, training of fish farm operators in methods to prevent escapes, and other measures. However, as noted above, it is virtually impossible to ensure that no cultured individuals will escape from marine enclosures. Therefore, it is important to also consider the second general approach.

General approach 2: Reduce opportunities for reproduction

Although ecological consequences (competition, predation, disease transfer) can arise from any escape event, direct genetic consequences only occur if the escaped individuals survive to maturity and successfully reproduce. For a variety of reasons, the fraction of escapees in the spawning population might be smaller than the fraction they comprise of the total population at the time of the escape event. Several approaches can potentially be useful here:

1. Recapture escapes before they have an opportunity to reproduce. However, empirical data (see Appendix A) suggest that mop-up operations are seldom effective in recapturing a significant fraction of escapes.
2. Locate culture operations well away from areas used by natural populations. Knowledge about the dispersal capabilities of escaped individuals can be used to suggest a minimum distance between culture operations and natural populations.

3. Use only sterile fish. Sterility in most fishes can be imposed with high efficiency and low cost, and has the potential to significantly reduce risks from escaped individuals. Some methods of imposing sterility have been associated with an increase in skeletal deformities.
4. Use a highly domesticated population for culture. Escapes from such a population might have a greatly reduced probability of surviving to maturity and successfully reproducing if they do survive. However, this strategy involves some important trade-offs regarding fitness; see discussion under the Managing Risk section below.

Another important general consideration is that, in real-world management applications, what are intended as absolute thresholds (e.g., parameter values that should never be exceeded) can become viewed as targets to manage for. For example, a program that takes strong steps to ensure that a critical metric never drops below 1,000 could have very different consequences for natural populations than a program that sets 1,000 as a target, in which case a significant number of outcomes with values less than 1,000 could be expected.

Monitoring (see Monitoring subsection in Managing Risk section and see Appendix A) is an essential component of any adaptive management strategy to minimize risks. Although monitoring by itself does not reduce risks, it can provide information that allows scientists and managers to modify biological or physical features of the aquaculture operation to reduce risks. The advent of large numbers of highly variable genetic markers in recent years has facilitated development of powerful methods for monitoring effects of aquaculture escapes on natural populations. For example, Tringali (2006) described a model that uses DNA fingerprints taken from aquaculture broodstock to identify cultured progeny after they are released (or escape) into the wild. In this two-step process, large samples of wild-caught individuals are screened for a small number of gene loci, and this process eliminates most wild-born individuals as potential offspring of known captive crosses. The second step screens additional loci on the smaller subset of individuals to complete the parentage assignment process. This approach has been used for postrelease genetic tracking of cultured red drum (*Sciaenops ocellatus*) in Texas.

Loss of Diversity within Populations

Genetic variation provides the raw material for evolution. All else being equal, populations with low levels of genetic variability have less capacity to respond to stressful conditions or environmental changes.

The primary factors affecting the amount of variation in a population are the rate of mutation (which creates new genetic variants, or alleles) and the effective population size (N_e), which determines the rate at which variation is lost by chance events (genetic drift). Migration also plays a role, by spreading new mutations among populations connected through gene flow. Managers cannot control the mutation rate, but they can have some control over N_e, which is generally smaller than the total number of breeders (N) because of unequal sex ratio or a skewed distribution of reproductive success (i.e., some individuals producing a large number of offspring and many others producing few or none). The genetic diversity loss rate is inversely proportional to N_e and increases rapidly as N_e declines. For example, in each generation the expected proportional increase in inbreeding (Δf) and the expected proportional loss of heterozygosity at neutral genes are both equal to $1 / (2N_e)$. In short, mutation creates new genetic variability, migration spreads the variation among populations, and a large effective size slows the rate at which genetic variability is lost by drift.

N_e also plays an important role in facilitating the action of natural selection. Natural selection is most effective in large populations, in which slight differences in survival probability averaged across many individuals make the outcome of selection highly predictable. In small populations, however, genetic changes promoted by natural selection can be overwhelmed by those arising from random genetic drift, and favorable alleles can decline and deleterious alleles can increase in frequency just by chance. As a consequence, small populations not only lose genetic variability at a high rate, they also have a higher probability of drifting away from their adaptive peak through random events. Gambling provides an analogous situation. In a large casino, even if the house margin is small for every individual bet, steady profits are almost inevitable because the ability to average across many, many chance events ensures that the house eventually comes out ahead. In a small gambling operation, however, the same small house margin will not provide the same buffer against the occasional lucky individual, and as a consequence, short-term profits (or losses) will be much more variable. Therefore, smaller gambling operations generally must use higher house margins, just as small populations must have stronger selection differentials if selection is to predictably overcome random genetic drift.

How large should effective size be for a healthy natural population?

There is no simple answer to this question, but several rules of thumb have been proposed in the scientific literature; these collectively have been referred to as the 50/500/5,000 rules.

1. Empirical data from domesticated livestock indicate that inbreeding levels of about 1% can be sustained for a number of generations. $N_e = 50$ produces a 1%/generation increase in inbreeding, so this criterion has been proposed as a minimum short-term standard to guard against the most serious effects of inbreeding. One caveat is that the response of a new population to inbreeding is largely unpredictable, so results for domesticated species might not be typical of most natural populations.
2. Theory and some empirical results from *Drosophila* spp. suggest that if N_e is consistently 500 or larger, the rate at which the population generates new additive genetic variability by mutation should equal or exceed the rate at which variation is lost by genetic drift. A caveat is that the general applicability of this rule for other species has not been rigorously demonstrated.
3. A much larger effective size (≈5,000) might be required to avoid what has been termed "mutational meltdown." A small fraction of mutations are beneficial, and some are lethal or nearly so, but the vast majority are slightly deleterious, meaning that they produce a slight reduction in fitness. In large populations, natural selection can effectively remove these slightly deleterious alleles, or at least keep them at very low frequency, but in small populations they can drift to high frequency by chance or even become fixed. Over time this creates a considerable fitness drag on the population, which can compromise its viability. For selection to be efficient in dealing with slightly deleterious mutations, N_e has to be much larger than the value required simply to maintain levels of genetic variability. It should be noted, however, that this model assumes a completely closed population, and rather modest levels of immigration from other natural populations might help alleviate some of the fitness decline due to mutational meltdown.

Among the most serious threats to within-population genetic diversity that could arise from marine aquaculture are those due to what has come to be known as the Ryman-Laikre effect (see Table 3 for a description of terms). Ryman and Laikre (1991) showed that in assessing the effects of fish culture on genetic diversity, it is not sufficient to know only N_e in the cultured or wild population; instead, it is necessary to consider the effective size of the cultured-wild system as a whole (N_eT). N_eT is a function of effective size of the captive (N_eC) and wild (N_eW) spawners and the quantity x, which is the fraction of the offspring generation that is progeny of individuals that reproduce in captivity (see Appendix A for details). The Ryman-Laikre effect refers to a reduction in N_eT that occurs when N_eC is relatively small and x is relatively large, so that offspring of the cultured individuals make up a relatively large fraction of the total population (Figure 3). When this happens, many individuals in the population will be closely related and this leads to higher levels of inbreeding and reduces N_e. Note that the genetic consequences of the Ryman-Laikre effect only occur if progeny of cultured individuals pass their genes on to the next generation. Therefore, the relevant definition of the term x should be the fraction of the total population of successful spawners that is derived from captively reared individuals.

The Ryman-Laikre effect raises two general concerns regarding genetic diversity within natural populations. First, it can reduce N_eT to a low enough absolute level that it raises concerns for population viability (see previous section). For marine populations with large effective sizes, this is unlikely to occur unless x is large and N_eC very small. A much more likely consequence for marine species is that N_eT, although still relatively large, can be reduced to a small fraction of its original value.

Some of the main consequences of the Ryman-Laikre effect can be summarized as follows (see Appendix A for more details):

1. N_eT is a type of weighted harmonic mean, and as such is most strongly affected by the small values (which usually is N_eC).
2. If N_eC is very small or N_eW is very large, N_eT generally will be closer to N_eC than to N_eW.
3. If N_eW is small (less than 500–1,000), then a variety of scenarios with moderate x and N_eC values will ensure that N_eT / N_eW is at least 0.5 (i.e., wild N_e reduced by no more than half).
4. If N_eW is large ($>>10^4$), then the best way to avoid reducing N_eT / N_eW to less than 0.1 is to ensure that x is very low ($<<0.1$) or N_eC is relatively high ($>>100$).

Table 3. Key terms that quantify the Ryman-Laikre effect.

N_eC	Effective size of the individuals reproducing in captivity
N_eW	Effective size of the individuals reproducing in the wild
N_eT	Effective size of the captive-wild system as a whole
x	The fraction of successful spawners that were produced in captivity
$1 - x$	The fraction of successful spawners that were produced in the wild
N_eT / N_eW	The ratio of the overall effective size (after considering the Ryman-Laikre effect) to the effective size of the original wild population

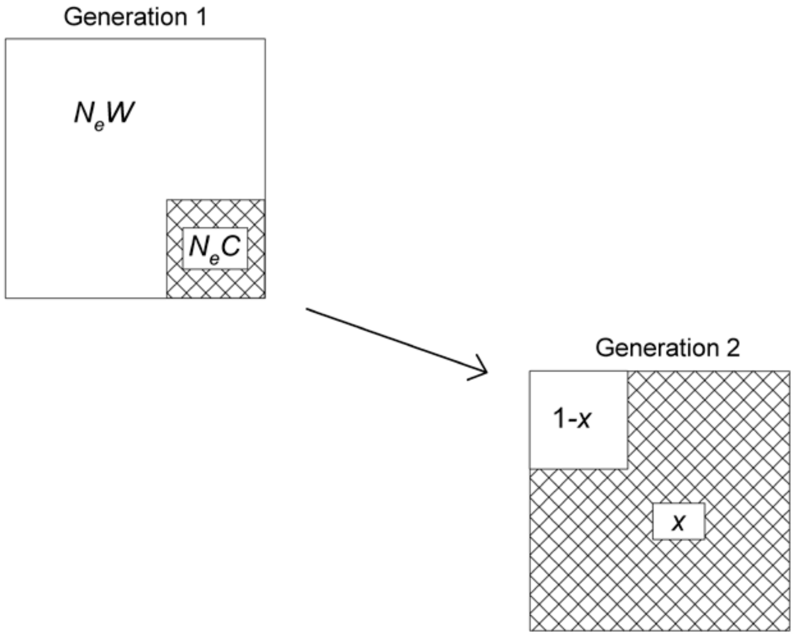

Figure 3. Schematic diagram of the Ryman-Laikre effect, which can reduce overall effective population size in a combined captive/wild system. The effect is strongest when the effective size of the captive population in the parental generation (N_eC) is relatively small compared to the wild population (N_eW) and offspring of the captive parents make a relatively large contribution (proportion x; lower right) to the overall population in the next generation.

What considerations apply specifically to marine aquaculture?

The Ryman-Laikre method was originally intended to apply to a situation referred to as supportive breeding, where a fraction of adults from a natural population are taken into a hatchery to increase their productivity (e.g., to provide more harvest opportunities). The original concept, then, was of a single population with two phases (captive and wild). This scenario might directly apply to some aquaculture operations, if broodstock is sourced from local wild populations. In other cases, the cultured stock might be highly domesticated or derived from a different population. In these situations, the Ryman-Laikre effect is less relevant, because if progeny of cultured fish make a substantial enough contribution to the overall population to trigger concerns about inbreeding, the local population would be so swamped with maladapted genes that viability would likely be seriously compromised (discussed below under Loss of Fitness).

Some marine species are capable of reproduction in net pens or similar containment facilities. Although fertilized eggs typically would not be considered “escapes,” they can potentially have similar genetic consequences for natural populations. Commercial operations will generally try to avoid these situations, as they represent a reduction of marketable product; however, there might not be sufficient financial incentives to completely eliminate the problem, so the potential consequences for natural populations should be evaluated.

Regarding the 50/500/5,000 rule, an effective size of 50 is clearly too small for long-term sustainability of isolated natural populations. For marine species, more reasonable minimum criteria might be in the 500–5,000 range. However, evaluations that focus on a fixed, critical value of N_e do not consider the consequences of proportional reduction in N_eW. For example, many scenarios that satisfy the criterion that $N_eT \geq 500$ could result in reductions of N_eW by several orders of magnitude (see Appendix A, Figure A-9). Is it sufficient to ensure that an aquaculture program does not result in N_eT less than, say, 500 if the effective size of the wild population was 1 million before the program began? Even if an effective size of about 500–1,000 is sufficient to provide for most medium-term evolutionary processes, the total number of alleles that can be maintained in a population with effective size of 10^6 is vastly larger than the number that can be maintained in a population with $N_e = 10^3$. This rich store of diversity provides the raw material for evolution and could be important in dealing with future environmental changes. Therefore, proportional reductions in N_eW should be considered, along with the absolute levels of N_eT, in evaluating risks to within-population diversity. From this perspective, the wild populations that are at greatest risk from the Ryman-Laikre effect are those with very large effective sizes.

Another key factor in determining the magnitude of the Ryman-Laikre effect is the ratio N_e / N (effective size to census size). In cultured populations, it is commonly the case that a relatively few parents produce most of the offspring, even when efforts are made to equalize reproductive success. Therefore, the ratio N_eC / N can be substantially less than 1, which means that simply using the number of spawners as an estimate of N_eC would provide an overly optimistic assessment. On the other hand, several studies that have used genetic methods to estimate N_e in marine species (fish and invertebrates) suggest that the ratio N_eW / N can be very low (perhaps 10^{-3}–10^{-5}) in some species. If this is the case, even very large marine populations might have relatively low N_e. If N_eW is already several orders of magnitude smaller than the census size, then there is less risk that historic N_eW will be greatly diminished through the Ryman-Laikre effect.

What strategies can help minimize risks to within-population diversity?

In addition to the general strategies discussed above, two specific actions can help reduce possibilities for a significant Ryman-Laikre effect.

1. Ensure that escaped cultured individuals do not make up a significant fraction of the natural population (i.e., keep x to a small number). The Ryman-Laikre effect disappears entirely (aquaculture has no net effect on N_eT) if individuals that reproduce in culture have the same per-capita production of successful natural spawners as do individuals that reproduce in the wild. For large wild populations, N_eC / N_eW will generally be a small number, which puts constraints on how large x can be and still avoid a substantial reduction in overall effective population size. In general, substantial reductions in wild N_e are possible if x is larger than about 10%.
2. Maintain a large N_eC. Because overall N_eT converges on N_eC as x increases, some protection against the most serious losses of diversity also can be achieved by ensuring that N_eC is as large as possible. A large number of breeding individuals is a necessary but not sufficient condition for large N_eC; it is also necessary to consider the ratio of effective size to census size in captivity (N_eC / N). Careful evaluation of the relative contributions of different breeders to the surviving progeny can provide important information about this critical ratio.

If the aquaculture broodstock is developed from local individuals, it is important to ensure not only that N_eC is large, but also that the breeders are representative of the population as a whole with respect to important phenotypic, life history, behavioral, etc. traits, which can be associated with local adaptations and are important for long-term population persistence. These traits are typically influenced by a combination of genetic and environmental factors. Random collection of a sufficient number of individuals across multiple spatial and temporal strata can help achieve this objective. However, it should be recognized that sample sizes of broodstock that are feasible to consider for most aquaculture operations (perhaps hundreds at most) are too small to expect that they can simultaneously be completely representative of the population for a large number of traits. Inevitably, not all diversity in the population can be captured in a finite collection of captive parents. Furthermore, attempts to broaden the diversity in the broodstock collection risk incorporation of multiple populations into a single cultured population, if subdivision occurs within the species in the target area. This risk is discussed in the next section.

Of these two strategies, the first (keep reproductively successful escapes to a minimum) is by far the most effective way to ensure that aquaculture does not cause major reductions in N_e. Increasing N_eC serves mainly to minimize the adverse consequences of escapes that cannot be avoided.

How long will it take for natural processes to restore lost genetic diversity?

If aquaculture operations lead to loss of genetic diversity within a wild population, but the problems that caused the loss are permanently fixed, how long will it take for original levels of genetic diversity to be restored by natural processes? In an isolated population, new genetic diversity has to be generated entirely by mutation, which is a slow process. For example, if an isolated population with $N_e = 100$ lost all its variation, even for markers with very high mutation rates like microsatellites it would take 500 generations or so before most of the diversity was restored; for a larger population with $N_e = 1{,}000$, the process would take 2,000 or more generations (Figure 4). In areas of the genome with lower mutation rates, the process could take orders of magnitude longer time. From the shape of the curves in Figure 4, panel A, it is also apparent that in general a great deal of time would be required to restore (by mutation alone) even a fraction of lost genetic variation.

Most marine populations are not completely isolated, however, and in this case new genetic variation can enter populations via migration, which can greatly speed up restoration of genetic variability. One scenario is depicted in Figure 4, panel B, which considers a small local population size ($N_e = 50$) that is initially at mutation-drift equilibrium (and hence has low levels of genetic variability). This might represent a local population for which genetic diversity has been reduced (for example, by overharvest or by the Ryman-Laikre effect). In this example, at generation 101 the local population starts to receive 5% immigrants each generation from a metapopulation with a total N_e of 1,000. Under these conditions, heterozygosity in the local population rises rapidly and approaches the new equilibrium value within about 10 generations—demonstrating that migration can bring in new genetic variation much faster than it can be generated by mutation. The good news about this is that levels of connectivity typical of many marine species can greatly speed up the rate at which genetic variation is restored in a local population following a bottleneck. On the other hand, high levels of connectivity could also facilitate spread of localized genetic effects of fish culture to a wider geographic area.

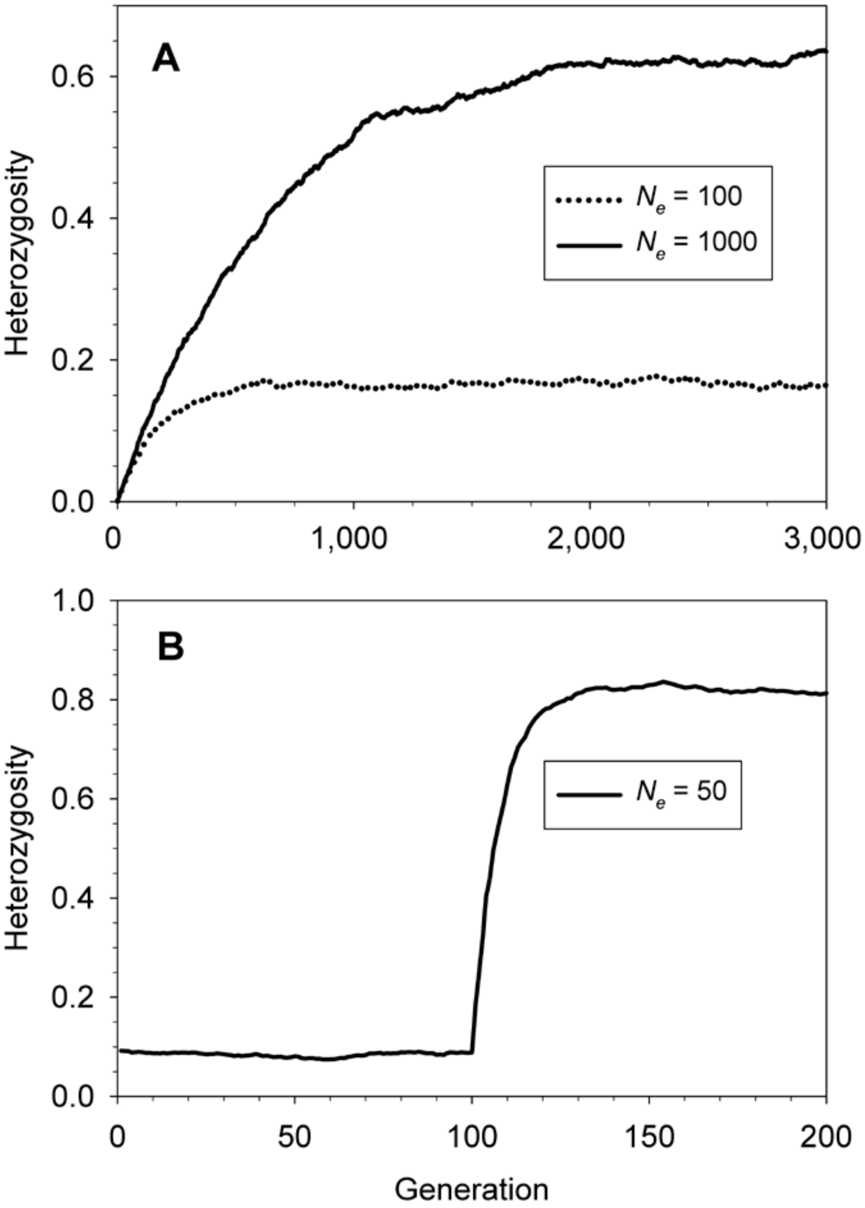

Figure 4. Rate of increase in heterozygosity in natural populations after factors reducing N_e are eliminated. Results are based on simulated data for 20 gene loci, each with 20 possible allelic states, and assuming a mutation rate of 5×10^{-4}, as is typical for highly variable markers like microsatellites. Panel A shows isolated populations with two different effective sizes ($N_e = 100$ and $N_e = 1{,}000$). Heterozygosity is regenerated entirely by mutation following a complete loss of genetic variation. Results are averaged across 100 different replicates for each N_e. Panel B shows a local population of $N_e = 50$ in a metapopulation with $N_e = 1{,}000$, with initial level of heterozygosity reflecting a balance between mutation and genetic drift for $N_e = 50$. At generation 101, immigration at 5% per generation starts to bring new alleles into the local subpopulation. The level of genetic variation quickly rises to approach the level found in the metapopulation as a whole.

In summary, the following points are the most important considerations for maintaining diversity within populations:

1. The amount of neutral genetic diversity maintained in a local population is determined by a balance between loss of diversity (by genetic drift, and other factors) and generation of new variation (by mutation or immigration).
2. N_e determines the rate of genetic drift. N_e is generally less than census size (N) and, in many marine species, perhaps orders of magnitude less.
3. Isolated populations probably require N_e of at least 500–5,000 for long-term persistence. However, most marine populations are not completely isolated and even low levels of migration can bring new variation into a local population.
4. For captive-wild systems, effective size of the cultured-wild system as a whole (N_eT) is a function of effective size in the captive (N_eC) and wild (N_eW) phases and the relative contribution of cultured individuals to the overall population (x).
5. N_eT can be reduced by the Ryman-Laikre effect if a relatively few captive parents (low N_eC) produce a relatively large fraction of the next generation in the wild (large x).
6. The most reliable strategy to minimize the Ryman-Laikre effect is to ensure that x remains relatively low ($x \ll 10\%$). Maintaining a large N_eC helps to place an upper limit on how large the reduction in effective size can be for escapes that cannot be avoided.
7. If a nonlocal or highly domesticated source is used for aquaculture broodstock, levels of gene flow that might trigger substantial Ryman-Laikre effects would also trigger more serious concerns for loss of fitness due to swamping of the wild population with maladapted alleles.

Loss of Diversity among Populations

Genetic differences among populations arise from two major processes: genetic drift and natural selection. With little or no migration, genetic drift occurs independently within each population and they tend to diverge over time. Migration tends to counteract this process of divergence by mixing alleles among populations. Most natural populations have achieved some sort of balance between migration and drift, whereby the diversifying effects of drift are offset by the homogenizing effects of migration. However, current levels of divergence can also reflect historical factors, such as recolonization from ice age refugia. Whereas genetic drift is a random process, natural selection can consistently favor different genotypes in different localities, which over time leads to local adaptation. If selection is strong enough, local adaptations can persist even in the face of moderate levels of gene flow.

Genetic diversity among populations confers resilience to natural systems on a variety of temporal and spatial scales. In the long term, genetic differences among populations increase the chances that at least some will be able to respond and adapt to future changes in the biological and physical environments. In the short term, a diverse array of natural populations helps buffer the species as a whole from natural fluctuations in environmental conditions. This short-term benefit is easily understood as an analog of the portfolio effect. An investment portfolio heavily weighted toward a few stocks might occasionally produce outstanding returns but also will be prone to large negative swings. A more balanced portfolio has less chance of a huge return but provides more predictable earnings over time.

Similarly, a species with diverse natural populations that express a range of phenotypic and life history traits will generally have some populations that have normal productivity even under anomalous environmental conditions. In contrast, a species that has been constrained to one or a few widely distributed life history strategies will tend to have boom or bust cycles, depending on whether the populations are in or out of synchrony with environmental conditions in that year. For example, Schindler et al. (2010) documented portfolio effects in Bristol Bay, Alaska, sockeye salmon. The considerable diversity among populations in age structure and run timing appears to have reduced temporal variability in total adult returns to Bristol Bay over five decades. The authors concluded that if these stocks did not express this life history diversity, the variability in adult returns over this period likely would have been more than twice as large. The portfolio effect speaks to the importance of conserving populations that are not currently particularly distinctive or productive, as they might be important under future environmental scenarios.

The major concern regarding loss of among-population diversity is that widespread escapes from one or a few aquaculture broodstocks will lead to replacement of numerous, locally adapted populations with a smaller number of relatively homogeneous ones. Such replacement could erode local adaptations and reduce diversity among populations. A reduction in life history diversity—a simplification of the natural populations' portfolio—could limit the capacity to respond to future environmental change. In many marine species, diversity among populations has evolved over many hundreds or thousands of years and cannot easily be regenerated once lost.

What considerations apply specifically to marine aquaculture?

Conventional wisdom is that because of large population sizes, a general lack of apparent barriers to dispersal, and environments that are perceived to be relatively homogenous, most marine species are not as strongly structured as are terrestrial, freshwater, or even anadromous species. Although each of these points has some empirical support, information is beginning to accumulate that challenges the generality of this conclusion. For example, evidence has been found for genetically based differences in life history among geographic populations of highly mobile species such as Atlantic cod. Absent direct information to the contrary, therefore, it would be prudent to assume that local adaptations might exist in the target species.

Many (perhaps most) species that are prospective targets of marine aquaculture can disperse widely, either as adults or eggs/larvae or both. As a consequence, escapes from even a single locality have the potential to affect many distant populations, with a homogenizing effect on diversity. Furthermore, aquaculture escapes might disperse more widely than typical natural fish because they don't have an identifiable home range or territory. This effect is well documented in some hatchery and aquaculture programs for Pacific and Atlantic salmon; the extent to which it applies to most marine species remains to be determined.

Molecular genetic data are available for natural populations in a wide range of species. In the vast majority of cases, these data reflect genetic variation at what are presumed to be largely neutral gene loci (i.e., genes that are not strongly affected by natural selection). Empirical studies show that, although these molecular genetic data provide a great deal of information about population connectivity, they generally are not reliable indicators of differentiation at genes related to local adaptation. For two reasons related to population size (N), this discrepancy between neutral and selected loci could be particularly important for marine species.

First, the magnitude of neutral genetic differentiation (for example, as measured by genetic distance or the metric F_{ST}) is determined by the absolute number of migrants per generation (mN_e), whereas the ability of migration to counteract local adaptation depends on the migration rate (m). If N and N_e are large, then mN_e can be large enough to produce low F_{ST} values even if m is very small. For example, a low $F_{ST} = 0.01$ for a species with $N_e = 10^6$ implies a very small migration rate ($m = 0.000025$), which would not prevent development of local adaptations if selective regimes differed among local habitats.

Second, as previously noted, selection is most efficient in large populations, which reinforces the idea that marine species with large populations could be locally adapted, even with modest selective differences and even if neutral markers show little evidence of differentiation.

What strategies can help minimize risks to among-population diversity?

As with all genetic risks, the most reliable way to minimize adverse effects on among-population diversity is to ensure that few individuals escape. Other actions that can minimize risks include the following:

1. Pay careful attention to locations of broodstock collection. Sourcing broodstock from too local a scale risks incorporating related individuals and hence elevating levels of inbreeding; collecting too broadly risks mixing individuals from different populations.
2. Keep the geographic scale of areas affected by strays smaller than the area occupied by a single population.
3. Obtain as much information as possible about the ecology and life history of the target species. In the absence of this information, it will be difficult to quantitatively assess risks and develop approaches to minimize them.
4. Regularly monitor for physically marked cultured individuals and genetic markers in nearby wild populations. Physical marks or tags on cultured individuals can greatly enhance the ability to monitor escapes and molecular markers can help to track genetic impact on natural populations. Although these methods by themselves do not reduce risks, they can provide vital information that allows better experimental design and management of the program.

How long will it take for natural processes to restore lost population structure?

If aquaculture operations lead to loss of genetic diversity between populations, but the problems that caused the loss are permanently fixed, how long will it take for original levels of population subdivision to be restored by natural processes? Something like the original population structure would be restored when the new system reached an equilibrium between migration and genetic drift. How long this would take depends primarily on the size of the populations and the original level of divergence (Figure 5). Many marine species have weak population differentiation (low F_{ST} values) and could reach the new equilibrium relatively quickly unless they are very large. For example, a species with original $F_{ST} = 0.001$ that was homogenized by human activities could regain half the original value within about 10 generations, assuming N_e for individual populations was no larger than about 10,000. In contrast, for a species with large populations ($N_e \approx 10^5$–10^6) and original $F_{ST} = 0.01$ (still quite low but within the range of many marine species), after normal levels of gene flow were restored, it would take thousands of generations to achieve an appreciable fraction of the original population differentiation.

Results discussed above apply to genetic diversity that is selectively neutral (but which might form the basis for future response to selection). Natural selection could potentially lead to faster restoration of diversity among populations, albeit at a demographic cost to the population.

In summary, the following points are the most important considerations for maintenance of diversity among populations:

1. Diversity among populations provides a short-term buffer against environmental fluctuations and, in the long term, enhances the evolutionary potential of the species.
2. Escapes from aquaculture operations have the potential to erode differences among natural populations that have evolved over long time periods.
3. Understanding the ecology and life history of the target species is important to developing effective strategies to minimize risks to among-population diversity.

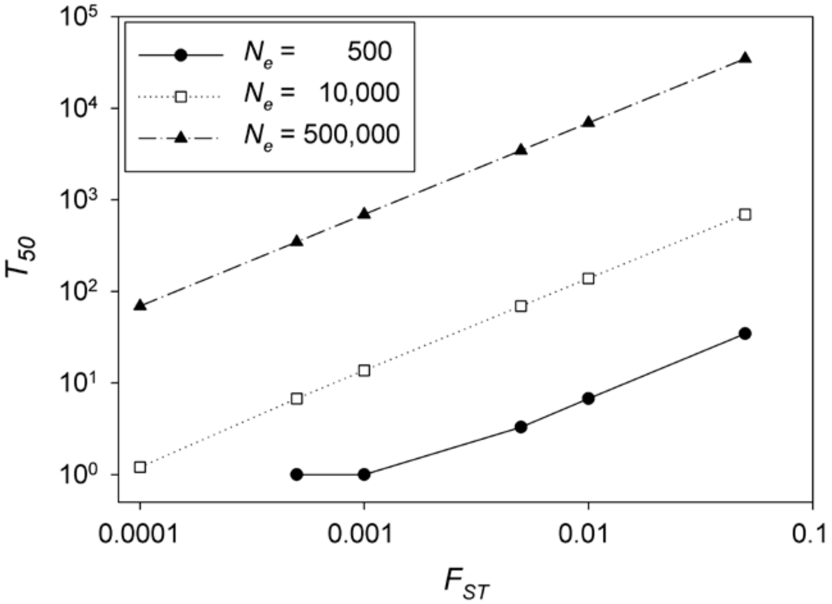

Figure 5. Estimated time (T_{50}, in generations) to reach 50% of the equilibrium F_{ST} value, assuming that anthropogenic activities have completely eliminated population structure ($F_{ST} = 0$) and that, at time 0, levels of migration (m) are restored that will eventually produce the equilibrium F_{ST}. For a given F_{ST}, the equilibrium migration rate depends on N_e in each subpopulation (500, 10,000, 500,000). Note the log scale on both axes. Data are based on Equation 3 (Appendix A) assuming equilibrium $F_{ST} = 1 / (1 + 4mN_e)$.

Loss of Fitness

Aquaculture can lead to loss of fitness in wild populations when captive and wild individuals interbreed. The fitness loss can occur through either or both of two mechanisms. Domestication leads to genetic changes associated with the culture environment, and these changes can be passed on to wild populations by escapes or intentional releases. Domestication can occur even when local populations are used to source broodstock. Outbreeding depression can occur when genetically differentiated populations interbreed—as might happen, for example, if nonlocal populations or highly domesticated stocks are used for broodstock and some individuals escape into the wild. Outbreeding depression does not directly depend on effects of fish culture, but its likelihood of occurrence can be greatly enhanced by stock transfers associated with aquaculture operations. These two mechanisms are discussed below.

Domestication

In captive populations, two related processes produce selective regimes that differ substantially from those experienced in the wild. First, intentional or inadvertent selection for traits that promote survival, reproduction, or both in captivity can increase fitness in the captive environment. For example, program operators might intentionally select for traits considered desirable, such as size, flesh quality, or feed conversion efficiency. Even without intentional selection, captive environments can exert strong inadvertent selective pressures for phenotypes that perform well in captivity. Traits that often are influenced by this type of selection include behavioral traits that increase feeding rates under captive conditions and earlier spawning time (because progeny of these adults grow faster and outcompete others in their cohort).

The second major way that selective regimes differ in captivity is through relaxation of selection that occurs in the wild. This does not require positive selection toward adaptations to captive life; rather, it arises in a successful captive program because large numbers of individuals survive that would not have survived in the wild. Although mortality in the wild might be largely random, some fraction is mediated by natural selection, which weeds out individuals with maladaptive phenotypes each generation. In a benign captive environment, individuals with these maladaptive phenotypes can survive to reproduce, leading to a gradual erosion of fitness of captive individuals when they are returned to the wild environment.

These combined effects lead to genetic change in a population that generally makes it more fit in the captive environment but less fit in the wild. Note that domestication, which is a form of natural selection, does not require (nor generally involve) any genetic change to individual fish; rather, it occurs across generations as individuals with certain phenotypes (and their associated genotypes) survive and reproduce at different rates than they would in the wild. Because of the profound changes in selective regimes and mortality patterns between captive and wild environments, some level of domestication is unavoidable. Indeed, genetic adaptation to captivity has been documented in a wide range of species, even though the exact mechanisms leading to domestication are less well understood.

Outbreeding depression

When two individuals mate, the fitness of their offspring can be strongly affected by the degree of genetic relatedness of the parents. Animal and plant breeders (including Charles Darwin) have long known that matings between close relatives (full or half siblings, or even cousins) often produce offspring with reduced fitness; this phenomenon is known as inbreeding depression. Conversely, if the parents are too divergent genetically, offspring fitness can also be reduced, through a phenomenon known as outbreeding depression. Over time, most natural populations have probably achieved a balance between the two forces, resulting in an intermediate level of outcrossing that is consistent with viability. Occasionally, natural processes create conditions under which strongly diverged populations come into contact, with some level of outbreeding depression a likely consequence. Although outbreeding depression has not been as well studied as inbreeding depression, numerous empirical examples have been found involving natural populations. Furthermore, anthropogenic changes to natural ecosystems, including aquaculture, can create conditions in which individuals from genetically differentiated populations can interbreed at rates that are orders of magnitude higher than would occur naturally.

Two general mechanisms can produce outbreeding depression, either separately or in conjunction. First, local adaptations can be diluted when maladapted genes are introduced from a genetically divergent population. Second, favorable combinations of genes that have been shaped by natural selection to perform well in the local environment can be disrupted by recombination (genetic shuffling) that occurs following a hybridization event.

What considerations apply specifically to marine aquaculture?

Most marine aquaculture operations create culture conditions that depart dramatically from those experienced in the wild and this should promote rapid domestication. As discussed above (Lessons Learned section), some degree of domestication is generally desirable from a production standpoint, which creates a tension when evaluating the possible consequences of domestication for natural populations. Use of local broodstocks will not avoid domestication, but it will avoid additional losses of fitness arising from translocation of genetically divergent populations. Some aquaculture operations will involve both factors (broodstock derived from a nonnative source and strong domestication for market-related reasons).

As is the case for the other genetic risks, whether aquaculture escapes will actually lead to reduced fitness of wild populations (and if so by how much) will depend in a complex way on a variety of factors, including the number and life stage of escapes, their probability of surviving to maturity, and the probability that mature escapes will successfully reproduce with wild individuals.

How long will it take natural processes to restore lost fitness?

If aquaculture leads to loss of fitness in a wild population, but the problems that caused the loss are permanently fixed, how long will it take natural processes to restore original levels of population fitness? In general, if the forces leading to loss of fitness (domestication, outbreeding depression) are eliminated or reversed, natural selection should gradually increase fitness in the wild population. However, several factors make it difficult to make quantitative predictions about the nature and extent of the new levels of fitness.

First, evolution is irreversible, in the sense that it is essentially impossible to recreate the exact conditions that led to a prior evolutionary state. Thus although selection might eventually produce something like historic levels of fitness of a natural population that had been strongly affected by aquaculture, exactly recreating the original population status is generally not an achievable objective.

Second, selection can operate in many different ways, both in reducing and restoring fitness in the wild. This can be contrasted with evolutionary changes of neutral alleles, which can be predicted in a statistical sense based on well-developed population genetics theory. The same body of theory can predict responses to selection, but with so many different types of selection to consider, the range of possible outcomes is enormous. Furthermore, the strength and direction of selection can change on short (ecological) time scales.

Third, basic evolutionary theory tells us that the rate of genetic change depends on the amount of genetic variation, the strength of selection, and population size. If strong domestication pressures cause rapid change, then the novel selective pressures are relaxed, the rate at which fitness increases will not necessarily be as rapid, because the forces acting to restore fitness might not be as strong.

Finally, by definition natural selection requires that less fit individuals produce relatively few or no offspring that survive to reproduce, and this means that selection imposes a demographic burden on the population. Thus although natural selection can move a maladapted population back toward its adaptive peak, during that process the population could be at significant risk and be particularly vulnerable to random demographic and environmental variability.

What strategies can help reduce risks to fitness of natural populations?

Assuming that a certain number or fraction of escapes is unavoidable, then two general strategies are available to attempt to minimize the fitness consequences of these escapes. In Strategy 1, efforts are made to keep the captive population as similar as possible genetically to the wild population. This necessitates using local fish for broodstock and making efforts to reduce the rate of domestication. Under this strategy, it would be difficult to achieve some of the benefits to production or cost efficiency that can result from intentional selection for traits that are desirable in cultured populations but probably maladaptive in the wild.

Strategy 2 takes the opposite approach: make the cultured population substantially different genetically from the local wild population, which might be accomplished by using nonlocal (and perhaps not locally adapted) sources for broodstock or intentionally selecting for traits that are considered beneficial in culture. The logic for this strategy is that if the captive population is highly domesticated or otherwise maladapted to local conditions, individuals that do escape have a low probability of successfully reproducing.

Strategy 2 thus seeks to minimize the number of genetic interactions between captive and wild individuals, but has the unfortunate consequence that interactions that do occur are likely to produce offspring with greatly reduced fitness in the wild. Conversely, Strategy 1 seeks to minimize the adverse effects of interbreeding events that do occur, but has the unfortunate consequence that such interactions are likely to be more frequent than those under Strategy 2. The overall consequences for natural populations depend in some complex way on the product of the number of interbreeding events and the severity of each. Unfortunately, at present we are just beginning to understand how to quantitatively evaluate the inherent trade-offs in these two strategies, which is necessary before on can determine which strategy will be less detrimental to wild populations. Figure 6 is a conceptualization of how such an evaluation might be carried out. In this figure, the curves show possible relationships between a measurable outcome (e.g., fitness of the wild population) and the degree of genetic differentiation between cultured and wild individuals.

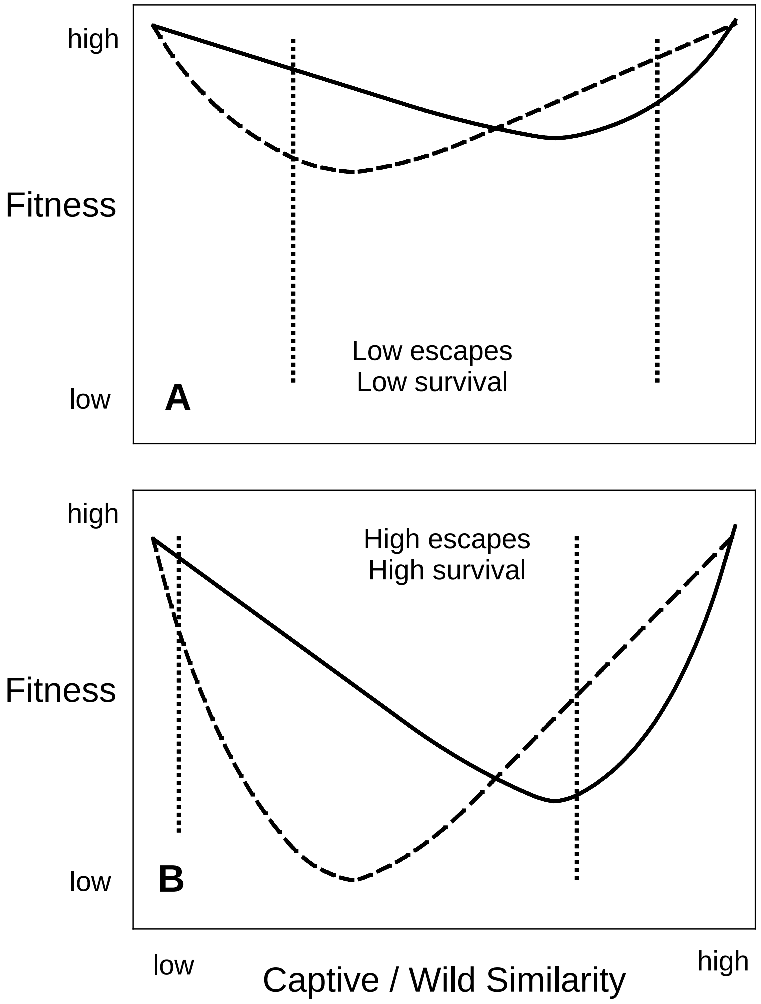

Figure 6. Hypothetical relationship between fitness of a wild population and the degree of genetic similarity between the wild population and a captive population from which some individuals routinely escape. The solid and dashed curves illustrate some of the possible shapes of this relationship; the vertical lines represent practical limits to how different or how similar the hatchery population can be made compared to the wild. All else being equal, higher rates of escapes or higher survival of escapes will lead to greater reductions in fitness (panel B) than will lower rates of escapes or survival (panel A).

In theory, under ideal conditions there should be no fitness loss if captive and wild fish are genetically identical, or if they are so divergent that no interbreeding is possible. Values of hatchery-wild differentiation between these extremes should show some level of reduction in fitness, but the shape of this relationship is unknown. The two vertical lines illustrate the practical limits to what can be achieved. The vertical lines on the right indicate that it is impossible, in the benign selective regimes in culture, to produce fish with identical adaptations to wild fish. The vertical lines on the left indicate how different any given cultured and wild populations might be under realistic conditions.

Under this framework, the following approach could be used to reach a decision about whether to pursue Strategy 1 or Strategy 2:

1. Determine the shape of the curve (probably through modeling, informed by some empirical data) and how it changes with various parameters considered;
2. Determine where the vertical lines fall with respect to inflection points on the curve (probably based on existing information); and
3. Determine which strategy leads to higher fitness.

For example, in Figure 6, panel A, if the “similar” strategy can achieve a level of captive/wild similarity indicated by the vertical line on the right and the “different” strategy can achieve a captive/wild difference as indicated by the vertical line on the left, then the “similar” strategy leads to higher fitness if the dashed curve describes the true relationship, while the “different” strategy leads to higher fitness if the solid curve describes the true relationship. If constraints for both strategies are shifted to the left (Figure 6, panel B), then the “different” strategy is preferable regardless of the shape of the relationship. Alternatively, one could assume a fixed value for one strategy and ask how extreme the other must be to become better. The key question would then become whether it is feasible to achieve the required level of captive/wild similarity or difference. If this type of analysis were conducted over a wide range of conditions, it might emerge that, given the practical situations likely to be faced by marine aquaculture, one strategy is consistently preferable to the other.

The height of the curves in Figure 6 will depend on the level of escapes and how successful they are in reproducing (compare Panels A and B). The extreme case of Strategy 2 is to ensure that the broodstock is sterile or otherwise incapable of reproducing in the wild. This is discussed in the Sterilization subsection below.

In summary, the following points are the most important considerations for maintenance of fitness:

1. Domesticated aquaculture populations typically have low fitness in the wild and the fitness reductions can be even greater if the cultured population is not derived from local sources.
2. Low fitness of cultured individuals in the wild has two contrasting consequences for natural populations:
    - Domesticated escapes are less likely to survive to maturity, and if they do are less likely to successfully reproduce; but,

    - Domesticated escapes that do manage to reproduce in the wild can have particularly deleterious consequences for fitness of natural populations.
3. Research currently underway should provide some insights into whether strong domestication of aquaculture broodstocks is likely to reduce or increase risks to fitness of natural populations.

Other Risk Factors

This document focuses on genetic risks. However, a comprehensive risk-benefit analysis should also consider a number of related risks and benefits. For completeness we briefly list some important risks below (see Waples and Drake 2004 for a more thorough discussion).

Competition and Predation

Aquaculture escapes can prey on wild populations and also compete with natural individuals for food or territory. Escapes that mature can compete with natural adults for spawning sites and access to mates. If the number of escapes is sufficiently large, their presence might impose other forms of selection that alter characteristics of wild populations important to survival and reproductive success. Effects on wild populations can occur even if the escaped individuals do not complete a life cycle in the wild—for example, if they destroy spawning nests of wild individuals or divert mating efforts by wild individuals into unproductive spawnings.

Disease and other Mortality Factors

Diseases and parasites are pervasive in the marine environment and are part of the ecological and evolutionary histories of all marine species. However, because of high rearing density, aquaculture operations can greatly magnify the incidence of naturally occurring diseases and parasites, and movements of eggs, fish, or fish products into new geographic areas can expose natural populations to new pathogens for which they have little or no natural resistance. Risks from introduced pathogens and parasites include temporary epidemics, long-lasting population reductions, and even extinction of wild populations. For example, Burreson et al. (2000) used molecular methods to show that the parasite *Haplosporidium nelsoni* (popularly known as MSX), which has decimated populations of the eastern oyster (*Crassostrea virginica*) along the Atlantic coast of the United States, most likely originated from translocations of Pacific oyster, *C. gigas*, from Japan. Risks posed by disease agents are harder to quantify than those posed by competition or predation, as a single individual transferred to a recipient population can have dramatic consequences. Disease agents also can be spread by water, independent of any releases of cultured individuals.

Low Power to Detect Adverse Effects

Because natural variability in most natural populations is relatively high, the ability to detect deleterious fitness effects of aquaculture escapes can be low, even in the most ambitious, well-designed program. This means that by the time adverse effects are detected, they might have already occurred for many years and might have long-lasting consequences for natural populations.

Programmatic Inertia

Experience with fish hatchery programs indicates that, once begun, hatchery programs can be difficult to stop for political or social reasons, even if there is compelling biological evidence regarding their ineffectiveness or their adverse impacts on natural populations. Therefore, a key question in considering whether to initiate a new commercial aquaculture operation is: Can the operation be terminated or modified if a biological evaluation indicates its costs or detrimental effects are greater than its benefits?

Risks to Natural Populations from other Anthropogenic Activities

Although this technical memorandum focuses on effects of marine aquaculture, it is important to remember that natural populations of marine species are also affected by a variety of other anthropogenic activities, including fishing, pollution, and habitat loss and degradation. A brief summary of some other anthropogenic factors follows.

Humans harvest a wide variety of marine species for food, and for some of these species (e.g., Atlantic cod), significant exploitation has occurred for a millennium or more. Concerns are growing not only for depletion of individual species, but also for major changes to marine ecosystems. A recent review of the status of global fisheries (Worm et al. 2009) found that in half of the well-studied ecosystems, the mean exploitation rate had declined to a level that should allow reaching maximum sustainable yield; however, nearly two-thirds of the world’s fish stocks still need rebuilding. Furthermore, the last decade has seen increasing recognition that size-selective fisheries might be causing evolutionary changes in life histories of some heavily exploited fish populations (e.g., Kuparinen and Merilä 2007).

For terrestrial species around the world, loss and degradation of habitat is perhaps the most pervasive threat to biodiversity. In contrast, the role that offshore marine habitats play in population dynamics of marine species is poorly understood. The best studied component is benthic habitats that can be damaged by bottom trawls, which are widely used in many U.S. fisheries. Anthropogenic changes to nearshore habitats, which are vital to juvenile rearing for many species, are better studied, and anthropogenic changes here (such as dredging, draining and filling wetlands, and armoring shorelines) can profoundly affect demographic and ecological processes in marine ecosystems (Levin and Stunz 2005). In tropical areas, rising water temperatures and declining pH due to increased CO_2 concentrations are putting increasing pressure on reef-building corals and other marine organisms with carbonate skeletons.

Relatively little attention has been given to pollution as a major risk factor for commercially and ecologically important marine fish species. Some exceptions include nutrients, sediments (Fabricius 2005), and trash (plastics and other marine debris, Gregory 2009). Little is known about the impacts of toxic chemical contaminants, which are complex and expensive to study. Effects of pollution are likely to become increasingly important in the future, given that 1) society is releasing chemicals into the ocean at a rate that exceeds the capacity of scientific institutions to study and understand their ecological impacts, 2) many toxics are pervasive and persistent in the marine environment and can be difficult or impossible to mitigate once released, and 3) the extent to which toxics will interact with other large-scale ecological forcing pressures (e.g., climate change and ocean acidification) is largely unknown.

Managing Risk

An important management consideration is to identify characteristics of marine aquaculture programs that help to ensure sustainability not only for these programs but also for wild populations. In this section we discuss a number of different tools that might be used to help manage genetic risks associated with marine aquaculture. This is not intended to be a comprehensive list; rather, the objective is to provide potentially useful examples, some of which are still under development and need further evaluation for practical usefulness.

Fault Tree and Risk Assessment Pathway

Kapuscinski et al. (2007) and Senanan et al. (2007) developed a fault tree and risk assessment pathway to assist in identifying and evaluating genetic risks associated with releases or escapes of transgenic individuals into the wild. We have modified these tools to assist in evaluating and monitoring genetic risks associated with escapes of aquaculture individuals into marine environments. The fault tree (Figure 2) describes the sequence of events leading to introgression of genes from escaping cultured individuals into the gene pool of wild relatives. The corresponding risk assessment pathway (Figure 7) guides the conduct of a systematic assessment of risk of gene flow from these cultured individuals into the wild population.

Aquaculture Genetics Management Plan

In the Pacific Northwest, the Hatchery Science Review Group has undertaken a comprehensive review of salmon hatchery operations in western Washington state (Mobrand et al. 2005), and efforts are currently underway to expand the evaluations to the entire Columbia River basin. An important outgrowth of the Hatchery Science Review Group effort has been development by NMFS of the framework for the Hatchery Genetics Management Plan, which provides a standardized format for describing the operation of each hatchery program for salmon and steelhead (*Oncorhynchus mykiss*) and the potential effects of each program on ESA-listed species. We have adapted the Hatchery Genetics Management Plan format to better reflect particular issues associated with marine aquaculture programs or operations in the Aquaculture Genetics Management Plan (AGMP, see Appendix B). Careful preparation of this document for each program will greatly facilitate application of the Kapuschinski et al. (2007) and Senanan et al. (2007) fault trees and risk assessment pathways, with emphasis on the genetic risks identified at the beginning of this section.

State and Federal Guidelines

Although the U.S. Fish and Wildlife Service frequently must deal with genetic issues associated with artificial propagation of fish species, and various guideline documents have

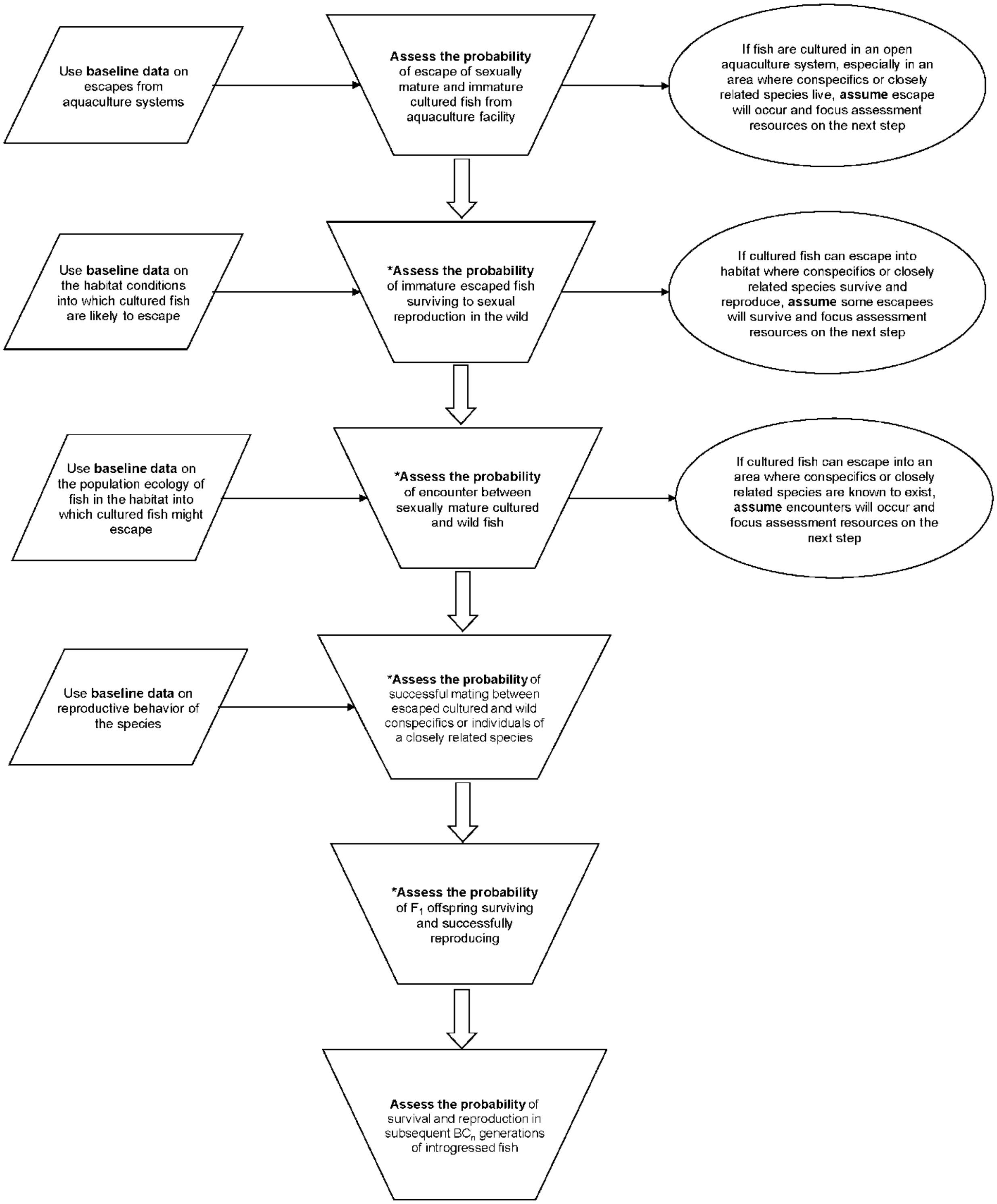

Figure 7. Pathway for conducting an assessment of gene flow. Asterisk (*) denotes an assessment step that requires empirical information on traits of the cultured organism. BC_n refers to the nth generation backcrosses between F_1 hybrids and wild relatives. (Adapted with permission from Kapuscinski et al. 2007, copyright Centre for Agricultural Bioscience International.)

circulated in draft form over the years, the agency has no formal policy guidelines on this issue.[2] Hard et al. (1992) produced a technical memorandum for NMFS that discusses in detail genetic and other considerations associated with artificial propagation of Pacific salmon. However, that document focused primarily on identifying situations under which artificial propagation might provide at least a temporary benefit to populations listed under the ESA. Thus although the general issues are similar to those considered here, the goals of using artificial propagation under the ESA differ substantially from those associated with most commercial marine aquaculture operations and stock release for enhancing commercial or recreational fisheries.

The published guidelines that are perhaps most directly relevant to the issues considered here are the Genetic Policy for the Release of Finfishes in Florida, produced by the Florida Fish and Wildlife Conservation Commission (FFAWCC 2007), which state (p. 4) that:

> It is the policy of the [commission] that all activities involving the release of fin fish shall be undertaken with full consideration of their impacts on natural biological diversity and in ways that do not threaten the state's natural biological heritage.

These guidelines cover intentional and unintentional releases and include considerable emphasis on aquaculture and marine stock enhancement. Genetic risks identified in the guidelines (loss of diversity within and between populations and loss of fitness and adaptive potential) closely parallel those considered here. The guidelines indicate that preliminary analyses should be conducted to determine whether it is necessary to complete a genetic management plan for the proposed program; the guidelines also provide a template for such a plan, which shares many similarities with the AGMP proposed here. The guidelines advocate an adaptive management approach to consideration of artificial propagation. Actions that might be called for depending on the circumstances include:

1. Developing a plan for genetic management of the aquaculture activity,
2. Identifying geographic boundaries of relevant natural stocks,
3. Preventing the translocation of nonindigenous genomes,
4. Minimizing potential impacts from propagation-related genetic changes (domestication) in cultured individuals,
5. Managing the proportion of cultured individuals in admixtures of cultured and wild individuals,
6. Monitoring recipient populations when genetic risks are deemed moderate to high, and
7. Undertaking action to eliminate detected genetic hazards.

The State of Alaska has also had genetic guidelines regarding aquaculture operations for more than three decades. The current genetic guidelines, which have been in place since the mid-1980s (ADFG 1985), were developed primarily to protect the state's abundant wild salmon populations, but have been applied more broadly. Specific policy provisions include the following:

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[2] D. Campton and S. Chambers, U.S. Fish and Wildlife Service, Portland, OR. Pers. commun., December 2009.

1. Live salmonids, including gametes, cannot be imported from other states.
2. Transport of stocks among major geographic areas within the state is prohibited.
3. Transport within regions can be considered after reviewing phenotypic characteristics and distance of transport.
4. Significant or sensitive wild stocks should be identified, and stocks cannot be introduced into areas where they might significantly affect these wild populations.
5. In watersheds with significant wild stocks, only broodstock derived from local populations can be used for stocking.
6. Establishment of drainages that serve as wild stock sanctuaries is encouraged.
7. A single donor stock cannot be used to found more than three hatchery stocks.
8. Broodstock collection should be representative with respect to run timing.
9. Cultured populations should strive to maintain effective population size of at least 400.
10. Cooperative research among university, state, federal, and private sector scientists to address key uncertainties is encouraged.

Atlantic Salmon in Maine

Following many decades of decline of Atlantic salmon in North America, the species has received federal protection in the United States and Canada. In the United States, all remaining natural populations (from Androscoggin River northward along the Maine coast to the Dennys River) are listed under the ESA as a distinct population segment (DPS). The DPS includes conservation hatchery populations, but not populations cultured in commercial aquaculture operations.

North America also hosts aquaculture operations for Atlantic salmon, with annual production in New England ranging to 16,000 metric tons over the last decade (Figure 8). Substantial production also occurs in British Columbia and the maritime provinces of Canada. In general, the industry rears juveniles in private freshwater facilities before transferring them to anchored net pens or sea cages for feeding in the marine environment until they reach market size. Unintentional escapes from the net pens and cages and amplification and release of pathogens and parasites are the primary risk factors for natural populations.

In recognition of these conservation concerns, the North Atlantic Salmon Conservation Organization passed the Williamsburg Resolution to “minimize impacts from aquaculture, introductions and transfers, and transgenics on the wild salmon stocks” (NASCO 2003). Because the U.S. populations are ESA listed, it was necessary for the U.S. agency permitting commercial aquaculture operators (U.S. Army Corps of Engineers) to consult with the U.S. Fish and Wildlife Service and NMFS to determine whether proposed actions were likely to adversely affect listed salmon. That formal consultation resulted in issuance of a Biological Opinion (NMFS 2003), which determined that the proposed aquaculture activities might adversely affect but were not likely to jeopardize the existence of the listed DPS.

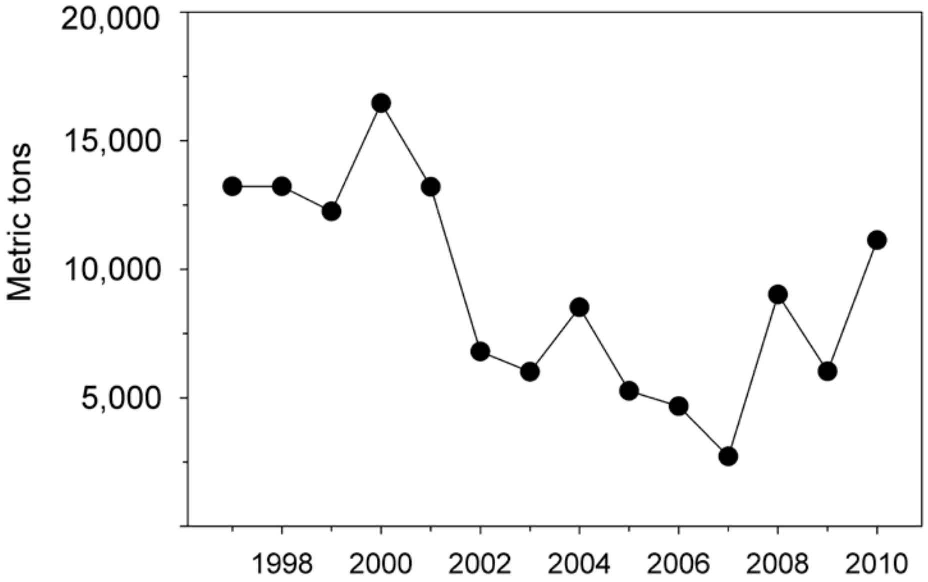

Figure 8. Recent aquaculture production of Atlantic salmon in New England. Data from Table 1.6.1 in USASAC 2011.

Issues associated with escaped farmed salmon that were considered in the Biological Opinion included redd superimposition, competition for food or space, and genetic introgression. Protective measures that were recommended to help ensure that the adverse effects do not rise to a level that would constitute legal jeopardy include: 1) use of only North American stocks for production, 2) implementation of containment measures to reduce escapes, 3) prohibitions on stocking transgenic salmon, and 4) 100% marking of all salmon placed in marine pens within the United States. Each of these requirements has been incorporated into permit requirements and is verified through annual third party audits.

The National Research Council conducted a review of the status of Atlantic salmon in Maine, and the resulting report (CASM 2004) contains a detailed discussion of genetic issues, including the Ryman-Laikre effect.

Genimpact

The European Union supported a multiyear project (Genimpact, online at http://genimpact.imr.no/) to evaluate the genetic impact of aquaculture activities on native populations. The overall objective was to bring together scientists and stakeholders to develop information that would be useful for policy makers. Specific goals were to 1) develop consensus statements on the genetic impact of farming activities and its implications for aquaculture management, stock conservation, and environmental safety and 2) integrate the scientific basis for the establishment of preventive measures for important aquaculture species. The final report (Svåsand et al. 2007) summarizes results of four workshops that covered topics such as genetics of domestication, monitoring, modeling, and management options to reduce impacts. Results of this work have been incorporated at various places in this document (e.g., Table 2).

Examples of Risk Assessment Tools

The All H Analyzer

Another outgrowth of the Hatchery Science Review Group effort was development of the All H Analyzer, or AHA model, which was designed to integrate consideration of effects of habitat, harvest, hatcheries, and hydropower development in a single model. The model deals with individual hatchery-wild population systems that experience harvest and various types of habitat conditions. The module that assesses evolutionary effects of hatcheries draws heavily on the work of Ford (2002) to assess the reduction in fitness of natural populations caused by domestication. The AHA model has seen widespread use throughout western Washington and the Columbia River basin and has some potential application to marine aquaculture. The AHA model was recently reviewed critically by the Recovery Implementation Science Team (RIST 2009), which concluded that the model can be useful in developing guidelines and evaluating relative impacts; however, the members cautioned that they "do not think that the AHA model can accurately predict the outcomes of specific hatchery or habitat actions in a quantitative way" (RIST 2009, p. 4).

The Offshore Mariculture Escapes Genetic/Ecological Assessment (OMEGA) Model

The OMEGA model (ICF 2012) was developed by NMFS and ICF International as a tool for use by scientists and resource managers to better understand potential negative impacts of farmed escapes on wild populations. The goals of OMEGA are to: 1) provide insights into risks associated with escapes from marine aquaculture, 2) identify research priorities, 3) explore options for design of sustainable aquaculture programs, and 4) inform policy and management decisions regarding genetic and ecological risks of aquaculture.

The OMEGA model includes three major components: biology of the cultured population, aquaculture facilities and operations, and biology of the wild population. Each component includes modules that describe specific assumptions used to model interactions of escapes and their effects on natural populations. The "wild" component describes recruitment, survival, growth, and age structure of the wild population. The model allows for the possibility that escapes might not survive, if they do they might not encounter a wild population, and if they encounter a wild population they might not reproduce successfully.

By evaluating different aquaculture operation scenarios, OMEGA allows the user to compare trends in total abundance of escapes and wild fish, as well as effects of the aquaculture program on survival of wild fish. OMEGA simulates a user-defined scenario of aquaculture escapes over a period of 100 years. Abundance, frequency, and size of escapes is defined by model inputs that specify the number, length of time, and size of fish held in offshore pens, and the likely magnitude and frequency of escape events.

OMEGA defines density-dependent life stages that mimic ecological interactions through competition for food and space. Effects of genetic and ecological interactions are calculated under a user-specified set of assumptions. These assumptions define the survival of escapes in nature, their likelihood of encountering conspecifics, the breeding success of escapes, and the consequence of interbreeding on the long-term survival of wild conspecifics. Calculation of loss of fitness in the wild population is based on the phenotypic fitness model described by Ford (2002).

Based on a demonstration of an earlier version of the model, we believe that OMEGA has the potential to provide useful information for managers dealing with marine aquaculture issues. Before widespread application, it will be important to evaluate performance under realistic scenarios, and caveats noted in the Lessons Learned section above about predictive capabilities for individual programs would also apply here.

Risk Trade-offs

Some risks are correlated in such a way that strategies to reduce one exacerbate another. In these cases, it is generally impossible to simultaneously reduce both types of risk, which sets up some inevitable trade-offs with respect to management options. Waples and Drake (2004) discuss a number of these trade-offs for programs that are designed to enhance natural populations. For aquaculture programs or operations, a major trade-off involves the choice of broodstock source. Using locally derived broodstock will tend to reduce genetic and ecological differences between cultured and wild individuals, but at the same time increase the likelihood that individuals that escape will survive to reproduce; using nonlocal or highly domesticated broodstock reduces the changes that escapes will reproduce successfully, but those that do would pass on very maladaptive genes to the wild population. Another trade-off arises if the decision is made to develop a local broodstock. Collection from too small an area helps minimize the risk of obtaining a population mixture, but increases the risk of founding the program on a narrow and perhaps nonrepresentative genetic base. This latter risk can be reduced by collection from diverse and widely separated geographic locations, but this increases the risk of mixing more than one gene pool in the program.

Monitoring

Monitoring genetic as well as ecological effects of aquaculture should be an integral component of any production program. A well-designed, integral monitoring program with participation by the aquaculture operation (or agency conducting stock enhancement) and the appropriate regulatory agencies is the most effective means of detecting escaped individuals in the wild and their effects on natural populations; such a program can also provide information about effectiveness of measures to reduce this risk, such as changes in confinement technology, and can permit rapid implementation of remedial responses (Kapuscinski et al. 2007, Senanan et al. 2007). Furthermore, a well-designed monitoring program can help maximize efficiency by reducing unnecessary or excessively expensive sampling efforts. Senanan et al. (2007) developed a monitoring and assessment pathway for releases of transgenic individuals into the wild that we have modified to help guide monitoring efforts associated with escapes of aquaculture individuals into the marine environment (Figure 9).

Considering the global scale at which marine stock enhancement and aquaculture programs are conducted (Leber et al. 2004), monitoring any sort of effects on natural populations currently plays only a very minor role. Nevertheless, there are some exceptions, and excellent

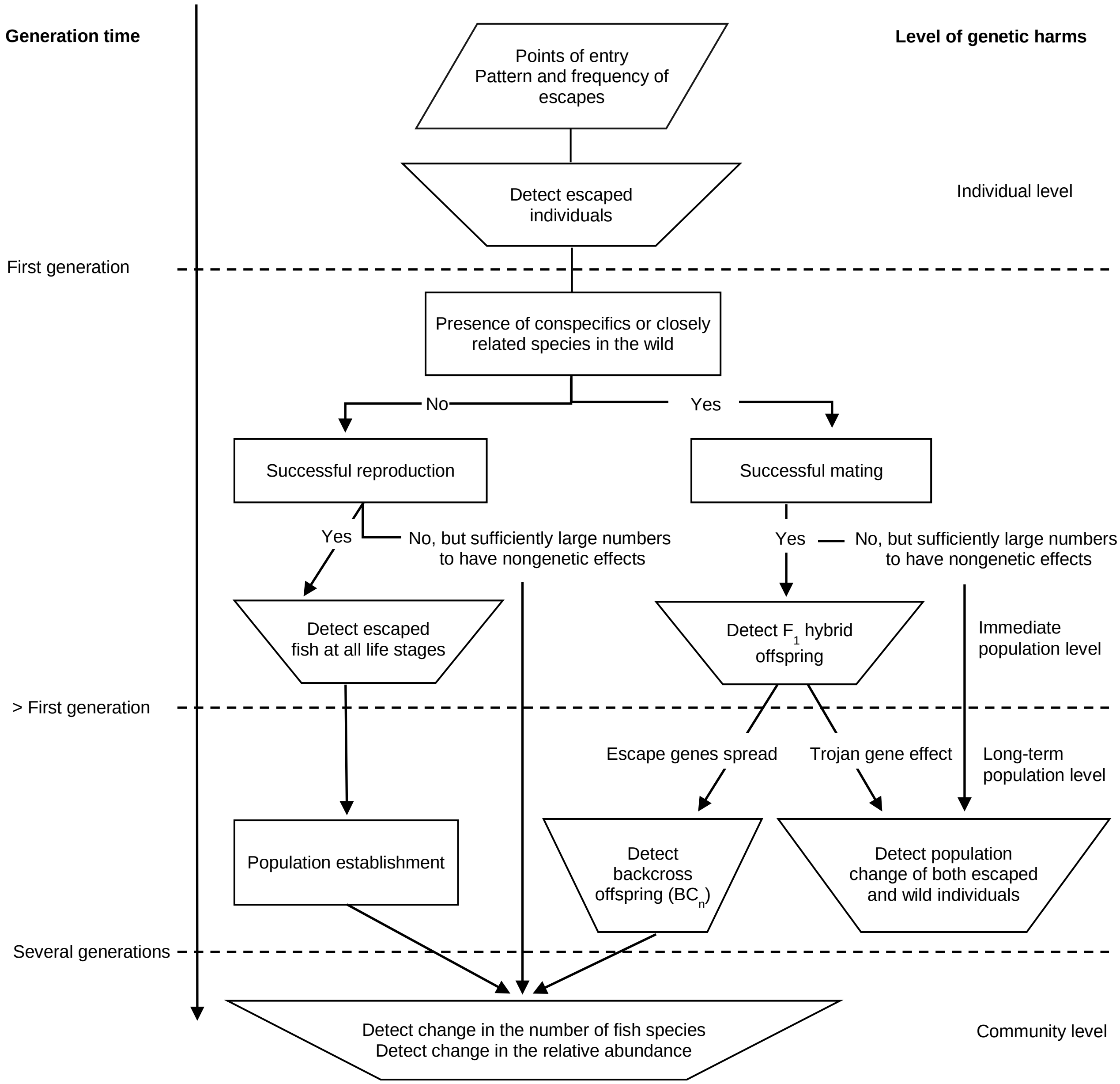

Figure 9. Monitoring should focus on specific measurable end points (illustrated by trapezoids) in the cascade of potential changes that might result from escape and spread of cultured individuals. Types of impacts are organized into individual, population, and community levels. Generation time refers to the number of generations after the escapes. BC_n refers to the nth generation backcrosses between F_1 hybrids and wild relatives. (Adapted with permission from Senanan et al. 2007, copyright Centre for Agricultural Bioscience International.)

examples of programs to monitor genetic effects on natural populations can be found for Atlantic cod in Norway (Svåsand et al. 2004), red sea bream (*Pagrus major*) and other marine species in Japan (Kitada et al. 2009), and red drum in the southeastern United States (several references cited in the Example section below). In the following, we highlight some features of an effective monitoring program.

Robust Monitoring is an Integral Part of Production

A robust monitoring program, integral to an aquaculture program, is characterized by clear, achievable, and measurable objectives. Such objectives inform a logically consistent risk assessment process to identify, prioritize, and select measurable end points that can help trigger appropriate remedial responses. Senanan et al. (2007) outlined an iterative approach for planning, conducting, and evaluating a monitoring program:

1. Define the monitoring end points;
2. Define parameters and measurable variables;
3. Design sampling schemes and test for adequate statistical power of sampling;
4. Choose and employ appropriate sampling methods;
5. Analyze, interpret, and store data;
6. Refine the monitoring design based on lessons learned from data collection and analysis; and
7. Inform risk management decisions.

Resulting data can help guide future risk assessments.

Identifying Remedial Responses

A robust monitoring program clearly identifies the remedial responses that would ensue when particular end points are reached based on monitoring. Unless actions to be taken as a result of particular outcomes are discussed and agreed to ahead of time, an effective and timely response to information gained through monitoring is unlikely. A full range of remedial actions should be clearly defined and associated with particular monitoring end points (Hard 1995b). These actions include, at a minimum:

1. Take steps to minimize escapes,
2. Expand spatial scope or temporal frequency of monitoring,
3. Consider transfer of production to an alternative facility with greater containment success,
4. Consider reproductive containment measures, and
5. Cease production.

Identifying Measurable End points

A robust monitoring program includes measurable end points that are tailored to the life history of the species under culture. Here we adapt six measurable end points identified by Senanan et al. (2007) for monitoring releases of transgenic organisms to the goal of monitoring the effects of escaped aquaculture individuals:

1. Presence of escapes,
2. Presence of F_1 hybrid offspring,

3. Presence of backcross hybrid offspring,
4. Presence of escaped individuals at all life stages,
5. Population change of wild individuals, and
6. Changes in the numbers of local fish species and their relative abundance.

The ability to detect escapes and their descendants can be enhanced considerably by marking all cultured individuals, either through physical tags or by using genetic marks. Modeling the consequences of escapes through genetic simulations is also likely to be useful in helping to refine monitoring methods, including the spatial scope and temporal frequency of monitoring.

Statistical Power

A robust monitoring program includes a realistic assessment of statistical power to detect undesirable effects. Hard (1995b) pointed out that genetic monitoring for changes in traits important to fitness and performance (e.g., life history characters) often will be complicated by the difficulty of 1) determining the source of changes in phenotypic variation (i.e., genetic versus environmental) and 2) low statistical power to detect such changes. One particularly pernicious, incorrect inference can occur when a failure to detect genetic differences arising between cultured and natural individuals leads to the conclusion that no such differences exist. This problem is likely to arise when monitoring is not systematic or during the initial phases of an aquaculture program with limited ability to contain escapement from the facility. Hard (1995b) recommended including an integral research component to production systems that can provide valuable information on the ways in which genetics versus environment contribute to key differences between cultured and wild individuals (i.e., the traits most sensitive to domestication in protective culture). Senanan et al. (2007) provided guidance on estimating power, sampling design, and sampling requirements to detect effects at various levels, including changes in wild population size or species composition.

Opportunities for Research

Coordinated production and research, along with rigorous monitoring, is essential to providing baseline information to detect changes resulting from aquaculture and identifying opportunities to evaluate sampling efficiency. For most cultured marine species, information on life history, genetics, and population structure is limited. Aquaculture programs provide an opportunity to improve this information through associated research. Studies in the following areas would be especially valuable in improving monitoring and reducing risks of aquaculture to wild populations (Senanan et al. 2007):

1. Patterns of escapes and efficacy of confinement measures;
2. Movement of the target species within local habitats;
3. Survival rate and reproductive success of escaped cultured individuals in the wild;
4. How fish density affects the above characteristics;
5. Genetic diversity of cultured stocks and potentially impacted populations;

6. Abundance of potential receiving populations and their temporal and spatial variation;
7. Fish community composition and natural variation;
8. Sampling efficiency for different locations, gear types, and environmental conditions; and
9. Modeling studies that can make use of data acquired by experiments, field studies, and monitoring and make predictions from a larger parameter space than established by data.

Frequently Asked Questions

What level of interbreeding with cultured individuals is completely safe for wild populations?

A value of 5% has often been proposed as an upper limit to the fraction of cultured individuals breeding in wild populations (e.g., as adopted by the State of Florida in its genetics policy, FFAWCC 2007). In fact, however, no level of interbreeding can be regarded as completely safe for natural populations if it continues indefinitely. That was the conclusion of a scientific panel (reported in Grant 1997) convened to consider this question with respect to straying of hatchery salmon. Even a very low level of interbreeding can be harmful if it continues long enough. This conclusion is based on two well-established facts from evolutionary biology: 1) most selective differentials (s) measured in the wild are relatively small (typically a few percent, and mostly less than 5%), and 2) migration of nonnative genes into a population can swamp local adaptations if the migration fraction (m) is larger than s. The Florida Genetics Policy acknowledged these points, but concluded from this that limiting migration rate to 5% would provide sufficient protection for adaptively maintained alleles. In practice, an upper limit often becomes a target that is actively managed for. If most realized rates of interbreeding of cultured and wild individuals are around 5% and continue for more than a few generations, the expectation is that large numbers of alleles that are currently selectively maintained will be lost or will drift to very different frequencies.

It is important to remember that the rate of migration does not necessarily equal the rate of genetically effective migration (gene flow). The genetic risks discussed here and in the previous section apply to situations in which the escaped aquaculture individuals survive to reproduce and successfully transmit their genes to subsequent generations. In some cases, the rate of gene flow can be much lower than m, but the degree to which this is true is likely to vary considerably among species, populations, geographic areas, and particular features of the aquaculture program.

Is it better to use a highly domesticated stock for aquaculture (because escapes are likely to have low fitness in the wild), or is it better to keep the aquaculture stock as similar as possible to local wild populations? Or would an intermediate strategy be best?

Unfortunately, there is no simple answer to this question. The net effects on natural populations will depend in a complex way on the number of interbreeding events, how detrimental each event is, and the time period over which they occur. Strongly diverged aquaculture populations might have fewer per capita interactions with wild populations (e.g., because they differ markedly in behavior, life history, or other phenotypic traits), but each interbreeding event that does occur is likely to be more detrimental to the wild population. If the cultured population is kept more similar to local natural populations, each interbreeding event is likely to be less harmful, but there could be many more such events. The net effects in each case must be some function of the number of interbreeding events and how bad each one is. It seems likely that either strategy might be better under particular circumstances. In theory, either extreme strategy could be less detrimental than an intermediate one that involved a moderately domesticated population with strong captive wild interactions (Figure 6, see also Lorenzen 2005, Baskett and Waples in press, and discussion in Appendix A).

Is it necessary to derive local broodstocks in each location, or can proven and successful broodstocks be imported from other areas?

This is another question for which there is no simple answer. With regard to genetic effects on natural populations, the trade-offs between using local broodstocks that are similar to local natural populations and divergent broodstocks that are strongly domesticated or derived from different areas are discussed in response to the question above. A variety of additional factors, such as potential for disease transfer, should also be considered if importing broodstocks is under consideration. If the target species is not native to the local area, then there are few concerns regarding interbreeding with local populations, unless interspecific hybridization is a possibility (which often is the case among fishes). However, importing nonnative species has caused ecological problems in many of the world's ecosystems, so proposals of this type should receive special scrutiny.

Our knowledge about fish culture and its effects on natural populations has improved considerably in recent years. If aquaculture programs follow new and improved guidelines, can't they avoid the problems of the past?

It is true that our understanding of how to run effective and efficient fish culture operations has grown considerably, as has understanding about how to reduce some of the most serious threats these programs pose to natural populations. However, it is a mistake to think that these advances are capable of removing all risks to natural populations. The environments experienced by cultured and natural individuals differ so dramatically in so many ways that some level of domestication in individuals used in commercial culture or in stock enhancement is inevitable. In fact, substantial domestication is likely a prerequisite for profitable commercial aquaculture production of many marine species. Unless containment is 100% effective, the results of domestication can affect natural populations. Therefore, the key question is not whether improved methods can eliminate all risks, but whether aquaculture operations can be managed in such a way that the resulting risks to natural populations do not rise above a level deemed to be acceptable. Scientists can help evaluate the levels of risk associated with different types of programs and their likely consequences for natural populations, but policy makers must determine whether the resulting risks are acceptable from a societal standpoint.

How many breeding individuals must be maintained in a cultured population?

In aquaculture, individuals that mature and spawn are generally not marketable and so represent a cost of production, which efficient operations will try to minimize. From the aquaculture standpoint, the minimum number of broodstock to maintain is probably determined largely by concerns for minimizing effects of inbreeding. Assuming cultured populations can withstand sustained inbreeding levels of 1–2% per generation, this would require a minimum of about 25–50 individuals per generation, based on the relationship $\Delta f = 1/2N_e$. Because effective population size can be much smaller than census size due to uneven sex ratio and highly skewed reproductive success, the number of breeders required to keep inbreeding levels below 1–2% could be much higher than 25–50. For example, Gold et al. (2008) used genetic parentage analysis to evaluate 13 spawning events involving 27 female and 18 male red drum in a Texas marine enhancement program. The mean effective number of parents contributing to a single spawn was only 2.6 and the estimated effective number of parents for the 2003 cohort as a whole was 29 to 47, depending on the release site. An important factor reducing effective size compared to the theoretical maximum was failure of many females to spawn.

Whether the aquaculture breeding population size is large enough to adequately account for potential effects on natural populations depends on a variety of factors discussed under the Genetic Risks to Natural Populations from Aquaculture subsection of the Characterizing Risks and Benefits section above.

If diversity is important, what is wrong with breeding between aquaculture and wild individuals—doesn't that create more diversity?

Breeding between cultured and wild individuals can affect both types of diversity: diversity within populations and diversity between populations. The consequences of these effects will vary depending on several factors.

Diversity among most natural marine populations has developed over hundreds or thousands of years. Interbreeding with cultured individuals cannot easily increase this diversity, but it can reduce it rapidly. For example, if escaped individuals survive and widely disperse, they could interbreed with a number of historically distinct wild populations. This can introduce nonnative genes into wild populations at rates much higher than would occur naturally, which in turn can reduce historic differences in genetic and life history traits between populations and make them all more similar to a single, cultured population.

The effects of interbreeding with cultured individuals on diversity within a single population are more complicated to analyze. Depending on the circumstances, interbreeding with cultured individuals can increase, decrease, or have no net effect on genetic and life history diversity within a wild population. Interbreeding with cultured individuals is most likely to increase diversity in a wild population that has undergone an extreme bottleneck. In this situation, cultured supplementation can increase the overall population size and minimize further losses of diversity. If the cultured population is closely related to the wild population, it also could help restore some variability that had been lost from the wild component. However, as discussed above regarding the Ryman-Laikre effect, it is more likely that interbreeding with aquaculture individuals will reduce genetic diversity in the local population, perhaps by a substantial amount.

If the cultured population is genetically different from the wild population, breeding between the two populations can indeed increase diversity within the combined population, at least temporarily. However, this increased diversity can come at the cost of reduced fitness. For example, a domesticated cultured population can diverge genetically from a wild population in many ways that will make it less able to survive in the wild. Interbreeding with such a cultured population could initially increase genetic diversity of a wild population, but the added diversity would include much genetic variation that reduces fitness in the wild. Therefore, the net effect could be a substantial genetic load to the wild population. Similar considerations apply to cultured populations that are derived from local wild populations from a different geographic area.

Even if aquaculture programs reduce diversity within and among populations and natural fitness, won’t natural selection restore these qualities over time?

This is probably true in at least some cases, given evolutionary time scales (hundreds to thousands to millions of years). However, aquaculture has the ability to dramatically change, in relatively short time periods, population characteristics that have evolved over many generations. Restoration of a dynamic equilibrium between mutation, migration, genetic drift, and selection can take a very long time, even if the risk factors are eliminated (see examples in Characterizing Risks and Benefits section above). Furthermore, the outcome is far from certain. Populations that are substantially affected by these factors might find themselves in a race to re-evolve something like their original genetic characteristics before they go extinct due to loss of genetic variability and reduction in fitness.

Example

Here we use a proposed marine aquaculture program for a hypothetical species (Species X) to illustrate how some of the risk assessment and risk management tools discussed above can be implemented in a programmatic evaluation. Species X's biology is loosely based on that of the red drum, which has been the subject of aquaculture and marine stock enhancement efforts for more than 20 years in the Gulf of Mexico and the Atlantic Ocean. Extensive evaluations have been conducted for some of these programs and this provides an opportunity to tune the example to realistic biological scenarios. Information presented below uses a simplified format based on the AGMP (Appendix B). For simplicity, we focus here on genetic issues rather than the full complement of topics that would be covered by an AGMP. The following information is based on material provided by the applicant seeking the permit.

Information from the AGMP

Section 1. Background Information

- The proposed site is in U.S. continental shelf waters of the Gulf of Mexico.
- Objective is commercial production in cage culture.
- Species X is large (can exceed 20 kg) and long-lived, with indeterminate growth. Based on information available for red drum, we assume a generation time of approximately 12 years, a maximum life span of 30–50 years or more, and maturity at 35–80 cm (age 3–6). We also assume that female batch fecundity increases exponentially with weight and can exceed 10^6 for large individuals.
- The expected production level is 1,500 tons per year, assuming 40 net pens, each holding about 50,000 fish that are marketed at 1–2 kg.
- Expected escapes are 1% of production, or 20,000 fish/year.
- The application is for a renewable permit for 10 years. The intent is to continue operations indefinitely as long as they are profitable and meet management and conservation guidelines.
- This is a commercial operation and has no plausible benefits to the natural populations, except perhaps indirectly via reducing harvest pressure. Performance standards and performance indicators for genetic risks are discussed in Section 8 below.

Section 2. Relationship of Program to other Management Objectives

The aquaculture program for Species X will be operated consistent with NOAA Aquaculture Program guidelines for marine finfish aquaculture. The AGMP for this program has been developed with attention to these guidelines and state regulations permitting aquaculture operations.

Section 3. Facilities

Broodstock are spawned in closed freshwater systems. Techniques for year-round spawning are well established using manipulation of temperature and photoperiod. After hatching, larvae are shipped in plastic bags to 1-acre receiving ponds. Fingerlings are moved within 30 days (at ≈3 cm) to larger ponds for growout to stocking size (about 20–25 cm).

Transfer to marine net pens occurs twice a year, being adjusted to production, harvest, and fallowing cycles in the marine net pens. Net pens are larger than commonly used in nearshore aquaculture, and approximate 2,500 m^3 with a maximum density of 7.5 kg/m^3. Care is taken that net structures are flexible, attached to a rigid frame, and anchored in ways that meet the technical standards demanded by open water operations. Oxygen levels are monitored to avoid hypoxia, which is related to the oxygen consumption of the fish and the water flow in the cages. Feed is delivered through automatic, submerged feeding channels. Feces and waste feed are monitored to optimize production and minimize impact on marine benthos and food webs. The net pen operations are marked such that ship traffic and the general public are diverted from the site.

Sections 4-6. Broodstock

For purposes of comparison, this proposal includes two different broodstock scenarios.

- Option 1: Mature adults are collected from natural habitats within 20 km of the culture site. At least 50 adults with approximately 1:1 sex ratio are maintained at all times. Each year, about one-fourth of the spawners are returned to the wild and replaced with new individuals. During the spawning season (late summer/fall), group spawning occurs in tanks and resulting eggs are collected and cultured as described above.
- Option 2: Fingerlings are obtained from a commercial provider and reared as described above. The commercial provider uses approximately 200 adults per year but replaces only about 10–25% per year. The commercial broodstock was developed two decades ago and originated from several localities on the east and west sides of Florida.

Section 7. Incubation and Rearing

Content for this section is to be provided by the applicant in the associated AGMP.

Section 8. Effects on Natural Populations

Relatively little detailed information is available on the biology of natural populations of this species. Based on data for red drum and other marine finfish, it is assumed that local populations are only weakly differentiated, perhaps following an isolation-by-distance pattern. Spawning areas have not been identified but are likely inshore, as eggs and larvae are rarely found in offshore waters. Sport and commercial fisheries are managed on the basis of regional stocks that bear an uncertain relationship to natural populations. The species is not considered overfished according to Magnuson-Stevens Act guidelines. Natural population sizes in the Gulf of Mexico are thought to be in the range 10^5–10^7 mature individuals.

Performance standards (and associated performance indicators) designed to address genetic risks include:

- Performance standard 1: Maintain adequate genetic diversity in broodstock. Performance indicator 1: Effective population size of at least 50 per generation.
- Performance standard 2: Mark all fish to facilitate identification and recovery of escaped fish. Performance indicator 2: Fraction of farmed fish without visible marks in a random sample.
- Performance standard 3: Keep fraction of escapes below 1% of total annual production. Performance indicators 3: Fraction of losses by life stage that are unaccounted for, and size and longevity of damaged sections of net pens that can allow escapes.
- Performance standard 4: Limit fraction of natural spawners that are aquaculture escapes to less than 1% in any local area. Performance indicator 4: Fraction of escaped fish found with natural spawners.

Section 9. Monitoring

Routine monitoring includes the following:

- Performance standard 1: Regular observations of the numbers of fish participating in spawning activity.
- Performance standard 2: Regular random samples of farmed fish to determine fraction with illegible marks.
- Performance standards 3: Regular inventories of stock to quantify unexplained losses and regular underwater inspections of net pen integrity.
- Performance standard 4: No monitoring planned. We expect that any farmed fish that do escape will have only a small chance of successful reproduction.

Section 10. Research

In the aquaculture program, routine data collection (for estimation of survival and growth rates to each life stage in culture) would provide opportunities for research into performance and possible adaptation in captivity. Research on genetic risks would likely differ between option 1 and option 2 for broodstock scenarios (see Sections 4–6 above), as one would expect no (or very limited) genetic difference between local wild and captive fish in option 1, whereas genetic differences from historical sources as well as from local wild populations would be expected under option 2. In the first scenario, the main research question would be to test the null hypothesis of no genetic difference between wild and captive fish, whereas in the second scenario, the magnitude and nature of genetic changes occurring during broodstock development, as well as the level of gene flow from farm escapes to wild populations, could be starting points for research.

Analysis

The general lack of specific biological information for the target species complicates the analyses. In this example, information is used for red drum and other marine finfishes, but it should be recognized that this additional source of uncertainty heightens the risks associated with the proposed program. The facilities appear to generally meet industry standards. The freshwater operations function under a separate permit and are not considered in detail here, except the spawning protocols.

Monitoring Performance Indicators

The proposed action (regular observations of the numbers of fish participating in spawning activity) is unlikely to be adequate to meet performance indicator 1 (maintain $N_e \geq 50$). Genetic analyses (e.g., Gold et al. 2008) have shown that, on any given day, only a small fraction of adults contribute gametes in group spawnings, but over the course of the season, a larger fraction of the population contributes to the offspring. This information is difficult or impossible to obtain by simple observation of spawning activity (which, in any case, is likely to occur at night). A reasonable assumption is that N_e of the hatchery component is unlikely to be more than about 50% of the number of spawners and could be considerably less.

Periodic random samples should suffice to determine whether a significant fraction of the population has lost its distinguishing marks (performance indicator 2). However, more information is needed on exactly how the marks will be applied, as well as evidence from tag retention studies. Performance standard 3 is more challenging to evaluate. Although the proposed monitoring measures should provide some useful information to determine whether the less than 1% escapes goal has been met, the proposal does not contain enough information to assess the likelihood that containment measures are sufficient to keep escapes below this level. This would be easier to evaluate if the applicant had provided data for previous years demonstrating that comparable operations have resulted in sufficiently low levels of escapes.

Regarding performance standard 4, since no monitoring is planned, it seems the applicants are assuming that the level of natural spawning will be sufficiently low if performance standard 3 is met. However, the two standards measure different things: Standard 3 refers to the fraction of the cultured population that escapes, whereas standard 4 refers to the fraction of natural spawners that are escaped fish. So even if escapes are limited to 1% of the farmed fish, the effects on natural populations could be large or small, depending on the size of the natural population. If 1% of the farmed fish escape and spawn naturally, that would be approximately 10^4 farmed fish interacting with an estimated 10^5–10^7 natural fish, or about 0.1% to 10% of the wild population. If natural abundance is at the low end of the estimated range and the expected number of fish escape and breed, the result could be significant introgression of farmed genes into the natural population. If natural abundance is near the top end of the estimated range, then 1% escapes would not represent a sizeable fraction of all spawners, unless all the escapes spawned in a limited geographic area where they could represent a larger fraction of a local spawning population. As the proposal did not include any discussion of the probability that escaped fish would survive to reproduce, we consider a range of scenarios below.

Loss of Diversity within Populations

Here we review information necessary to evaluate the effective size of the combined wild-farmed system, assuming some farmed fish escape and spawn. Under broodstock option 1, 50 spawners are used per year. Assuming a ratio of about 0.1–0.5 for effective size:census size, this means that the offspring in a yearly cohort would be produced by about N_b = 5–25 effective parents. Under option 2, a total of 200 spawners are used per year, resulting in an estimated N_b = 20–100 effective parents. These are yearly estimates of effective size. In broodstock option 1, 25% of the spawners (12–13 fish) are replaced each year, and over a 12-year generation, this means that about 200 total individuals are used. Therefore, we also considered a scenario with $N_eC = 100$ (assuming N_eC / N_C = the upper limit of 0.5). For option 2, if we assume that 10–15% of the broodstock are replaced each year, the program would use about 500 different spawners over a generation, suggesting a maximum N_eC of 250. The wild population is thought to number 10^5–10^7 mature individuals. For natural populations of marine fish, estimated N_e / N ratios are generally no higher than 0.1 and can be orders of magnitude lower. This suggests that N_e in the wild component could be in the range 10^3–10^6, which is consistent with estimates for red drum (Turner et al. 2002).

We use equation 1 to evaluate the expected effective size of the total system (N_eT) and compare it to the control situation (N_eW = effective size of the unsupplemented wild population).

$$N_eT = \frac{1}{\dfrac{x^2}{N_eC} + \dfrac{(1-x)^2}{N_eW}} \qquad (1)$$

The above analysis suggests that yearly values of N_eC = 5–25 (for option 1) and 20–100 (for option 2), $N_eW = 10^3$–10^6, and x = 0.001–0.1, while generational values of captive effective size might be as high as $N_eC = 100$ (option 1) or 250 (option 2). Note that under option 1, broodstock are removed from the wild population, so N_eW is adjusted to account for this. Inserting these values into the above equation produces the results shown in Table 4.

One point to note is that under almost all of the scenarios shown in Table 4, the overall effective size would satisfy the criterion of Tringali and Bert (1998), who proposed that N_eT not be allowed to drop below 500. The only scenario that fails to meet this standard is scenario A for option 1 ($N_eT = 351$). Note also that if x is as small as 0.001, any changes to overall N_e from the Ryman-Laikre effect are expected to be very small, even for the low range of N_eC values (scenario H). However, under several of the possible scenarios, although N_eT remains relatively large, it is reduced to only a fraction of its original value. This result occurs, for example, when escaped farmed fish comprise 1% of successful spawners ($x = 0.01$) and N_eW is large initially (10^6) and N_eC is small (5–20): N_eT would then be about 5% of its original value for option 1 and 17% for option 2 (scenario F). Assuming $x = 0.1$ (upper end of the estimated range), N_eT will not be more than about 3% of its original value when wild population size is large (scenario C and scenario D).

These results illustrate the point that large wild populations are at the greatest risk from proportional reductions in N_e due to the Ryman-Laikre effect, even when the resulting N_eT is still moderately large. These reductions might have no immediate fitness consequences; however, a

Table 4. Results of applying equation 1 to various combinations of parameter values for the proposed aquaculture program for Species X, under two different options for sourcing broodstock.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Option 1								
X	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.001
N	1,000	1,000	10^6	10^6	1,000	10^6	10^6	10^6
N_eW	950	800	$\approx 10^6$	$\approx 10^6$	950	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$
N_eC	5	100	5	100	5	5	100	5
N_eT	351	899	500	9,920	951	47,664	5.0×10^5	8.3×10^5
N_eW / N_eT	0.351	0.899	<0.001	0.010	0.951	0.048	0.505	0.835
Option 2								
X	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.001
N_eW	1,000	1,000	10^6	10^6	1,000	10^6	10^6	10^6
N_eC	20	250	20	250	20	20	250	20
N_eT	763	1,176	1,997	24,504	1,015	1.7×10^5	7.2×10^5	9.5×10^5
N_eW / N_eT	0.763	1.176	0.002	0.025	1.015	0.167	0.725	0.954

population with $N_e = 10^6$ can maintain many more rare alleles than one with $N_e = 500$, and this might be important for long-term viability and evolvability of the population. Would such a reduction be acceptable? Science alone can’t answer this question; it also requires consideration of societal values and tolerances to different kinds of risk.

Loss of Diversity among Populations

This risk is hard to quantify, given the lack of information specific to the target species. In general, concerns increase when there is disparity between the geographic scale of population genetic structure and either a) the geographic scale over which broodstock are collected, b) the geographic scale over which escaped fish stray, or both. The application does not provide sufficient information on any of these parameters for a rigorous evaluation of risk.

In general, marine fish species with similar life histories show relatively weak population genetic structure, a result also reported for red drum by Gold et al. (2002). However, as noted above, large populations can have very low migration rates (e.g., $m < 0.001$) and still exchange enough migrants to maintain similar allele frequencies. Furthermore, several empirical examples now call into question the conventional assumption that most marine species with high dispersal capability are not likely to exhibit strong local adaptations.

Loss of Fitness

Commercial aquaculture involves rearing to market size under artificial conditions in captivity, so substantial fitness differences (compared to wild fish) can be expected to develop over time even in a population derived from local fish (as in option 1). For example, Saillant et al. (2007) found that juvenile growth rate is heritable in red drum and this trait likely would be under strong selection in aquaculture. If the broodstock source is not local or has been assembled from multiple different sources (as in option 2), then any fish that escape would be expected to perform even less well in the wild. This loss of fitness (of farmed fish in the wild) is something of a two-edged sword for evaluating risk. If the fitness loss is substantial, few escaped fish would survive to reproduce, but the few that did could pass on very maladapted genes to the natural population. With a more modest fitness loss, more escaped fish would survive to spawn naturally, but each interbreeding event with wild fish would probably not be as detrimental. The net fitness effects of these two scenarios are difficult to predict, which also makes it difficult to determine which option is less likely to cause major reductions in wild population fitness. However, this ambiguity applies to the genetic consequences of option 1 and option 2; there might be sound ecological reasons (e.g., concerns for spread of disease) for deciding whether importation of nonlocal broodstock is appropriate.

Comments

This hypothetical example includes a considerable amount of information on some aspects of the program but little information about other key features. This makes it difficult to make quantitative predictions of risks, but it also is probably typical of many types of situations that managers would confront in making decisions about appropriate uses of marine aquaculture.

Given the available information, a quantitative analysis was possible only for changes in effective population size, and this only by considering a wide range of possible values for some key parameters. The very qualitative analyses of risks of fitness and diversity among populations identified causes for concern that suggest a careful consideration of risks by the public regulatory agency might be warranted. All of the genetic consequences of escapes scale with their fractional representation in the natural spawning population, which depends not only on the number of escapes but also on their probability of surviving to reproduce. Therefore, efforts should focus on preventing the former and gathering information about the latter. Here are several specific steps that could be taken to help resolve some key uncertainties regarding the proposed project.

1. Characterize the genetic structure of the natural populations. Given the typically low genetic signal and various sources of random noise, it is important to include a temporal dimension to the sampling design (Waples 1998). Patterns that are consistent across two or more time periods are more likely to be robust indicators of population genetic structure.
2. Identify time(s) and place(s) of natural spawning by local populations.
3. Conduct surveys in the wild to map distribution of escaped fish. How does the distribution of escapes match the geographic scale from which broodstock are collected? How does it match the population genetic structure? Are escaped fish (especially mature individuals) found in natural spawning areas?
4. Determine (or clarify, if known) whether all farmed fish are marketed before they reach maturity, or whether at least some can mature in marine net pens. This will determine whether genetic leakage from spawning in net pens is an issue that needs to be considered.
5. Periodically inspect and certify containment systems, as well as fish handling procedures.
6. Develop a contingency plan to deal with significant escape events. Perhaps this could include an outreach/education program to ask commercial and recreational fishers to report frequency of marked farmed fish in catches.

Discussion

The major objective of this technical memorandum is to provide information to assist NOAA in fulfilling its marine aquaculture regulatory and policy mandates under existing laws. We emphasize that, although we focus here on genetic issues, this is intended to complement (and not substitute for) consideration of the numerous ecological issues associated with aquaculture operations, which are discussed elsewhere. Furthermore, although this document focuses on marine finfish, most of the issues discussed here apply more broadly to aquaculture operations for shellfish and other invertebrates, as well as freshwater finfish.

Like many federal agencies, NOAA has several and potentially competing responsibilities and legislative mandates for marine aquaculture, including enabling development of sustainable aquaculture and stewardship responsibility for living marine resources. Although the profitability of commercial marine aquaculture operations might be enhanced by the use of highly domesticated strains that grow fast, have high fillet yield, tolerate crowding, and resist disease under cultured conditions, in general this type of operation produces individuals that are maladapted to natural conditions, and if they interbreed with wild fish they can reduce viability of natural populations.

All the genetic consequences of marine aquaculture are proportional to the fraction of successful natural spawners that are escaped fish. Therefore, by far the best strategy to minimize opportunities for adverse genetic interactions is to ensure that farmed fish do not escape in the first place. Once fish escape, experience suggests that even concerted efforts to recapture them will be only marginally successful at best. Because no aquaculture system in the marine environment can ensure 100% containment through either physical or reproductive containment measures, it is important to consider the scale of operations permitted to provide adequate safeguards for natural populations, given some inevitable level of escapes.

Although escaped farmed fish can have ecological consequences for marine ecosystems as soon as they escape, they have direct genetic effects only if they survive to successfully reproduce and contribute genes to natural populations. Therefore, this report is concerned with situations in which natural populations of the targeted aquaculture species occur in or near the geographic vicinity of the aquaculture operation. For example, most of the genetic issues discussed here do not apply to Atlantic salmon aquaculture operations in western North America, as there are no native Atlantic salmon with which the escaped fish can interbreed. In these situations, genetic concerns are restricted to indirect effects, such as potential disruption of spawning activities, introduction of disease agents, or changes to selective regimes experienced by native populations of other species.

The factors that determine how likely escaped fish are to spawn with local natural populations are diverse and almost certainly vary widely across species and geographic areas (and perhaps over time as well). Because these factors have a strong influence on actual genetic risks posed by marine aquaculture, achieving a better understanding of how they function should be an important research priority.

In evaluating evolutionary consequences of marine aquaculture, it is important to consider their proposed duration. Effects that might be inconsequential if only a single pulse event is involved can become substantial if they continue repeatedly across years and generations. For example, an extreme, single-generation bottleneck of $N_e = 4$ and a less severe but more prolonged bottleneck of $N_e = 50$ for 13 generations both result in about the same total loss of genetic variability. This argues for careful consideration of proposed programs that are expected to operate indefinitely, with opportunities for continual genetic inputs to wild populations over considerable periods of time.

A key decision in any aquaculture operation is choosing the broodstock source and this issue is also of keen interest to regulators. It is useful to briefly contrast two extreme scenarios regarding the broodstock source: Strategy 1 uses a recently developed stock based on individuals collected from the local area, while Strategy 2 uses a productive but highly domesticated stock derived from nonlocal populations. With respect to ecological issues, there are reasons for concern about use of nonlocal broodstock (e.g., introduction of novel diseases or parasites). Use of a local broodstock is also indicated if the intention is to use the survival advantage provided by fish culture to help boost abundance in a local natural population (by direct supplementation). However, for commercial aquaculture operations that are intended to be closed systems, the relative genetic consequences of these two scenarios for natural populations are more complicated to assess (see Frequently Asked Questions).

This key topic also merits more detailed research and evaluation. One point that should be emphasized, however, is that use of a locally derived broodstock is not a panacea that eliminates genetic concerns for marine aquaculture. Results that have accumulated over the last decade show that even in conservation salmon hatcheries that use locally derived broodstock, hatchery fish typically show lower fitness in the natural environment than do wild fish. Furthermore, salmon programs release juveniles into the wild where they spend a good part of their life cycle, so even larger fitness decreases likely would be associated with marine aquaculture programs that propagate the entire life cycle in captivity.

All of the major genetic concerns associated with marine aquaculture have been recognized for at least 2–3 decades, especially with respect to artificial propagation of salmon, and a considerable body of empirical evidence has accumulated to demonstrate that each concern can and has been realized for natural populations. However, it is also the case that for any given type of program, observed effects on natural populations typically cover a wide range from no detectable effect to substantial changes. This means that, although genetic risks of artificial propagation can be characterized and assessed as described in this document, it typically will be the case that considerable uncertainty is associated with predicting the exact consequences of any particular program.

Therefore, it is important for scientists, managers, and policy makers to discuss how to deal with uncertainty and what is an appropriate way to assign burden of proof. Should proposed programs or operations be allowed to go forward unless it can be convincingly demonstrated that they will cause serious harm? Or should proposed programs not be allowed to proceed unless it can be demonstrated with a high degree of certainty that adverse effects will not occur? These questions will repeatedly arise in considering the most appropriate ways to implement marine aquaculture. They cannot be answered by science alone, but how they are answered can have a profound effect on the nature and magnitude of aquaculture programs that are considered permissible. The same general questions, it should be noted, are relevant to assessing the consequences of other anthropogenic actions that affect marine populations (such as fishing or habitat modification), as well as the consequences of propagation programs that release large numbers of individuals of other taxa into the wild (e.g., birds, insects, or trees).

To provide a context for evaluating effects of aquaculture, we also briefly considered some other anthropogenic activities that can have substantial effects on marine species. On a global scale, fishing probably has the largest effect, as harvest managers typically aim to reduce targeted species to half or less of their pristine biomass. Furthermore, substantial ecosystem effects of harvest can occur at removal levels well below those thought to lead to maximum sustainable yield. And bycatch of nontarget species and long-lasting, physical changes to marine environments caused by some harvest methods have substantial effects around the world. Anthropogenic changes to nearshore marine habitats are also pervasive, and these areas are important for early life stages of a wide range of marine species.

Human impacts to offshore habitats are not as obvious, and at present, we have only limited understanding of the consequences of those changes for natural populations. However, there are many reasons to expect that these pressures will increase in coming decades. First, the global human population continues to grow, particularly in vulnerable coastal areas. Second, marine pollution is an escalating problem, and new, potentially toxic, compounds are developed and placed into use faster than their biogenic effects (and interactions) can be adequately studied. Finally, global climate change brings the likelihood of warmer water, altered current patterns, and increasing acidification that could dramatically alter marine habitats and ecosystems in the twenty-first century.

Considering only aquaculture of marine finfish (the focus of this paper), global effects on natural populations probably are much smaller than for harvest or habitat modification. However, the footprint is much larger if one takes a broader view of artificial propagation in the marine environment to include marine net pens for salmonids (with large operations on several continents) and marine stock enhancement programs that involve juvenile releases (sea ranching), which are conducted on huge scales for hundreds of species worldwide. Furthermore, marine aquaculture programs have grown rapidly in some areas outside the United States and might do so within U.S. waters in the near future. In any given area and for any given ecosystem, therefore, marine aquaculture programs might have biological consequences that equal or exceed those of other anthropogenic activities. Finally, because marine aquaculture involves direct intervention in reproduction, growth, and survival of the target species, it has the potential to cause evolutionary changes that have long-lasting or permanent consequences for natural populations. For all these reasons, marine aquaculture programs merit careful evaluation on a case-by-case basis to determine the most appropriate regulatory response.

Glossary

[Editor's note: For cross-reference in the explanation of evolutionary terms, those also listed in the glossary appear in boldface.]

Allele. An alternative form of a **gene**. Each **diploid** individual has two alleles (one from each parent) for each gene locus.

Artificial selection. Differential survival or reproduction of individuals with different **phenotypes** in an artificial system (e.g., one controlled by humans). Artificial selection can be intentional or can arise indirectly from selective regimes in cultured environments that differ from those in the wild.

Assortative mating. A departure from random mating in which similar individuals mate among themselves either more often than would occur by chance (positive assortative mating) or less often (negative assortative mating). Positive assortative mating can lead to **inbreeding.**

Diploid. Having two copies of each type of chromosome; generally, one copy is derived from each parent. In contrast, haploid individuals have one set of chromosomes.

Domestication. Genetic changes associated with artificial propagation that affect fitness in captive and wild environments. Domestication results from a combination of two forces: selection for traits that are adaptive in the captive environment and relaxation of selection against traits that are maladaptive in the wild.

Effective population size (N_e). An index that determines the rates of many evolutionary processes in populations. The rate of **genetic drift** is proportional to $1/N_e$, which means that it is higher in small populations.

Escape. In the context of this document, an individual that has escaped from captivity and may or may not survive to reproduce in the wild (often also called an escapee).

Fitness. The capability of an individual to pass its **genes** on to the next generation. Relative fitness determines how successful a given individual will be compared to other individuals within the same population at the same time. Mean absolute fitness of a population is a reflection of its ability to consistently replace itself over time. Fitness of individuals and populations can depend strongly on the environment in which it is measured.

F_1, F_2. Used to denote the number of generations following an episode of interbreeding. The F_1 generation is progeny of the interbreeding event; the F_2 generation is progeny of F_1 individuals.

F_{ST}. A measure of the level of genetic differentiation among populations. F_{ST} values can range from 0 (no genetic differences among populations) to 1 (different populations fixed for different **alleles**), although for highly polymorphic markers (like microsatellites) the maximum F_{ST} can be considerably less than 1. Generally, marine fishes have lower F_{ST} values than do anadromous or freshwater species.

Gene. The basic unit of heredity in living organisms. A gene is a section of DNA that has predictable functions, such as coding for a specific protein or regulating cellular processes.

Genotype. The genetic makeup of an individual. The genotype at a single **gene** locus is specified by the two **alleles** an individual carries; a multi-locus genotype is specified by **alleles** at multiple **gene** loci.

Genetic drift. Random changes in **allele** frequency between generations. Genetic drift occurs in all finite populations but can be quite strong in small populations.

Genetic diversity. The range of **genes** and **genotypes** found in an individual, population, or species. **Heterozygosity** is a common measure of genetic diversity within individuals or populations; genetic distance and related measures quantify genetic diversity among populations.

Heterozygosity. A measure of **genetic diversity** within individuals or populations. An individual with two different **alleles** at a **gene** locus is a heterozygote (a homozygote has two copies of the same allele). The average fraction of individuals that are heterozygotes is a measure of heterozygosity.

Inbreeding. Mating between close relatives. Inbreeding is rare in large, randomly mating populations but is inevitable in small populations because everyone rapidly becomes related. **Assortative mating** that causes relatives to mate with each other more often than would occur by chance can also lead to high levels of inbreeding. Inbreeding can lead to **inbreeding depression.**

Inbreeding depression. A reduction in fitness caused by mating between close relatives, leading to reduced **heterozygosity** and expression of deleterious recessive **alleles.**

Introgression. Movement of genetic material from one species or population into another, as a result of interbreeding.

Natural selection. The process by which different traits become more or less frequent in a population, due to consistent effects on survival or reproduction of individuals that express those traits.

Outbreeding depression. Loss of **fitness** that results from matings between genetically divergent individuals. Outbreeding depression can occur through two different mechanisms: dilution of local adaptation (extrinsic outbreeding depression) or breakdown of coadapted gene complexes (intrinsic outbreeding depression). In the former, fitness loss typically occurs in the ***F_1*** generation; in the latter, it often will not occur until the ***F_2*** or later generations.

Phenotype. The observed state of an individual, as measured at one or more traits (e.g., age, size, number of fin rays, parasite load). The phenotype is the result of environmental variation combining with the **genotype** to affect phenotypic expression at one or more observable traits.

SNP. For *single nucleotide polymorphism*. A type of DNA variation in which, for a specific location in the genome, individuals differ by a single base pair in their DNA sequence. Most SNPs involve just two different base pairs, each representing a different **allele**. More than 1 million SNPs have been identified in the human genome.

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Appendix A: In-depth Material

Appendix A provides a more in-depth treatment of material discussed in the body of this report regarding the genetic effects of fish culture on wild fish populations. To aid the reader, to the extent feasible, this appendix uses the same headings found in the body, although not all sections there have corresponding entries here. We draw on several sources of information, mainly collected from experiences of 1) contained rearing of salmonid fishes to market size (fish farming); 2) rearing and intentional releases of salmonid fishes for stock augmentation, supplementation, or sea ranching; 3) captive breeding programs for endangered or at risk salmonid fishes; and 4) farming and sea ranching of marine fish species. All of these areas have been subject to recent reviews.

Sources of Information

Genetic Impacts of Escaped Farmed Atlantic Salmon (*Salmo salar*) on Wild Salmonid Populations

Ferguson, A., I. A. Fleming, K. Hindar, Ø. Skaala, P. McGinnity, T. Cross, and P. Prodöhl. 2007. Farm escapes. *In* E. Verspoor, L. Stradmeyer and J. L. Nielsen (eds.), The Atlantic salmon: Genetics, conservation, and management, p. 357–398. Blackwell Scientific Publishing, Oxford, UK.

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Genetic Impacts of Stock Enhancement and Sea Ranching

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Genetic Impacts of Aquaculture of Marine Species of Shellfish and Finfish

Bert, T. M. (ed.). 2007. Ecological and genetic implications of aquaculture activities. Kluwer Academic Publishers, Dordrecht, the Netherlands.

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Lessons Learned

General Lessons

Here we review the relevant information accumulated by the above references by formulating some general lessons learned from these activities. These might serve as starting points for discussion leading to the formulation of a regulatory policy for development of marine aquaculture.

Expect the unexpected

Virtually every new culture program presents a number of surprises and these unexpected events often can increase risks to natural populations. For example:

1. Diseases that are rare (and often undetected) in wild populations can become greatly magnified in fish farms or other dense concentrations of fish. This can happen in wild fish as well (VHS in the Great Lakes, for example).
2. Attempts to establish a broodstock based on a single target population can unintentionally incorporate individuals from other populations (Hedgecock et al. 2001).
3. Cultured populations can mature at unexpected times and seasons (Johnson and Pravecek 1996), as can wild populations.

4. Escaped farmed fish can lead to an increased rate of interspecific hybridization, through mechanisms that are poorly understood.
5. It is conceivable that escaped rainbow trout (*Oncorhynchus mykiss*) could help spread *Gyrodactylus salaris*, a freshwater parasite deadly to Atlantic salmon, to nearby rivers by moving between rivers through brackish water.

Program goals

Risks and benefits are best evaluated in the context of program goals. Points to consider are:

1. Risks to wild populations can be difficult to identify, detect, and measure. These assessments are more difficult in the absence of clear goal statements.
2. Similarly, potential benefits can only be evaluated in reference to stated goals.
3. If a program has multiple goals, it might not be possible to simultaneously achieve all of them.
4. Clearly articulated goals are also essential for program evaluation and adaptive management.

Adaptive management

Lessons about effects on natural populations are often slowly learned from experience.

1. The major risks of salmon enhancement programs were identified 2–3 decades ago (STOCS 1981, Ryman 1981 [fish gene pools], Hindar et al. 1991, Waples 1991). However, for many years these concerns had little effect on management, in part because of difficulties in making quantitative predictions about consequences of a specific program or activity. Recent studies (Berejikian and Ford 2004, Araki et al. 2008) provide an increasingly robust body of empirical evidence to demonstrate adverse effects on natural populations.
2. Additional issues are related to higher individual growth rate and other changes caused by the selective breeding programs and domestication during whole-life captivity.
3. Many of the effects of cultured fish on wild fish were not fully appreciated in salmon aquaculture despite demonstrated evidence for these effects.
4. Marking cultured fish often can be insufficient to detect effects when they are released or escape. Initiatives to mark farmed fish (and livestock) have been resisted because of expense, health and welfare issues to the animals, and uncertain benefits of marking.

Evidence for effects of farm fish on wild fish is important to obtain

The types of evidence most important to document include:

1. Baseline genetic and ecological characterization.
2. Genetic marks or physical tags on farm fish or other ways of accounting for escapes of farmed fish.

3. In the absence of this information, effects might not always be easily attributable to a particular factor.

Diversity of natural populations

The most reliable way to ensure long-term sustainability is to conserve a diverse array of natural populations or native races.

1. Ultimately, adaptation of natural populations to environmental variation is constrained by genetic variability in traits that affect fitness in the wild.
2. Most genes that influence fitness phenotypes have not been identified, and most such phenotypes are thought to be affected by several to many genes with small effect.
3. It is therefore crucial to conserve phenotypic diversity within and among natural populations.
4. One coarse but risk-averse approach is to conserve natural habitat quality (including minimizing opportunities for invasions by genetically modified organisms) so that natural populations can maintain sufficiently robust populations with adequate diversity to respond to future challenges.

Monitoring

An effective monitoring component is important but cannot compensate for the failure to implement risk-averse strategies.

1. Monitoring is designed to detect responses or effects but not necessarily to provide guidance on how to intervene.
2. The consequences of failure to implement risk-averse strategies cannot be overcome with monitoring alone, no matter how well designed.
3. A monitoring program will be most effective if designed and implemented at the outset of a production program and can be tailored to specific program requirements.

Lessons from Salmon Aquaculture

Escapes

In the marine environment, the most common rearing technologies consist of large nets or ponds capable of holding large numbers of fish above a certain body size. This technology is “open” in the sense that it allows communication with the outside world with respect to escaped fish, fish disease agents, and organic matter (fish feed and feces). Escaped farm fish is the primary concern when considering genetic impacts on wild populations, but fish disease agents can also be important, as they can cause changes in population size or selection regimes of wild populations.

Fish can escape from the net pens for various reasons (Jensen et al. 2010). They can be lost during routine handling operations such as net changing or harvest. They can escape through holes in the nets or containment devices made by human error (boats, propellers, etc.) or by predators attacking the nets. They can escape in big accidents where the nets themselves or the structures holding the nets are broken down by ice, severe storms, or during towing operations. Onshore ponds can be flooded during storms. Several fish-farming countries keep records of the number of fish escaping from containment and some records also keep track of causes of escape (Thorstad et al. 2008). Finally, fish can escape that are too small for the mesh sizes in the net pens; their number is poorly known.

Better control of fish and water is offered by tank rearing in freshwater hatcheries and production facilities. Both inflowing and outflowing water can be controlled, and total control of fish is achievable, although not necessarily achieved in practice. Escapes have occurred from hatcheries and tank rearing facilities, but are often easier to detect and can be more localized. In sum, escapes into the environment of farm fish can occur at all life stages and times of the year.

Recapture of escaped fish

Efforts to recapture escaped farm fish have had limited success. Points to consider are:

1. Large-scale escapes occur during bad weather conditions when recapture might be impossible.
2. Farmed fish can spread rapidly after they escape (Skilbrei et al. 2010), so an immediate response can be essential to the success of recapture efforts. This can be particularly important if (as has occurred in Norway) escaped fish have recently been medicated and are thus not suitable for human consumption.
3. Some escapes can go undetected by inadequate technology.
4. Recapture techniques are not often scaled to the necessary capacity and immediacy (e.g., how to catch the biggest escape events with up to 500,000 1–3 kg salmon on short notice).
5. In small populations having a high incidence of farm escapes, recapture can be affected by weirs at the river mouth or by selective fishing (for farm escapes) in the river, with obligatory release of accidentally caught wild fish.

Postescape survival

Captively bred fish can disperse over large areas. The intentional transfer of fish and fish eggs over distances that are well beyond the species' dispersal capability is leading toward homogenization of fish communities (Rahel 2000, Naylor et al. 2005) and potentially also affecting the genetic structure of species (Ryman 1981, STOCS 1981, Hindar et al. 1991). Apart from this, aquaculture programs can increase the dispersal rates and dispersal distances of fish populations. Hatchery-produced fish can (but not always) stray more than wild fish (Quinn 1993, Jonsson and Jonsson 2006), depending on the life stage and location of release. An experiment comparing farm smolts and local wild smolts released into a river mouth showed a higher straying rate in the farm fish (Jonsson and Jonsson 2006).

Fish escaping from fish farms can show very high straying rates. When farm salmon escape during the time of the year when natural smolts and adults migrate (late spring and summer), these escapes tend to migrate into rivers in the vicinity of the escape site (Hansen et al.

1987, Youngson et al. 1997, Whoriskey and Carr 2001). Autumn and winter escapes of farm salmon show a much wider dispersal (Hansen et al. 1987, Hansen 2006), being found in rivers located several hundred miles away. Fish escaping upon attainment of sexual maturity show a more limited dispersal and can be easier to recapture (Chittenden et al. 2011).

Marking

Adequate marking and monitoring are necessary to detect fish that do escape.

1. Many aquaculture operations operate on thin economic margins, which can limit their ability to devote resources to monitoring and detection of escaped fish.
2. Activities to help reduce risk of aquaculture production to wild fish should become an integral part of an aquaculture program or operation during initial planning and inception to provide a clear and consistent framework for assessing benefit and risk.
3. Early freshwater escapes often show wild-type morphology upon recapture.
4. Early seawater escapes (at smolt stage) show sea-ranched-type morphology.
5. Eggs and alevins of farm female origin could be identified by color pigment differences when farm fish were fed canthaxanthin as a colorant, but are harder to distinguish when farm fish are fed synthetic astaxanthin, which is the natural pigment (Lura and Sægrov 1991a, 1991b).
6. DNA microsatellite markers might not be reliable in distinguishing wild from farm salmon, as the variability among farm strains and wild populations make detection of origin (among potentially many different ones) difficult.
7. Microsatellite markers can be effective following large-scale escapes from a single farm/cohort (Glover et al. 2008), and in experimental setups where parents (escaped or wild) and their offspring are sampled.
8. By searching among 4,500 single nucleotide polymorphisms (SNPs) in wild populations and farmed strains of Atlantic salmon, Karlsson et al. (2011) were able to find a diagnostic set of 60 SNPs for identifying group membership (wild or farmed) in spite of considerable genetic variation among wild populations and among farmed strains.

Reproduction of escaped individuals

Reproductive capabilities of captively bred fish in the wild can vary widely. Experiments with releases of captively bred and reared salmonids at various life stages from eggs, parr, and smolt into the river to post-smolts and subadults released from oceanic sites show that the earlier life stages have better reproductive performance in the wild (Fleming et al. 1996, Einum and Fleming 1997). This suggests a within-generation, environmental effect of captive rearing.

Number of fish escaping and the proportion they make up in wild spawning populations do not necessarily decrease over time.

Official records of the number of farm fish escaping from Norwegian aquaculture suggest a decline in the relative number of farm fish escaping during 1993–2007 (Directorate of Fisheries 2012). This statement holds for either of the two species which have been farmed on a large scale during this period, Atlantic salmon (Figure A-1) and rainbow trout (Figure A-2). For Atlantic salmon, the trend is even stronger if unofficial escape records for the years 1988–1992 are included.

In comparison, a relatively new species in fish farming, Atlantic cod (*Gadus morhua*), suggests a similar decreasing temporal trend in number of fish escaping per ton produced. However, this species currently has a much higher number of fish escaping per ton produced (Figure A-3). For Atlantic cod, it is suggested that the higher rate of escape from net pens is caused by two behaviors not seen in salmonids; active biting at meshes in the nets, and active

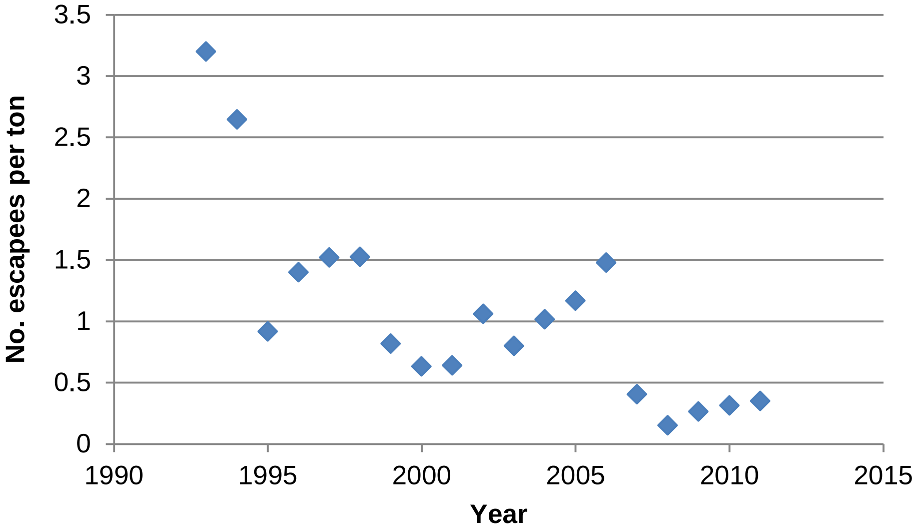

Figure A-1. Farm Atlantic salmon escapes, official Norwegian records.

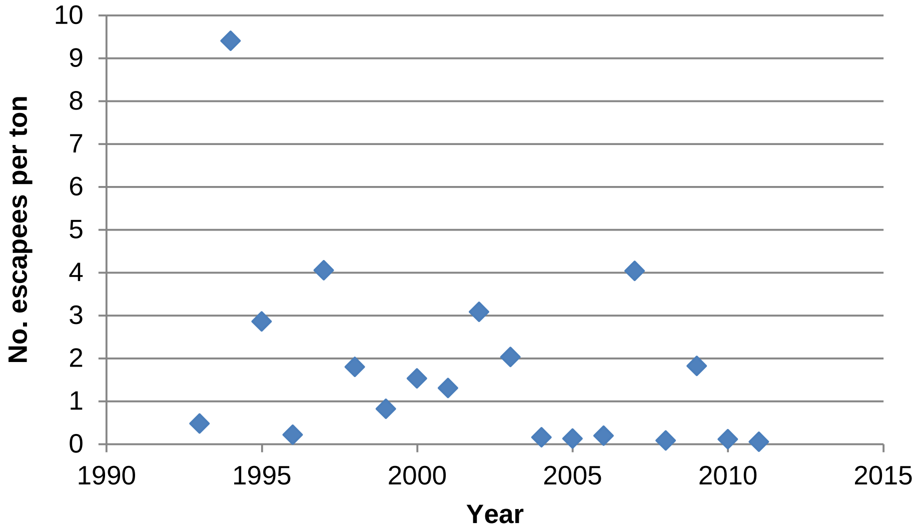

Figure A-2. Farm rainbow trout escapes, official Norwegian records.

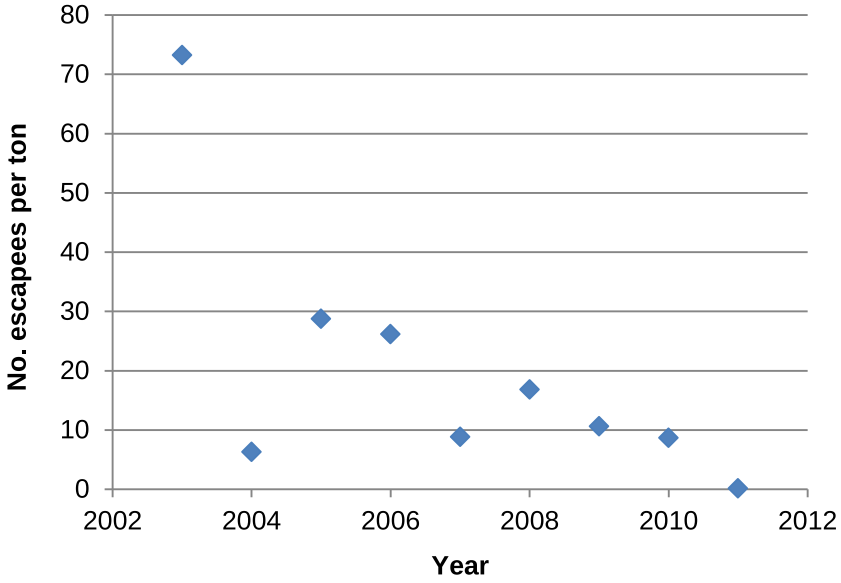

Figure A-3. Farm Atlantic cod escapes, official Norwegian records.

searching for holes by individual fish (Hansen et al. 2009). Moreover, being pelagic spawners, cod can spawn in net pens and thereby produce unknown numbers of escaped individuals that are not recorded in program statistics. These observations suggest that new species in aquaculture will pose novel challenges for containment.

Even though fish farmers have improved their ability to contain fish in net pens, especially following new technical standards from 2004 (Jensen et al. 2010), the total number escaping does not necessarily decrease. The main reason for this is the rapid increase in production itself. Norwegian official records indicate little change in the number of escaped Atlantic salmon from fish farms during 1993–2007, being 250,000 to 900,000 fish annually (Figure A-4). Unofficial records indicated that even higher numbers of fish escaped annually from farms during 1988–1992; an interdepartmental group estimated 1.6 million fish escaped annually. After a record low number in 2008 (111,000), the numbers of farmed salmon escaping have increased again with 225,000, 291,000, and 365,000, respectively, for 2009–2011. High numbers of escaped farm salmon have also been recorded elsewhere, including Scotland, Ireland, western United States, and Canada. It has been estimated that more than 396,000 Atlantic salmon escaped into the Pacific Ocean from farms in British Columbia, Canada, from 1991 to 2001 (Gaudet 2002). More than 595,000 fish were accidentally released from fish farms in Washington State from 1996 to 1998 (Noakes et al. 2000). Escaped farm Atlantic salmon have been found as far north as the Bering Sea (Brodeur and Busby 1998).

Recent developments in fish farming technology have led to increased use of very large cages in salmon farms, with one cage (net pen) holding 700 tons of fish and one farm holding 3,500 tons of fish (e.g., 2 million fish at 1.75 kg) or more. For comparison, the 2008 estimate of prefishery abundance of all wild Atlantic salmon in the North Atlantic was 3.5 million fish (ICES 2009). This suggests that a single catastrophic event, where all of the fish from a big cage escape, can have a large effect on natural populations.

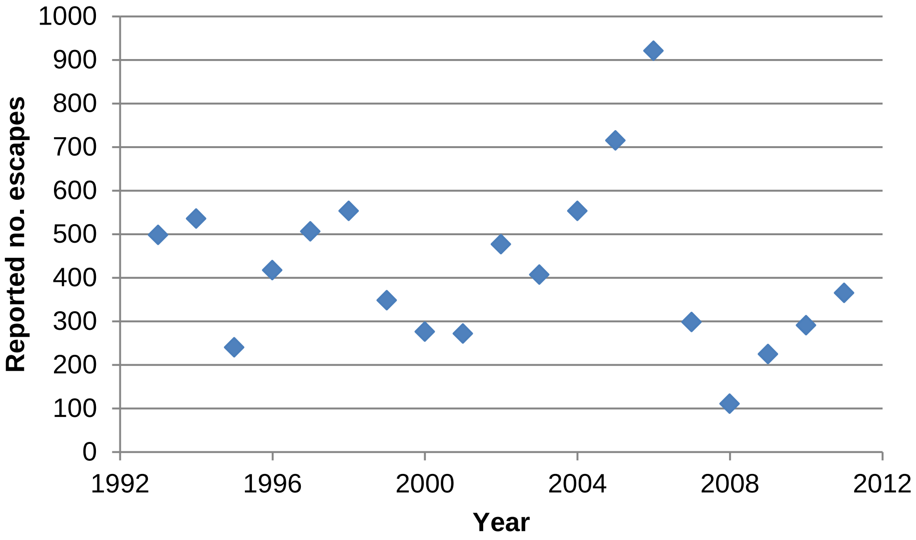

Figure A-4. Reported number (in 1,000s) of escaped farm Atlantic salmon by year from hatcheries and grow-out farms in Norway. (Adapted from Directorate of Fisheries 2012.)

Estimates of escaped farm fish in the catches of wild fish have been made from classification of farm and wild fish based on external morphology and scale characters (Fiske et al. 2006). The proportions of escaped farm Atlantic salmon in wild Norwegian spawning populations were highest around 1990 (>30% on average, among fish caught near spawning) and somewhat lower during recent years (Fiske et al. 2006 and Figure A-5). Estimates of the proportions of escaped farm salmon vary over space and time, being higher on the outer coast than in the rivers and in autumn catches than in summer catches within rivers (Figure A-5). Proportions can be particularly high in rivers near strong concentrations of fish farms (Fiske et al. 2006), and especially so in populations that have declined for reasons that might or might not have to do with fish farming activities.

On the east coast of North America, escaped salmon outnumbered wild fish by as much as 10 to 1 in some rivers. For example, after massive escapes in southwest New Brunswick, Canada in 1994, 1,200 farm salmon were counted entering the province's Magaguadavic River compared to 137 wild fish (Atlantic Salmon Federation 2004). Escaped farm salmon of at least partial European origin (as determined by DNA analyses) have also been found in the Magaguadavic River even though only farm salmon of local origin are licensed for use in Canada (Atlantic Salmon Federation 2005). A recent overview for eastern North America stated that escaped farmed salmon had been found in 54 of 62 rivers investigated within a 300 km radius of the aquaculture industry since 1984, including 11 rivers that contain endangered salmon populations (Morris et al. 2008). On average, escaped farmed salmon made up 9.2% of adult salmon entering rivers in this area. In Norway, there is some evidence that the actual number of escaped farm salmon can exceed the reported number considerably, at least in some years, but the extent of this disparity is uncertain in most cases because of the low numbers of marked farm fish. New technical standards for sea cages and initiatives to produce larger smolts for release in sea cages have likely improved this situation in recent years.

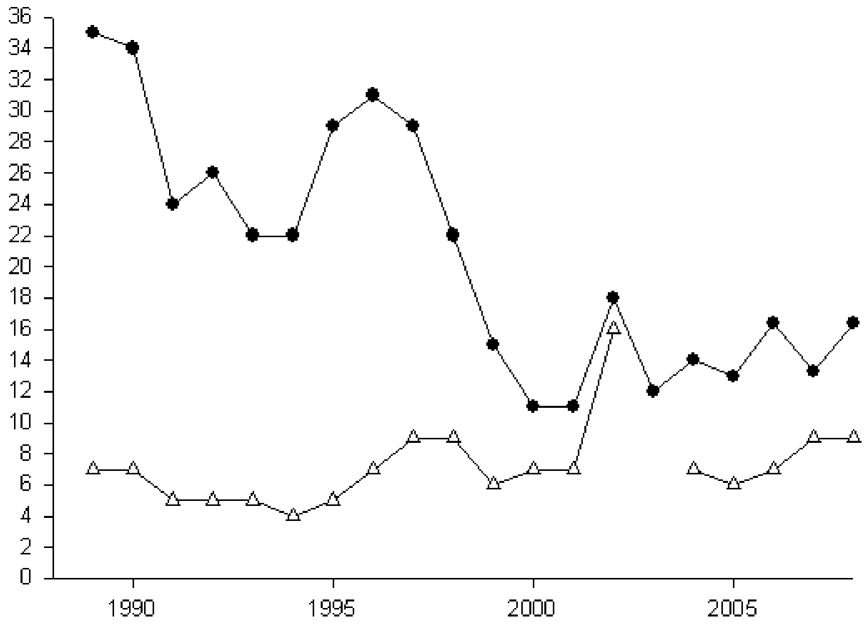

Figure A-5. Percent farm escaped Atlantic salmon (unweighted averages) by year in river fishing during summer (Δ) and in experimental catches during autumn shortly before spawning (●) in Norwegian rivers. (Adapted from Hansen et al. 2007 and SACASM 2009.)

Joint Lessons from Salmon Aquaculture and Hatcheries

Long-term sustainability

Little evidence exists to show that artificial propagation can improve the long-term sustainability of natural populations. Artificial propagation can alleviate short-term risk of extinction, and there are several examples involving long-term captive culture of fish which demonstrate that artificial propagation can maintain broodstocks for several generations in protective culture. This proven track record indicates that artificial propagation can help to preserve valuable natural genetic resources of a population at risk of extinction for short periods while causes for decline are being addressed. However, how long such programs can perpetuate these populations is not known for certain and, even if very long-term propagation were feasible, it is not known how long a population can be propagated in protective culture and retain the ability to be viable in the wild environment (Waples and Drake 2004). Furthermore, even if artificial propagation could produce more naturally reproducing adults than those produced naturally, it is not yet known whether artificial propagation would result in a net benefit to a natural population. As stated elsewhere, unless supplementation of wild production is a direct goal of aquaculture production, benefits to wild populations from aquaculture programs can often be limited to relaxed harvest pressure on wild fish due to affordable aquaculture products on the market.

Genetic changes associated with artificial propagation are inevitable (Table A-1). Artificial selection programs intentionally select for genetic changes in traits that are of economic importance. Some of these traits are likely also important performance traits in the wild. Other captive breeding programs do not intentionally select for altered performance in the

Table A-1. Compilation of genetic changes between cultured and wild salmonid populations in phenotypic traits. Sources: Hindar et al. 1991 and references therein, their Table 1; Ferguson et al. 2007; Fraser 2008 and references therein, his Table 5.

Trait	**Change in cultured populations**
Egg size	Lower in cultured populations (opposite seen)
Egg survival	Equal or lower in cultured populations
Juvenile survival	Lower in cultured populations (opposite seen)
Juvenile physical fitness	Lower in cultured populations
Body morphology	Variable results
Territorial behavior	Weaker in cultured populations
Aggression	Variable results between studies and species
Dominance	Higher in cultured populations
Concealment behavior	Lower in cultured populations
Wariness	Lower in cultured populations
Predator response/avoidance	Lower in cultured populations
Juvenile growth rate	Higher in cultured populations
Precocious male maturity	Lower in cultured populations
Age (and size) at smoltification	Lower to equal in cultured populations
Ocean survival	Lower in cultured populations
Ocean and river recapture rate	Lower in cultured populations
Seawater growth rate	Higher in cultured populations
Adult body size	Variable results
Adult run timing	Variable results
Age at sexual maturity	Higher in selected cultured populations
River recapture rate	Lower in cultured populations
Straying rate	Higher in cultured populations
Return rate	Lower in cultured populations
Male spawning performance	Lower in cultured populations (opposite seen)
Female spawning performance	Lower in cultured populations
Disease resistance	Variable results (dependent on origin)
Interspecific hybridization	Increases among offspring of cultured fish

wild but can inadvertently do so by the fish being adapted by natural selection to the captive environment, by sampling only a limited genetic variation when creating and maintaining the captive broodstock, by excluding natural selection to a natural environment, and by excluding sexual selection during spawning. Moreover, some of these programs introduce genetic differences in performance traits between captively bred and local wild fish by basing the captive stock or stocks on fish of nonlocal origin.

Farm salmon differ genetically from wild salmon in morphological, behavioral, and ecological traits that are affected by domestication, even where the origin is local. Fleming and Einum (1997) compared a seventh generation strain of farm salmon in Norway with its principal founder population from the wild—the River Namsen population. The fish were reared in a common environment and compared for several fitness-related traits. Farm salmon showed more robust bodies and smaller fins. Farm juveniles were more aggressive in a tank environment, but wild juveniles dominated in a stream-like environment. Farm juveniles were also more risk-prone, reappearing from cover soon after a simulated predator attack (see also Johnsson et al.

2001). Growth performance in farm juveniles was higher than in wild juveniles (see also Thodesen et al. 1999).

Similar results were obtained in comparisons between another strain of farm salmon and two wild populations (Einum and Fleming 1997). These results suggest that farming generates rapid genetic change due to genetic drift and intentional and unintentional selection in culture, and that some changes involve important fitness-related traits. The higher growth rate of farm salmon also carries over in the wild where farm and farm-by-wild offspring have shown higher growth rates than offspring resulting from wild-by-wild crosses (Einum and Fleming 1997, McGinnity et al. 1997, 2003, Fleming et al. 2000) (Figure A-6).

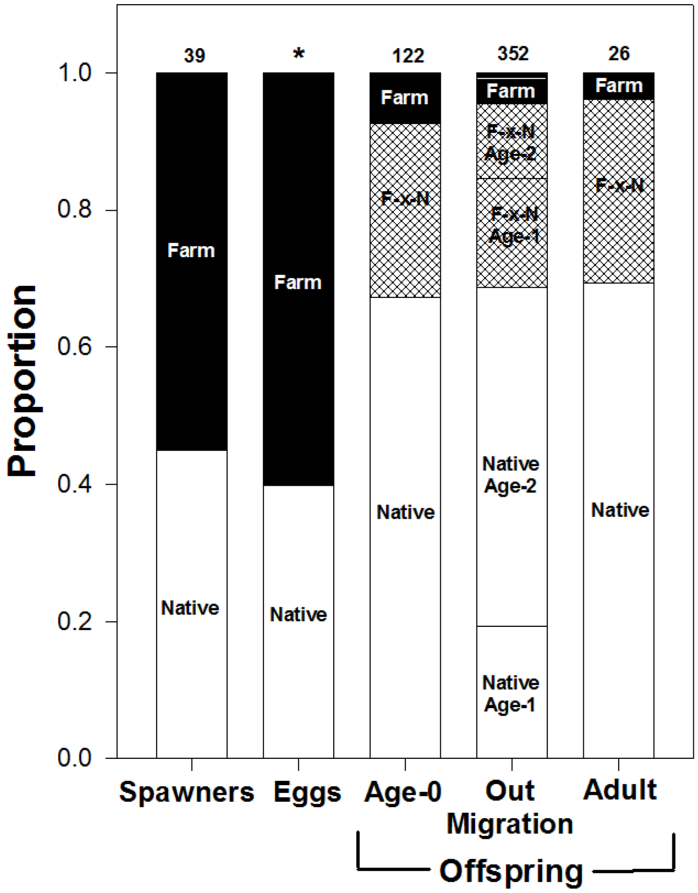

Figure A-6. Changes in the proportional constitution of the Atlantic salmon population in the River Imsa following the release of native wild and farm spawners. The number above each bar represents either the total population size (spawners and adult offspring) or the sample size examined at each life stage (age 0 and outmigration). Two age groups of outmigrants existed, age-1 and age-2, and are stacked on top of each other for each offspring type. In asterisk (*) bar, potential egg deposition was 19,443 for native females and 29,388 for farm females. Black bars = farm offspring, white bars = native offspring, and gray bars = hybrid offspring. (Adapted with permission from Fleming et al. 2000, copyright The Royal Society.)

Fitness

Fitness in the wild of captively bred fish declines with number of generations in captivity. In steelhead (*Oncorhynchus mykiss*), Araki et al. (2007) measured lifetime reproductive success of the first two generations that were reared in captivity and bred in the wild after they were released. By reconstructing a pedigree with DNA-microsatellite markers, they showed that genetic effects of domestication reduced subsequent reproductive capabilities by approximately 40% per captive-reared generation when fish are moved to natural environments.

Araki and coworkers also conducted a meta-analysis to compare their data with those from other hatchery stocks with known number of generations in hatcheries. This analysis suggested an exponentially declining curve at a 37.5% fitness decline per generation of captive rearing. Araki et al. (2007) argued that this continuing decline with generations in captivity supports genetic effects as the primary cause, as purely environmental effects should not accumulate over generations. There is also a tendency for nonlocal hatchery broodstocks to have lower relative fitness than locally derived stocks (Araki et al. 2008).

Genetic risks

Genetic risks associated with fish culture can be reduced but not eliminated entirely. The primary reason that genetic risks of cultured fish on wild fish cannot be completely avoided is because the risk of escape in open systems and even some closed recirculating systems is always present, if even at low levels. Reproductive containment is not 100% effective. Because broodstock selection and collection cannot retrieve the entire genetic and phenotypic repertoire of a wild population, even if sampling is random and reasonably representative, genetic divergence is assured. Furthermore, the characteristics of the culture environment intended to maximize in-culture survival to market size or reproduction impose distinctly different selective pressures on cultured fish than on wild fish. The opportunity for genetic change from domestication can be rapid in such situations (Waples and Drake 2004). Finally, not all risks can be minimized simultaneously. For example, efforts to minimize phenotypic divergence from wild fish (e.g., in body conformation or reproductive timing) can increase the likelihood of ecological interactions, including interbreeding, when escapes do occur.

Direct effects

The consequences for natural populations of interbreeding with cultured fish depend on a variety of factors. Escapes of cultured fish into the wild are best approached as an experimental perturbation with the potential for adverse genetic as well as ecological effects on wild populations. For most species, the life history consequences of interbreeding between divergent cultured and wild populations are poorly known. Life history characters in most species studied to date indicate that many of these characters affect reproductive fitness in the wild, are influenced by environmental as well as genetic factors, are often correlated with other characters in complex ways that reflect trade-offs in performance at different life stages or in different ecological activities, and often have “plastic” expression (i.e., the phenotype produced by a given genotype depends on the environmental context, so that often no single genotype is superior in all environmental settings).

Given these uncertainties, with potentially severe consequences for natural populations, a risk-averse approach might have the following elements: 1) potential for escapes and their long-term genetic consequences are considered during the early stages of program planning; 2) if concerns for these consequences are substantial, a number of remedial actions can be considered; 3) production could be initiated at a reasonably small scale and conducted in areas that permit rigorous monitoring of potential recipient wild populations; and 4) regular evaluation and monitoring can facilitate prompt remedial action (Hard 1995b).

Indirect effects

Reduced population size and other indirect genetic effects have been associated with fish culture. Indirect genetic effects do not require interbreeding or exchange of genes; rather, they occur because of altered selective regimes (which can affect local adaptations) or reductions in population size (which also reduces the effective population size [N_e] and can erode genetic variability). These indirect genetic effects can occur between species that usually do not meet, such as between Atlantic salmon and native species in the Pacific Ocean and between rainbow trout and native species in the Atlantic Ocean, as well as between farmed and wild populations of the same or closely related species.

In a meta-analysis, contrasting the marine survival and abundance of wild salmonids in areas of salmon farming with nearby wild populations in areas without salmon farming, Ford and Myers (2008) demonstrated a significantly lower marine survival or abundance in wild populations near fish farms. Their analyses included effects of Atlantic salmon farming on wild Atlantic salmon in Scotland, Ireland, and Canada; on wild sea-run brown trout (*Salmo trutta*) in Ireland; and on wild coho salmon (*Oncorhynchus kisutch*), pink salmon (*O. gorbuscha*), and chum (*O. keta*) salmon in Canada. In many cases, the reductions in survival or abundance of wild fish were greater than 50%. The estimates of the mean effect in the meta-analysis were significant and negative, suggesting that salmon farming has reduced the survival of wild salmon and trout in many populations and countries. In another analysis, Vøllestad et al. (2009) found stronger reductions in the catches of wild Atlantic salmon in western Scottish and western Norwegian rivers than in other regions of Scotland and Norway, and implicated intense fish farming as one possible cause. A more detailed analysis of one-sea-winter Atlantic salmon in Norway suggested that the negative temporal trend of returns was stronger in the presence of salmon farms on the migration route of the smolts in coastal/fjord areas (Otero et al. 2011).

Ford and Myers (2008) did not explicitly study the mechanisms by which salmon farming affects wild populations, but noted that stronger effects on wild populations were found in the Atlantic than in the Pacific (British Columbia, Canada) and that one possible cause could be interbreeding with escaped farm salmon, in addition to other impacts. Either of the whole-river experiments described below suggests that interbreeding between escaped farm salmon and wild salmon can reduce population size of the wild population (Fleming et al. 2000, McGinnity et al. 2003). A reduction in population size of the wild population can also be caused by pathogens and parasites, competition and displacement, predation, interspecific hybridization, or by any combination of these.

Long-term studies of stock and recruitment in steelhead populations suggest how different levels of hatchery-produced individuals in the population affect spawner-recruit relationships and population sizes (Chilcote 2003). Based on studies in 12 steelhead populations in Oregon, he found a negative effect on recruitment of increasing proportions of hatchery-produced individuals in the spawning population. At 50% hatchery steelhead among the spawners, the mean productivity of the population (measured as the number of recruits per spawner) was reduced by 63% relative to the same number of wild spawners (Chilcote 2003).

Effects caused by introduced pathogens and parasites can be temporary epidemics or long-lasting population reductions and even extinction of local wild populations. Effects on wild populations can be severe even for endemic parasites, such as salmon lice (*Lepeophtheirus salmonis*). Outbreaks of salmon lice on wild fish are connected with louse density in fish farms and sometimes with lice on escaped farmed fish (Bjørn et al. 2009, Revie et al. 2009). Consequences for wild fish include curtailed life history of anadromous brown (sea) trout, reduced individual growth rate, reduced individual and population fecundity, and increased seawater mortality. Atlantic salmon farming in the Pacific Ocean has been implicated in increased mortality of endemic pink salmon. Comparison of populations exposed to fish farms with populations not exposed to them in British Columbia have shown that population growth rate was lower for the exposed populations during sea lice infestations than for exposed populations before the infestations or for the unexposed populations (Krkosek et al. 2007).

Salmon lice have also been shown to transfer virus (infectious salmon anemia, ISA) between fish and have recently been shown to exhibit multiple resistance to therapeutics used in European fish farms. In some fish farming areas, the number of farm salmon and trout is now so great that even strong and efficient control of sea lice in the farms cannot prevent lethal infections of wild smolts by sea lice when migrating through the area. This has been indicated in modeling studies (Heuch and Mo 2001, Figure A-7 and Figure A-8) and more recently demonstrated by empirical and experimental studies in intense fish farming areas (Bjørn et al. 2009). Direct experimental evidence that sea lice–induced mortality can be substantial comes from releases of hatchery-reared smolts in Ireland, where emamectin-treated (providing lice protection) smolts showed increased survival and were 1.8 times as likely to return as adults compared to control fish (Gargan et al. 2012)

Two barriers limit natural spread of pathogens in anadromous species in the wild: 1) the change of habitat and associated osmotic and physiological transitions from freshwater to salt water and back again, and 2) homing behavior of anadromous salmonids. Both of these barriers can be broken down by translocation, genetic modification during domestication, and the deliberate and unintentional releases of salmonids by humans (Bakke and Harris 1998).

One example related to salmon translocations is introduction of the parasite *Gyrodactylus salaris* to Atlantic salmon populations in 40 Norwegian rivers. This parasite does not appear to be native to Norway, and was probably first imported to a central hatchery with salmonid eggs or juveniles from the Baltic in the 1970s (Johnsen and Jensen 1991). From the central hatchery, the parasite spread to other hatcheries and rivers with intentional transport and releases for stock enhancement and with aquaculture escapes. Long-term reductions in population size of affected Atlantic salmon populations average 85% (Johnsen et al. 1999). Support for the hypothesis that *G. salaris* is an introduced species has come from experiments showing higher resistance to the parasite in Baltic than in Norwegian Atlantic salmon (Bakke 1991) and molecular genetic studies (Bakke et al. 2007). Such long-distance translocations of cultured fish can result in

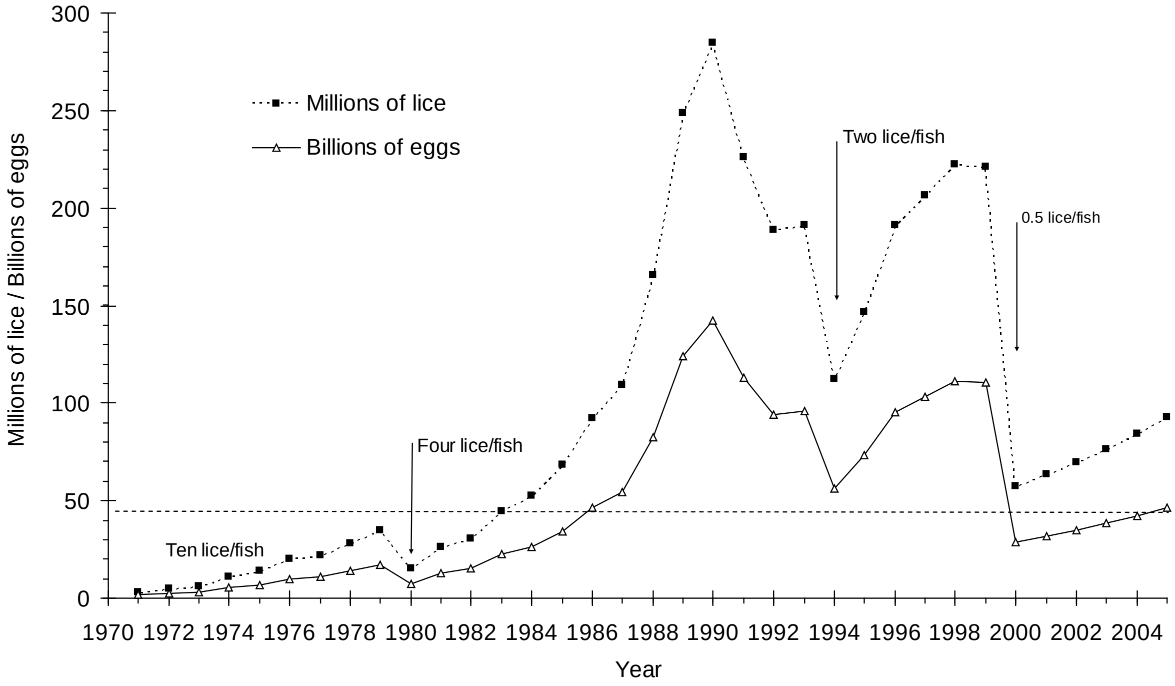

Figure A-7. Simulation of the number of sea lice produced in Norwegian waters as a consequence of growth in the salmon farming industry. Arrows indicate target levels of the number of sea lice per fish in fish farms. (Reprinted with permission from Heuch and Mo 2001, copyright Inter-Research.)

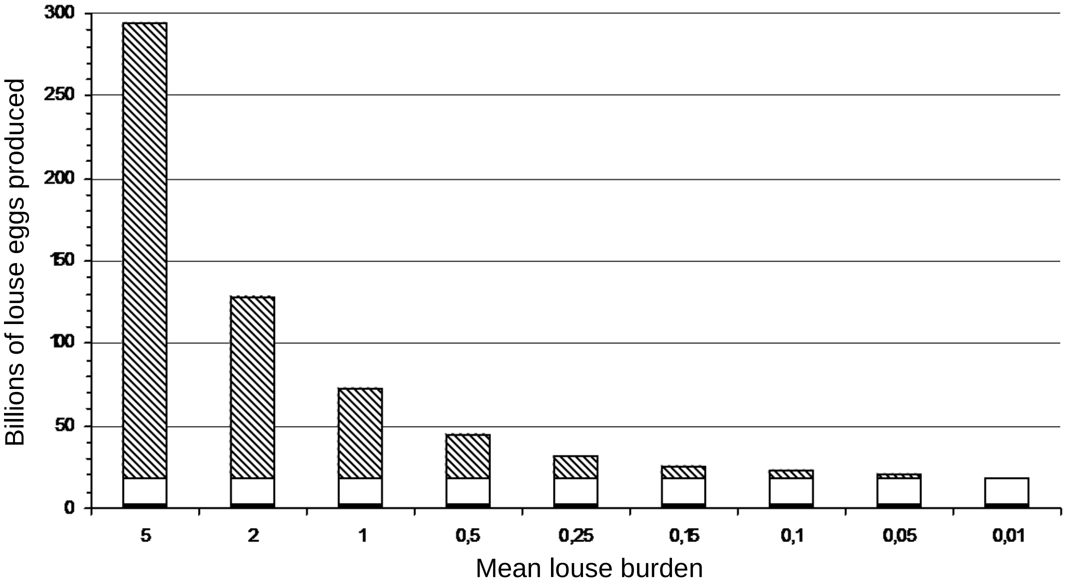

Figure A-8. Simulation of the infection pressure of sea lice at different target levels of the number of sea lice per fish in fish farms. Black indicates wild salmonids, white indicates salmonids escaped from farms, and diagonally lined indicates farmed salmonids. (Reprinted with permission from Heuch and Mo 2001, copyright Inter-Research.)

translocations of pathogens that might reduce wild population sizes and thus have indirect genetic effects on wild fitness.

Dispersal of cultured salmonids and products are also heavily implicated in the spread of whirling disease *Myxosoma cerebralis* (Modin 1998, Bartholomew and Reno 2002), a sporozoan disease that can affect many anadromous salmonid species, and furunculosis, caused by the bacterium *Aeromonas salmonicida salmonicida.* Furunculosis was introduced to Norwegian fish farms in 1985 with smolts from Scotland, and spread rapidly from the first few infected farms to reach 550 fish farms (70% of the total) by the end of 1992 (Johnsen and Jensen 1994). More than 250,000 salmon escaping during 1988–1989 were from farms infected with furunculosis. The disease was found among spawning salmon the following autumn, first among farm escapes and later also among wild fish. By 1992 furunculosis had been registered in 74 Norwegian rivers. In four rivers, the disease reached epidemic proportions (Johnsen and Jensen 1994). Long-term effects of furunculosis do not seem to be as strong as for some parasitic diseases.

Some viral diseases such as ISA often lead to high mortality and were previously not considered to spread easily between populations. ISA was detected in Norwegian fish farms in 1984, and subsequently in Canada (1996), Scotland (1999), and the United States (2001). In 1999 clinical ISA was detected for the first time in wild Atlantic salmon and in escaped farmed Atlantic salmon entering the same river (Lovely et al. 1999, Bouchard et al. 1999). Three wild broodstock fish in the Magaguadavic River died from the disease. They were found after cohabiting a trap in the river with escaped farm salmon, some of which were infected with ISA. This suggests 1) that wild salmon are susceptible to ISA and 2) if it was transmitted to the wild fish from infected farmed fish, then infected farmed fish do pose a risk to wild salmon (Carr and Whoriskey 2002).

Spawning interactions potentially affecting population size include destruction of nests of early spawning wild females by later spawning farm females (Lura and Sægrov 1991b). In New Zealand, spring-spawning rainbow trout have been found to destroy the nests of autumn-spawning brown trout (both species are introduced). Another type of spawning interaction is interspecific hybridization. Hybridization rates are often elevated where species meet after human translocation and also increase following escapes of farmed fish (Youngson et al. 1993, Hindar and Balstad 1994). When interspecific hybrids survive but are infertile, the effect is an increase in competition for food and space by intermediate phenotypes and a likely reduction of population size of the species providing eggs and potentially both parental species.

All of the above-mentioned factors that reduce population sizes also have the potential to alter selective regimes, for example, by favoring more disease-resistant individuals when new infectious agents are introduced, by favoring resident fish in the presence of increased mortality for migrants, or by favoring individuals that compete effectively in the presence of faster-growing, aggressive offspring of escaped farmed fish. Moreover, high numbers of escaped farmed fish at certain life stages can lead to altered selection from density itself, for example, during juvenile stages where fish escape directly into juvenile rearing habitats. To our knowledge, little research has been done in this field, the most relevant being empirical and experimental studies of character displacement and character release in fishes (Robinson and Wilson 1994, Schluter 1994), and the concept of limiting similarity of coexisting species (MacArthur and Levins 1967). At the molecular level, changes in MHC I genetic variation have been implicated in Irish brown trout following the buildup of salmon aquaculture (Coughlan et al. 2006).

Genetic effects

Genetic effects have occurred in native populations following invasion of intentionally or accidentally released cultured fish. In an earlier review, Waples (1991) grouped genetic effects of fish releases into two classes: direct and indirect. Direct genetic effects are those caused by interbreeding and include changes in allele frequencies, in genetic diversity within and between populations, and in adaptive traits. Indirect genetic effects can be caused by any factor that causes a reduction in population size of the wild population (thereby increasing genetic drift) or alters the selective regime experienced by the wild population. They were dealt with in the previous subsection.

At the same time, a review of the literature on the genetic effects following releases of nonnative salmonid populations (Hindar et al. 1991) provided two broad conclusions:

1. The genetic effects of (intentionally or accidentally) released salmonids on natural populations are typically unpredictable; they vary from no detectable effect (on genetic constitution) to complete introgression or displacement.
2. Where genetic effects on performance traits have been detected, they appear always to be negative in comparison with the unaffected native populations. For example, reduced total population size has been observed following introductions of exogenous populations, and also reduced performance in a number of traits, which can explain such population declines (e.g., lower survival in fresh and sea water).

More recent experience from salmon farming has indicated genetic effects of escaped farm fish in empirical and experimental studies. Molecular genetic changes were demonstrated in the wild salmon population in the Glenarm River, Northern Ireland, resulting from the spawning of escaped farmed salmon (Crozier 1993). A follow-up sample was taken from the river 7 years later (Crozier 2000). Overall genetic variation across eight allozyme loci indicated that the wild population remained significantly different from the preescape population and from the immediate postescape population. The presence of an allele not having been previously detected in this population suggested that further incursion(s) of farmed salmon might have taken place. Molecular genetic changes to native populations, following escapes of farm salmon at the juvenile and adult stages, were also shown in three rivers in Ireland by using mitochondrial DNA and nuclear DNA markers (Clifford et al. 1998a, 1998b).

Analyses of historical and contemporary scale samples in Norwegian rivers using DNA microsatellites demonstrated that genetic changes had occurred in three rivers (the Opo, Vosso, and Eio, Skaala et al. 2006). These changes could be tied to intrusion of escaped farm salmon in the Vosso (Sægrov et al. 1997) and likely also in the other two rivers. No changes in the genetic profiles were found in four other rivers (the Namsen, Etne, Granvin, and Hå), even if expected in two of them. Small reductions in genetic distances among populations were observed in the contemporary samples compared with the historical samples, indicating a reduction in population differentiation over time, likely due to immigration of escaped farmed salmon. A recent, enlarged study of microsatellite diversity over 3 decades in Atlantic salmon populations in 21

Norwegian rivers corroborated these findings by showing highly significant genetic changes over time in 4 of the populations and a significant reduction in spatial genetic structure over time (Glover et al. 2012).

Experimental demonstration that escaped farm Atlantic salmon affect wild populations comes from controlled, whole-river experiments in Norway and Ireland. In the River Imsa, Norway, a lifetime, natural experiment was carried out using fifth generation farm salmon spawners released together with local wild fish in the river (Fleming et al. 2000). In the Burrishoole River system in Ireland, eggs of artificially made crosses of first and second generation offspring of farm salmon, wild salmon, first and second generation hybrids, and first generation backcrosses were planted in the river (McGinnity et al. 1997, 2003). In both the Imsa and the Burrishoole experiments, lifetime reproductive success (spawning to adult return, or eggs to adult returns) was considerably lower in farm fish than in wild fish, and changes in the wild population were found that are likely deleterious to the population. Details of the Imsa experiment (Fleming et al. 2000) and the Burrishoole experiment (McGinnity et al. 1997, 2003), both here following the summary of Ferguson et al. (2007), are given in the two subheadings below.

The Imsa experiment—In the River Imsa in Norway, farm and native adult Atlantic salmon had similar prespawning migration patterns and nesting locations, though farm females spawned before native females. Both types of males began courting females shortly after release; however, native males were more active doing so and retained less of their testes unspawned. The findings from supplementary experiments in a controlled, artificial spawning arena paralleled those from the river, indicating that farm males were competitively and reproductively inferior, obtaining fewer spawnings and having 24% of the breeding success (i.e., number of live embryos parented) of native males. Farm females also showed a reproductive inferiority (e.g., fewer nests, lower egg survival), achieving just 32% of the breeding success of native females.

During September-October the following year, offspring from spawnings in the river were sampled by electrofishing the River Imsa. The proportion of farm to native genotypes had shifted dramatically from that at release (56% farm) to this stage, with farm genotypes now composing slightly less than 20% of the population (Figure A-6). Moreover, most of the farm genetic representation was in the form of hybrid offspring between farm females and wild males. Pure farm offspring comprised less than 8% of the 0+ parr.

Based on the breeding success in the arena experiment, the early survival of farm genotypes was estimated to be 70% that of native genotypes. Thereafter, there was no significant evidence of differential freshwater survival, as farm genotypes composed 18% of the smolt population. There were, however, indications of resource competition in freshwater, as there was considerable diet overlap among native, farm, and hybrid offspring. Moreover, the total production of smolts was 28% below that expected based on the potential egg deposition and the 16-year stock-recruitment relationship for the Imsa (Jonsson et al. 1998). For native females, smolt production was 31–32% below that expected in the absence of farm females. This effect can reflect competitive asymmetries, as native parr were smaller than farm and hybrid parr due to differences in growth rate and parental spawning dates. There were also indications of displacement of native parr further upstream. The approximate 30% depression in smolt production observed was the second largest in 16 years of records (Jonsson et al. 1998). Moreover, it occurred despite the absence of competition from older salmon cohorts and during a period that was favorable for smolt production, at least at a broad geographic scale.

As smolts, the offspring types showed distinct behavioral and life history differences. Farm smolts descended earlier and at a younger age than native smolts, with hybrids being intermediate. Hybrid smolts were also longer and heavier than native smolts, while farm smolts weighed less for a given length than their counterparts. Despite these differences, there were no significant differences in marine survival to maturity, the overall lifetime success of farm fish relative to wild fish being 16%. All adult recaptures were made in the coastal fishery or the River Imsa, and no fish were reported straying into other rivers. The mean age at maturity of hybrid salmon (3.4 years) was significantly less than that of native salmon (4.2 years) because of differences in their age at smolting and poor survival of native age-1 smolts.

The Burrishoole experiment—This experiment, comprising three cohorts (1993, 1994, 1998) of Atlantic salmon, was undertaken in the Burrishoole River system in western Ireland. This involved multiple families of the following seven groups: native wild (all cohorts), farm (all cohorts), F_1 hybrid wild × farm (male and female reciprocal groups, 1993 and 1994 cohorts), F_2 hybrid wild × farm (1998 cohort), BC_1 backcrosses to wild (1998 cohort), and BC_1 backcross to farm (1998 cohort) (Table A-2). As the aim of the experiment was to look at genetic differences without the confusion of behavioral differences, eggs and milt were stripped from mature adults and artificially fertilized (see McGinnity et al. 2003 for further details). Fertilized eggs were incubated to the eyed stage in the hatchery with cumulative mortalities being recorded. The highest egg mortality occurred in the F_2 hybrid group (median 68%), which was significantly higher than all other groups (e.g., wild 3%).

Table A-2. Lifetime successes of wild, farm, and hybrid groups of Atlantic salmon in the Burrishoole system, Ireland, averaged over several cohorts where available. Survival of the wild group is taken as 1.0. Where another group is not significantly different from the wild group, it is also given a value of 1.0. When significantly different, then the actual survival relative to the wild group is used. Data for marine survival of F_2 hybrids are not available and were set at 1.0. Data from McGinnity et al. 2003.

Group	Fertilization to eyed egg	Eyed egg to smolt[a]	Eyed egg to smolt[b]	Smolt to adult	Lifetime success[a]	Lifetime success[b]
Wild	1.00	1.00	1.00	1.00	1.00	1.00
BC_1W	1.00	0.89	1.00	1.00	0.89	1.00
F_1HyW	1.00	0.73	1.00	0.58	0.42	0.58
F_1HyF	0.87	0.50	0.63	0.61	0.27	0.33
F_2Hy	0.34	1.00	1.84	n.a.	(0.34)	(0.63)
BC_1F	1.00	0.79	1.59	0.39	0.31	0.62
Farm	0.79	0.41	0.76	0.07	0.02	0.04

[a] This assumes that displaced parr have the same survival as parr of the same group remaining in the experiment river, that is, that the river is not at its parr carrying capacity and spare habitat is available for displaced parr.
[b] This assumes that displaced parr emigrating from the experimental river do not survive, that is, that the river is at its parr carrying capacity.

Aliquots of each family were maintained in a hatchery tank until 11 months as a control on the field experiments. No significant differences in survival among groups were found to this age. However, given that total mortality was less than 10% under "protected" hatchery conditions, there was little opportunity for detectable differential survival. These hatchery controls serve to demonstrate that all groups were potentially equally viable and that the differential survival apparent in the wild was the result of genetic or maternal differences.

Farm salmon showed significantly lower representation than wild in the samples of 0+ parr of all three cohorts from the experiment river at the end of the first summer (Table A-2); "hybrids" (i.e., F_1 and F_2 hybrids and BC_1 backcrosses) were intermediate or not significantly different from wild fish. During the period from May 0+ to September 1+ (i.e., second year), the highest proportion of emigrant parr, taken in the experiment trap, was from the wild group and the lowest from the farm group, with "hybrids" intermediate in representation (in all three cohorts). In the river 0+ parr, it was found that farm parr were largest in size, wild parr smallest, and "hybrids" intermediate, as expected from the selection of farm strains for increased growth rate. Thus downstream migration was inversely proportional to parr size and proportional to cohort density over the three cohorts, indicating competitive displacement of wild parr by the larger farm and "hybrid" fish. Although displaced wild parr were found to survive downstream under the experimental conditions used, such survival would not occur if suitable unoccupied habitat were not available. This would be the case, for example, when a river is at parr carrying capacity or where the spawning area debouches directly to sea, as might be typical for escaped farm salmon spawning in some circumstances.

Smolt output was assessed in two ways. First, as the actual numbers of migrants taken in the experiment trap, which assumes that emigrant parr do not survive, that is, the river is at its parr habitat carrying capacity. In spite of displacement of the wild parr, the farm group produced significantly fewer smolts for the 1993 and 1994 cohorts, but was not significantly different for the 1998 cohort. The "hybrids" had variable representation among cohorts due to differential emigration as a result of different planting densities. The second estimate of smolt output assumed that emigrant parr have the same survival downstream as parr of the equivalent group remaining in the experimental river. This scenario is equivalent to the intrusion of farm salmon into a river with parr habitat in excess of that required by the wild population. With the exception of the F_1 wild mother hybrid group of the 1993 cohort and the F_2 hybrid group, all groups had significantly lower smolt production relative to wild. Again farm salmon consistently had the lowest smolt production relative to wild in all three cohorts (34%, 34%, 55%).

Adult salmon returned from sea after one and two sea winters (1SW and 2SW). In the 1SW returns, all groups, except the BC_1 backcross to wild, showed a significantly lower return relative to wild. In the 2SW returns, all groups, except farm of the 98 cohort, showed a proportionately greater return. However, the Burrishoole population is primarily a 1SW stock and the wild 2SW return was only 2.5% of the total return. Farm salmon have been bred for late maturity, a trait with high heritability under such conditions (Jónasson et al. 1997). Overall the farm group showed a 0.3% return compared with 8% for wild smolts. Egg deposition is likely to be the limiting factor in salmon recruitment, and taking account of the differential egg production of 1SW and 2SW females (Mangel 1996) shows that total potential egg deposition was significantly lower than wild for all groups except BC_1 backcross to wild. Overall the concordance of the results in the three cohorts considerably increases confidence in the findings.

Lifetime success in the Burrishoole experiment—estimated as egg-to-adult survival and taking that of wild fish as 100%—varied from 2% in farm-by-farm offspring to 27–89% in various hybrid- and backcross groups, with backcrosses to wild fish being the highest among these (Table A-2). One conclusion of the study was that repeated interbreeding of escaped farm salmon with wild salmon had a cumulative negative effect on the wild population and could in the long run lead numerically weak populations into an extinction vortex.

A recent experiment in Canada, designed to study potential local adaptation to acidified rivers in Atlantic salmon populations and whether or not repeated interbreeding with farm salmon influenced this adaptation, found mixed evidence for reduced local adaptations by interbreeding (Fraser et al. 2008). Wild juveniles had higher survival in acidic water than farm salmon or wild-by-farm hybrids. In contrast, the backcrosses and second generation wild-by-farm hybrids performed equally well if not better than wild salmon in acidic water for the life stages studied. Follow-up studies on farm-wild hybridization across divergent wild populations and multiple traits found evidence that hybrid fitness decreased with increasing divergence between the hybridizing populations, but these studies also had limited ability to predict changes in specific traits (Fraser et al. 2010).

When interbreeding between genetically different populations results in a reduction in fitness relative to both parental genotypes, it is often referred to as outbreeding depression. The mechanisms responsible for outbreeding depression fall into two different categories: 1) local adaptation, where the hybrid population lacks adaptations to its environment; and 2) coadaptation, where the hybrid population contains combinations of alleles at different loci that are not adapted to each other (Templeton 1986). Outbreeding depression can occur in the first hybrid generation, among their offspring, or even in later descendants (Lynch 1991). The degree of fitness loss seems to depend on how distant a cross is (extent of genetic differentiation between the parents).

In the Burrishoole experiment (McGinnity et al. 2003), significantly higher egg mortality occurred in the F_2 hybrid group (median 68%) than all other groups (e.g., wild 3%). Since the first generation backcrosses, which used aliquots of the same eggs as F_2 hybrids, showed significantly lower mortality (8%), this high F_2 hybrid mortality is not due to maternal or egg quality effects and most likely reflects outbreeding depression (McGinnity et al. 2003). Another case of outbreeding depression in Atlantic salmon is provided by the crossing of anadromous and landlocked Atlantic salmon (Sutterlin et al. 1987), where lower early survival rates and morphological abnormalities were found in hybrid (landlocked by anadromous) offspring.

One experiment with temporally isolated populations of pink salmon from the same stream provides an instructive case of outbreeding depression. Across its endemic range, this species has a rigid 2-year life cycle and breeds only once, followed by death. Thus two populations exist in the same river, one spawning in odd-numbered years and the other in even-numbered years. Gharrett and Smoker (1991) produced hybrids between two such temporally isolated populations by fertilizing eggs of one population with cryopreserved sperm from the other. They observed good return rates and increased variances in body size among first-generation hybrids, suggesting that hybrids were more genetically variable than purebred groups. Second-generation hybrids, however, produced very low return rates, suggesting that outbreeding depression had occurred.

This outbreeding depression was not likely caused by the two populations being adapted to different environments; in the long run, the environments in odd- and even-numbered years in the same river should be alike. Rather, the outbreeding depression seems to have been caused by disrupting coadapted gene complexes that evolved independently in the two populations.

Genetic technologies

Breeding programs and applications of genetic technologies are poorly developed for most aquaculture species, and the potential consequences for wild populations have not yet been carefully evaluated. How fish respond to intensive culture in protective environments remains unclear for most species. This is treated in some detail in the next subsection.

Lessons from Marine Stock Enhancement

The lessons learned from detailed studies of salmonid fishes can have relevance for a number of other species that are used in aquaculture or intended for such use. However, the salmonid fishes constitute a small number of species among the roughly 20,000 fish species and a limited palette of life histories, particularly compared to marine fishes. Also, increased focus on marine aquaculture and the long-term studies of some marine species in particular invite a comparison between salmonids and marine fishes.

A recent report of the genetic impact of aquaculture on native populations in the European Union (Svåsand et al. 2007, http://www.genimpact.imr.no) gives summary information on economically important finfish and shellfish in European aquaculture. In Table 3 (body of this report), we compared information on Atlantic salmon with information on the marine fish species treated by Svåsand et al. (2007). To supplement and compare the information about each species, we also extracted data from FishBase (http://www.fishbase.org) and the primary literature.

Key life history characteristics of marine fishes

Much less is known about the basic biology of most marine species compared to salmon. Although culture of marine finfish offshore is now technically feasible, few marine fishes have been successfully cultured to maturity and many of the basic environmental requirements of marine fishes for successful growth, development, and reproduction are not well understood. In Europe, the development of aquaculture production seems successful for European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus auratus*), while there are still challenges to culturing well-known species such as Atlantic cod and Atlantic halibut (*Hippoglossus hippoglossus*).

Marine fishes are very numerous and exhibit a wide diversity of life histories; furthermore, in general, there is considerably less knowledge about the basic biology of these species (genetics, ecology, abundance) than for salmonids. Marine fishes typically:

1. Can have very small eggs to large eggs,
2. Exhibit reproductive behaviors from broadcast spawning to mouth brooding,
3. Are pelagic to benthic spawning,
4. Exhibit internal or external fertilization,
5. Display no parental care to extensive parental care,
6. Range from young to old age at sexual maturity,
7. Express variable interspawn interval and senescent periods,
8. Can be r- or K-selected,
9. Show considerable variation in body size, and
10. Show considerable variation in population size.

Despite the great diversity in these life history traits, it seems that those species that are relevant marine culture species look remarkably similar to salmon: the marine species treated in Table 3 are migratory, large-sized, show high age at sexual maturity, and are often piscivorous. In many cases, these species are also increasingly produced in intensive culture before the impacts of cultured fish on wild populations are understood.

In general, on an equivalent spatial scale, marine species show lower levels of molecular genetic differentiation among populations than do anadromous or freshwater species. Comparisons of a number of fish species using enzyme electrophoresis show that genetic structure (i.e., amount and distribution of genetic variation) differs between marine, anadromous, and freshwater species. Gyllensten (1985), in a comparison of 19 fish species, found that the average fraction of the total gene diversity allocated between localities (called G_{ST} or F_{ST}) increases in the order marine (0.016), anadromous (0.037), and freshwater (0.294) species, whereas the total gene diversity (average heterozygosity, called H_T) is higher in marine (0.063) than in freshwater (0.043) and anadromous (0.041) species.

Ward et al. (1994), in a study of 113 species, obtained the same qualitative but slightly different quantitative results, some of which are referred to in Table 3. They suggested that the average degree of genetic differentiation between subpopulations within species in the various environments could be interpreted as marine subpopulations exchanging between 10 and 100 times more migrants per generation than freshwater species, using Wright's (1943) relationship between F_{ST} and the number of migrants N_em, $F_{ST} = 1 / (1 + 4N_em)$, where N_e is effective population size and m is migration rate in an island model of migration.

Evidence for local adaptations of marine species

Local adaptations have for a long time been recognized in freshwater and anadromous fishes, for example in salmonid fishes by Ricker (1972), Taylor (1991), and Garcia de Leaniz et al. (2007). Given the homing tendency of migrating salmonids and the estimates of high genetic differentiation between subpopulations in molecular genetic markers, this comes as no surprise.

In marine fishes, on the other hand, pelagic egg or larval stages coupled with low differentiation between subpopulations in molecular genetic markers suggest that the gene flow between localities is too high for strong local adaptations to develop. Recent research employing a broader panel of genetic markers, however, suggests that marine fishes might be more genetically structured than previously thought, and that accompanying ecological differences provide strong evidence for local adaptations in a number of species (Hauser and Carvalho 2008).

Opportunities for natural reproduction of farmed fishes

Many marine fishes have been shown to spawn successfully in captivity, including net cages. Successful spawning in cages where eggs, larvae, or juveniles are capable of escaping to the surrounding water column poses the opportunity of genetic interactions between cultured and wild fish if those escaped individuals survive to reproductive maturity (Jørstad et al. 2008). If spawning in culture is frequent—and especially if it is undetected—it is feasible for propagule pressure from the program to pose an unacceptable risk to wild populations in the area, particularly if these populations are depressed in abundance or genetic diversity.

The very high fecundity of many marine species means that a small number of broodstock can potentially have a large genetic impact on natural populations. Females of many marine fish species are capable of producing a large number of small eggs or larvae, which in the wild are often broadcast into the water column with no maternal care. If survival of eggs or larvae in culture to market size or to maturity is sufficiently high, relatively few adults can be considered or sampled for aquacultural broodstock. In haddock (*Melanogrammus aeglefinus*), it was recently shown that the potential for inbreeding was high during communal breeding of this gadoid fish (Trippel et al. 2009). Such practice substantially increases the probability of genetic differentiation between cultured and wild fish, thereby increasing adverse genetic consequences of interbreeding whenever it does occur.

Detecting the genetic effects of cultured marine fishes on natural populations

Steps to detect these effects include:

1. Document presence and if possible estimate abundance of escaped fish,
2. Document presence of F_1 hybrid offspring,
3. Document presence of backcross hybrid offspring,
4. Document presence of escaped fish at all life stages (larval, juvenile, preadult, adult),
5. Determine whether there has been a population-level change of wild individuals, and
6. Determine numbers of local fish species and their relative abundance.

To our knowledge, interaction studies of marine cultured fish species with wild conspecifics are still limited. In European aquaculture, Atlantic cod are probably among the best studied species, primarily because of studies of genetically marked cod juveniles released to the wild (Bekkevold et al. 2006, Jørstad et al. 2008).

Effects of escaped fish

A good deal of evidence suggests that significant numbers of fish have escaped from marine net pens in the Mediterranean, but few studies have rigorously or quantitatively addressed this issue, and in general program operators are not required to document escapes (Hansen and Windsor 2006, Dempster et al. 2007). One study (Bahri-Sfar et al. 2005) found that European seabass of western Mediterranean origin that were farmed in the eastern Mediterranean and subsequently escaped had established naturally reproducing populations, but these appeared to be distinct from the local eastern seabass populations.

Freshwater environments are generally regarded as being more prone to invasions than marine environments. This is likely connected to the fragmented and inaccessible (island) nature of many freshwater environments, where many fish communities are far from being ecologically saturated. Another category of environments prone to invasions are those that are disturbed by humans. Among the marine environments, those occurring along the coast and in estuaries are most likely to fall into this category.

Studies of biological invasions have been more common in the freshwater environment than in the marine environment. Lessons from freshwater fishes suggest that invasive species have some ecological characteristics in common. This has been used to perform risk assessment for alien fishes in the North American Great Lakes (Kolar and Lodge 2002). Whether this is useful for genetic risk assessment of species taken into fish culture is so far unknown.

Ecological roles and mechanisms of impact

Marine species offer a greater diversity of ecosystem roles and functions than salmonid fishes, which are all high in the food web, many of them being top fish predators. Incidentally, this is also the case for most of the marine fishes being developed for aquaculture, but not for the common carp (*Cyprinus carpio*), one of the major products of freshwater fish culture.

Mechanisms of impact that have been empirically verified by studies of salmonids include interbreeding between cultured and wild populations of the same or a related species, competition with and potential displacement of wild fish, reciprocal disease transmission between wild and cultured populations, and altered selective regimes for the wild population following contact with cultured fish. Other mechanisms that should be considered when developing guidelines for marine aquaculture include but are not limited to:

1. Predation,
2. Herbivory and detritivory,
3. Broadcast spawning,
4. Disease agents that are shared between species, and
5. Ecosystem modifiers.

Predation of escaped farmed fish on wild fish has not been described as a result of salmon aquaculture (other than anecdotally, to our knowledge), but is described from escapes of tilapia (*Oreochromis* spp.). Increased aquaculture production and escapes of marine piscivorous fish like cod likely will increase predation pressure on migrating smolts of salmonids.

Case studies of marine species for which captive breeding and culture programs have been developed

Table 3 (body of this report) provides a list of selected marine species for which genetic impacts of ranching and farming have been treated in the literature. Common to these species is that interaction studies are in their infancy, and predictions of impacts by marine finfish culture programs such as cod farming are largely based on the salmonid literature (Bekkevold et al. 2006).

Lessons from Agriculture

The long history of agriculture has provided a number of insights into how human production systems and natural populations differ and can lead to conflicts when cultured and natural organisms interact in the wild. The environmental impacts of agriculture are generally well-known, but they are often highly location specific and some agricultural effects on the environment can take a long time to appear. Agricultural impacts include the use of fertilizers and pesticides, irrigation and drainage, and intensive harvest, which contribute to increased pollutant loads, mechanical disturbance, and offsite effects through manipulation of soil and surface water; however, these impacts vary with the type of habitat converted to agricultural use. Consequently, understanding the myriad of environmental impacts of agriculture on the natural environment and the populations it supports is an exceedingly complex task.

Nevertheless, many of the risks posed by human production systems to natural systems have long been recognized and several lessons about the effects of agriculture on wild populations are clear (Tilman et al. 2001, Green et al. 2005, Millennium Ecosystem Assessment 2005). First, agricultural operations can reduce biodiversity through ecosystem simplification, loss of ecosystem services, and direct extirpation and displacement of wild populations. Second, risk to the genetic diversity within and among natural populations can occur through habitat loss or degradation or through genetic interactions between cultured and wild organisms. Third, because cultured organisms are typically adapted to the agricultural environment, interactions between them and natural organisms in the wild can lead to substantial losses of fitness in natural populations through the direct genetic effects of interbreeding and introgression, or through indirect genetic effects that result from changes in the regime of natural selection. Such fitness losses have long been predicted from evolutionary and life history theory and have now been documented repeatedly in a variety of taxa. Finally, the risks posed by cultured organisms to wild organisms can be reduced through careful siting and monitoring of agricultural operations, but they can seldom be avoided entirely. Moreover, reducing some risks can escalate others; for example, increasing the genetic divergence between cultured and wild organisms to facilitate agriculture can reduce the opportunity for interactions between them, but when interactions do occur the consequences could be much larger. Such interactions threaten the diversity of natural populations that are critical for long-term viability in the face of unpredictable environmental change.

Theoretical and empirical developments in the last few decades provide increasingly strong evidence that such effects on natural populations are real. However, a considerable amount of uncertainty remains, particularly when trying to anticipate the effects of specific aquaculture programs on particular populations in particular areas.

Aquaculture has focused on improving growth rate, feed conversion efficiency, early maturation, high larval quality, disease resistance, and product quality. A primary goal of the aquaculturist, like other plant and animal breeders, is to produce fish efficiently with desirable phenotypes, or more precisely, to increase the frequency of desirable phenotypes in the cultured population. The primary aims of a breeding program are to increase the survival and production of farmed animals and improve product quality, as determined by market forces (Gjedrem 2005).

Initially, desirable phenotypes are likely to be characterized by rapid growth, high flesh quality, ease of culture, controlled maturity, and high disease resistance. Experiments to improve growth and disease resistance in fish began by the 1920s (Embody and Hayford 1925). Thus selection in aquaculture tends to focus on growth rate, feed conversion efficiency, early maturation, high larval quality, disease resistance, product (flesh) quality, and domesticability (e.g., tameness, adaptation to captivity, reduced stress in confinement). Some of these traits also have large environmental components and their expression can vary considerably in different culture environments due to phenotypic plasticity (Gjedrem 2005).

Sterilization

Sterilization of salmon can be achieved through induction of triploidy (production of three sets of chromosomes). This has two potential benefits: 1) prevention of gene flow from cultured to wild fish, and 2) diverting energy that would have gone into sexual maturation into production of body mass (O'Flynn et al. 1997). Triploidy is achieved by preventing the disjunction of the second polar body from the newly fertilized egg, which can be done by giving a temperature or pressure shock after fertilization (Benfey et al. 1988, Quillet and Gaigon 1990). The result is a fish with two copies of the maternal chromosome, instead of one from each sex as in normal diploid individuals. Triploidy generally results in complete sterility in females, but not in males. If the sperm are irradiated before fertilization, followed by administration of heat or pressure shocking, only the two maternal chromosome copies will be functional, resulting in gynogenetic diploid (all-female) offspring (Kirpichnikov 1981, Quillet and Gaigon 1990). In the common carp, partial sterility has been achieved by genetic modification controlling the gonadotropin-releasing hormone (and thereby gonad development) by so-called antisense sequence (Hu et al. 2006).

All-female embryos can be produced for triploidization by using sex-reversed females as the functional "male" parent in the previous generation. The latter is achieved by first feeding female fry with a diet containing a particular concentration of male hormone, resulting in functional sperm-producing testes. Therefore a two-generation process is involved, where the second generation triploid fish are not hormonally treated. Molecular identification of the sex-determining locus of Atlantic salmon can facilitate production of all-female lines (e.g., Artieri et al. 2006).

In salmon farming conditions, the overall yield of triploids has been shown to be lower than diploids due to poorer marine survival (O'Flynn et al. 1997). In ranching experiments, Cotter et al. (2000) have shown substantially reduced river returns of triploid salmon when compared with diploids.

Although sterilization is rarely 100% successful, this approach has the potential to significantly reduce risks from escaped salmon (Cotter et al. 2000), common carp (Hu et al. 2006), and other aquatic species. The most common method of inducing sterility is to make individuals triploid by giving a heat or pressure shock to the eggs shortly after fertilization. Triploid fish grow and survive well (Burke et al. 2010, Benfey 2011), but show an increase in skeletal deformities. This has been used as an argument to discourage production of triploid fish with reference to animal welfare considerations. Recent results suggest that skeletal deformities can be reduced by adding more phosphorous to the diet of sterile farmed fish (http://www.imr.no/nyhetsarkiv/2011/mai/gode_resultat_med_steril_oppdrettslaks/en).

Trade-offs among Competing Risks

A trade-off in the consequences of encounter can arise from the relationship between the degree of divergence and the encounter rate. Fish that are more similar are likely to encounter each other at higher rates, with more opportunity for genetic interaction (especially interbreeding) but perhaps milder consequences of each interaction. By contrast, fish that are more divergent can encounter each other at lower rates but the consequences of encounter for fitness loss can be more serious. Consequently, encounters between cultured and wild fish are never risk free but the risk depends on the rate of encounter and the degree of divergence (Kapuscinski et al. 2007). The aquaculturist must recognize this relationship and the potential for consequences of encounter to grow if aquaculture goals lead to greater divergence between cultured and wild fish. This recognition is integral to the paramount consideration of developing sustainable marine aquaculture alongside healthy, viable natural populations in their marine habitats.

Characterizing Risks and Benefits

Potential Benefits of Artificial Propagation for Natural Populations

A. Reduce short-term extinction risks for endangered populations and
B. Help maintain a population at a safe level until factors for decline can be addressed

Bringing a population at risk of extinction into protective culture can reduce immediate risk of extinction by improving stage-specific survival generally or minimizing risk of mortality of reproductive adults or their embryos (Hard et al. 1992). In this way, captive propagation can significantly increase the number of individuals to be returned to the wild. Maintaining a population in the short term (at least several decades or generations of the species) through artificial propagation and helping to reduce short-term extinction risk are the potential benefits to natural populations that are best-documented in the empirical record. Zoos around the world maintain species and populations of a wide range of taxa that might be extinct in the wild without such programs. Frankham et al. (2002) contains a good discussion of genetic issues associated with such programs.

A number of examples can be cited from aquatic species. For example, by 1990 the last remaining sockeye salmon (*Oncorhynchus nerka*) from the Snake River were nearly extinct. This population, from Redfish Lake in Idaho, is particularly distinctive, as it spawns at a higher elevation (2,000 m) and has a longer freshwater migration (1,500 km) than any other sockeye salmon population in the world (Waples et al. 1991). The few (16) adults that returned in 1991–1996 were spawned and their progeny, together with some smolts collected when they were outmigrating from the lake, were reared to adulthood in captivity (Flagg et al. 2004). The major goal of this captive broodstock program was to perpetuate the gene pool for a short period of time to give managers a chance to identify and address the most pressing threats to the population. Considerable efforts have been made to conserve as much as possible of the genetic diversity that survived the bottleneck (Kozfkay et al. 2008), and a recent analysis (Kalinowski et al. 2012) estimated that the captive program had managed to retain approximately 95% of the original genetic variation over about five salmon generations. In 2010 well over 1,000 adult sockeye salmon returned to Redfish Lake—more than at any time in more than 50 years. Most of these were hatchery produced; it remains to be seen how the population will fare if and when the hatchery program is terminated. Nevertheless, captive propagation has helped to rescue a unique gene pool that was on the brink of extinction and give it a chance for survival.

The white abalone (*Haliotis sorenseni)* was listed throughout its range (southern California and northern Mexico) as endangered under the U.S. Endangered Species Act (NOAA 2001; 66 FR 29054, May 29, 2001). The species had declined to such a low density in the wild that natural reproduction was unlikely and extinction was predicted by 2010 (White Abalone Recovery Team 2008). Although recovery goals included eventual establishment of numerous self-sustaining populations in the wild, given the critical and immediate nature of the risks, captive propagation is viewed as a crucial component of near-term efforts. Molecular methods have shown that adults removed from the wild retain high levels of genetic diversity (Gruenthal and Burton 2005), and captive breeding will be conducted at multiple sites to help reduce risks of catastrophic failure (White Abalone Recovery Team 2008). Molecular techniques are being used to ensure broodstock integrity and in the future can facilitate assessment of outplanting efforts.

How long can populations be propagated artificially and still retain viability in the wild? This is a difficult question to answer. Some aquatic species have been intensively cultured for more than a century, and some facilities are perhaps that old. However, few populations or stocks have been continuously cultured for very long periods of time. For the past several decades in Japan, returns of approximately 50 million chum salmon per year have been maintained almost entirely by hatchery propagation, while natural habitats have been blocked or degraded and natural production has dwindled (Masuda and Tsukamoto 1998). These fish are spawned and their progeny reared for a short time in hatcheries, but they spend the majority of their life in the Pacific Ocean, so they are exposed to natural selection for part of their life cycle. Kaeriyama and Edpalina (2004) have recently raised concerns about long-term effects of these hatchery programs on fitness and sustainability of the natural populations.

C. Speed recovery by providing a demographic boost to an existing population

For populations that are depressed but not at high short-term risk, an increasingly popular strategy is to use artificial propagation to boost abundance and perhaps speed recovery. Is this strategy successful? The answer depends on what is used as a measure of success. Many of these supplementation programs can produce more adults than would result from wild reproduction by the same number of spawners. However, virtually no empirical data exist regarding the effects of artificial propagation on long-term viability of natural populations. For an empirical review of various ways to measure the success of supplementation efforts for Pacific salmon and steelhead, see Waples et al. (2007).

D. Reseed vacant habitat

Reintroductions to formerly occupied habitats have been attempted for a wide variety of plant and animal species. In general, probability of success is enhanced when the source population is wild, large numbers of individuals are released, the causes for the original decline have been addressed (Fischer and Lindenmayer 2000), and appropriate models have been used to assess the species' habitat requirements (Cook et al. 2010). Tettlebach and Smith (2009) describe efforts that have met with at least short-term success in using hatchery outplanting to restore a New York population of the bay scallop (*Argopecten irradians*) after it was nearly eradicated in the wild by a series of brown tide algal blooms.

E. Reduce harvest pressure on natural populations

Overfishing is a serious problem for many marine species, and existence of a consistent supply of high quality cultured product at a reasonable price could substantially reduce demand for (and profit from) wild harvests (Engelsen et al. 2004).

Genetic Risks to Natural Populations from Aquaculture

Unmonitored, large-scale releases of cultured individuals into the wild is a global issue that affects a wide range of plant and animal species in addition to fish (see Laikre et al. 2010 for a review). Bartley et al. (2004) list nearly 100 marine stock enhancement programs in developing countries, in addition to numerous programs in countries such as Japan and Norway that have a long history of sea ranching and fish farming, respectively. General discussions of genetic risks associated with fish culture can be found in Allendorf and Ryman 1987, Hindar et al. 1991, Waples 1991, Busack and Currens 1995, Campton 1995, Waples 1999, Brannon et al. 2004, Waples and Drake 2004, Fraser 2008, Naish et al. 2008, and Araki and Schmid 2010.

Escapes of cultured animals to the wild are the primary means by which aquaculture and production of domesticated individuals pose a threat to natural populations. Escape events can range from relatively constant leakage of small numbers of individuals to large catastrophic events involving tens of thousands of individuals, millions of gametes or larvae, or more (e.g., Jensen et al. 2010). Escapes from most shore-based, recirculating culture systems, such as those that have been used for salmonid culture in Iceland (Thorarensen and Farrell 2011), probably pose a negligible risk. The probability of escapes is highest for net pen cages in nearshore or open ocean sites exposed to storm events or without adequate safeguards against escapes in effluent.

Loss of diversity within populations

Crow and Kimura (1970) is a good general reference for standard population genetic principles, including the rates of genetic drift and increase in inbreeding. Detailed accounts of many of the issues discussed here in a conservation context can be found in Frankham et al. (2002) and Allendorf and Luikart (2007). Franklin (1980) proposed what became known as the 50–500 rule, based on empirical data that suggested the following: 1) N_e of 50 produces inbreeding at about 1% per generation [1% = 1 / ($2N_e$) = 1 / (2 × 50)], and this level of inbreeding can be tolerated in short-term captive breeding populations (e.g., cattle or sheep), but 2) a larger effective size ($N_e \approx 500$) is required to provide a balance between mutation and drift for quantitative genetic traits. Subsequently, others (e.g., Lynch and Gabriel 1990, Lande 1994, Lynch and Lande 1998) developed an idea first proposed by Wright (1931) and showed that an effective size much larger than 500 (perhaps 5,000) might be required to prevent a “mutational meltdown” through accumulation of slightly deleterious alleles.

Unless N_e is large, selection cannot operate effectively to eliminate slightly deleterious alleles, and they accumulate over time and can drift to high frequency just by chance. Furthermore, even if an effective size of about 500–1,000 is sufficient to provide for most medium-term evolutionary processes, the total number of alleles that can be maintained in a population with effective size of 10^6 is vastly larger than the number that can be maintained in a population with $N_e = 10^3$ (Ryman et al. 1995b). Ryman et al. (1995b) were probably the first to point out that orders of magnitude reductions in huge fish populations could have a substantial effect on allelic diversity, even if effective population size and heterozyosity remained very large. Waples and Naish (2009) discussed this issue and provided a hypothetical numerical example.

Most marine species have overlapping generations, which complicates calculation of N_e. Felsenstein (1971) and Hill (1972, 1979) developed methods to calculate N_e per generation in species with overlapping generations. Waples et al. (2011) developed a hybrid Felsenstein-Hill method that combines the best features of both approaches; the hybrid method uses age-specific survival and fecundity data (as are found in a standard Leslie matrix) and can accommodate overdispersed variance in reproductive success (as occurs in many marine species).

Ryman and Laikre (1991) provided the following expression for effective population size in a captive-wild system:

$$N_eT = \frac{1}{\frac{x^2}{N_eC} + \frac{(1-x)^2}{N_eW}} \qquad (2)$$

where N_eT = effective size of the cultured-wild system as a whole, N_eC = effective size of the individuals reproducing in captivity, N_eW = effective size of the individuals reproducing in the wild, x = the fraction of spawners in the offspring generation that were produced in captivity, $1 - x$ = the fraction of spawners in the offspring generation that were produced in the wild, and the Ryman-Laikre effect disappears entirely (i.e., there is no net change in overall effective size) under two special cases. In case 1, $x / (1 - x) = N_eC / N_eW$. In case 2, $x = 0$.

In Case 1, the ratio of captive to wild contributions is the same as the ratio of the effective sizes of the two phases. In this situation, the captive individuals do not contribute disproportionately to the overall population and N_eT is the sum of $N_eC + N_eW$. In stock enhancement, Case 1 would generally be considered an unsuccessful program because the hatchery has not enhanced population size; for marine aquaculture, where stock enhancement is not a goal, Case 1 still might be considered financially successful, as long as the numerical losses due to escapes were not large enough to compromise program goals. Case 2 implies that either

a) there is no aquaculture program, b) there is a program but there are no escapes, or c) some individuals escape but do not contribute any genes to subsequent generations. In these cases, N_eT is simply N_eW, less any reduction to N_eW that results from taking individuals from the wild into captivity.

The Ryman-Laikre effect (reduction in overall N_e) is largest when $x / (1 – x)$ is large compared to N_eC / N_eW, in which case N_eT is reduced compared to the sum of the wild and captive components ($N_eC + N_eW$). Marine species are potentially susceptible to large Ryman-Laikre effects because N_eW is typically much larger than N_eC (perhaps orders of magnitude larger), and high fecundity creates the possibility that x can be large even if only a relatively few spawners are used for broodstock (Tringali and Bert 1998, Hedgecock and Coykendall 2007). Tringali and Bert (1998) showed that if the objective is to ensure that $N_eT \geq 500$, the goal can be achieved with $N_eC \geq 50$ and $x \leq 17\%$; if N_eC is at least 100, the culture fraction can be as high as 30% without driving N_eT below 500. These results are not very sensitive to the initial value of N_eW provided that it is at least 500. However, even if N_eT remains above some absolute target, it still might be reduced to a fraction of the original value, as discussed below.

Figure A-9 quantitatively illustrates the Ryman-Laikre effect for scenarios that should be plausible for at least some marine species. In panel A, N_eC was assumed to be 100, which is about the upper limit for effective sizes associated with the best-studied marine stock enhancement programs. For any given value of x, N_eT quickly asymptotes at a rather modest value, regardless how large N_eW is. Panel B shows that, even with the aquaculture fraction as small as 0.1 (10%), effective size of the cultured-wild system as a whole can be a tiny fraction of N_eW. If N_eW is on the order of 10^6 or 10^7 (perhaps realistic for some of the species subject to aquaculture), an aquaculture operation with escapes that comprise 10% of the breeding population would probably reduce N_e of the wild population by 3 or 4 orders of magnitude.

Table A-3 shows how overall N_eT and N_eT / N_eW vary with different parameters that might be applicable to marine aquaculture programs. These examples assume that 200 captive individuals are used for broodstock, and that the ratio N_eC / N is either high (0.5) or low (0.05), leading to $N_eC = 100$ or 10, respectively. In this example, N_eW is assumed to be either 10^3 or 10^6 and the fraction of successful spawners that are of captive origin is assumed to be either 1% or 10%. If N_eW is relatively low (10^3), these parameters lead to little or no reduction in overall N_e, except for $N_eC = 10$ and $x = 0.1$, in which case wild N_e is reduced by nearly 50%. Results are considerably different for large wild populations ($N_eW = 10^6$), in which case the best case scenario ($N_eC = 100$; $x = 1\%$) leads to a 50% reduction in N_e and the other scenarios all lead to 90% or greater reductions in effective size. Of course, a captive contribution of 1 or 10% to successful natural spawners represents a lot more total escapes for $N_eW = 10^6$ compared to $N_eW = 10^3$.

The captive effective size used in Figure A-9, panel A ($N_eC = 100$) is as large or larger than values that have been reported for marine stock enhancement programs (Tringali 2006, Gold et al. 2008, Kitada et al. 2009, Gruenthal and Drawbridge 2012). However, if the species is long-lived and different individuals are spawned every year, it should be possible to increase N_eC to at least 500 or so over the course of a generation.

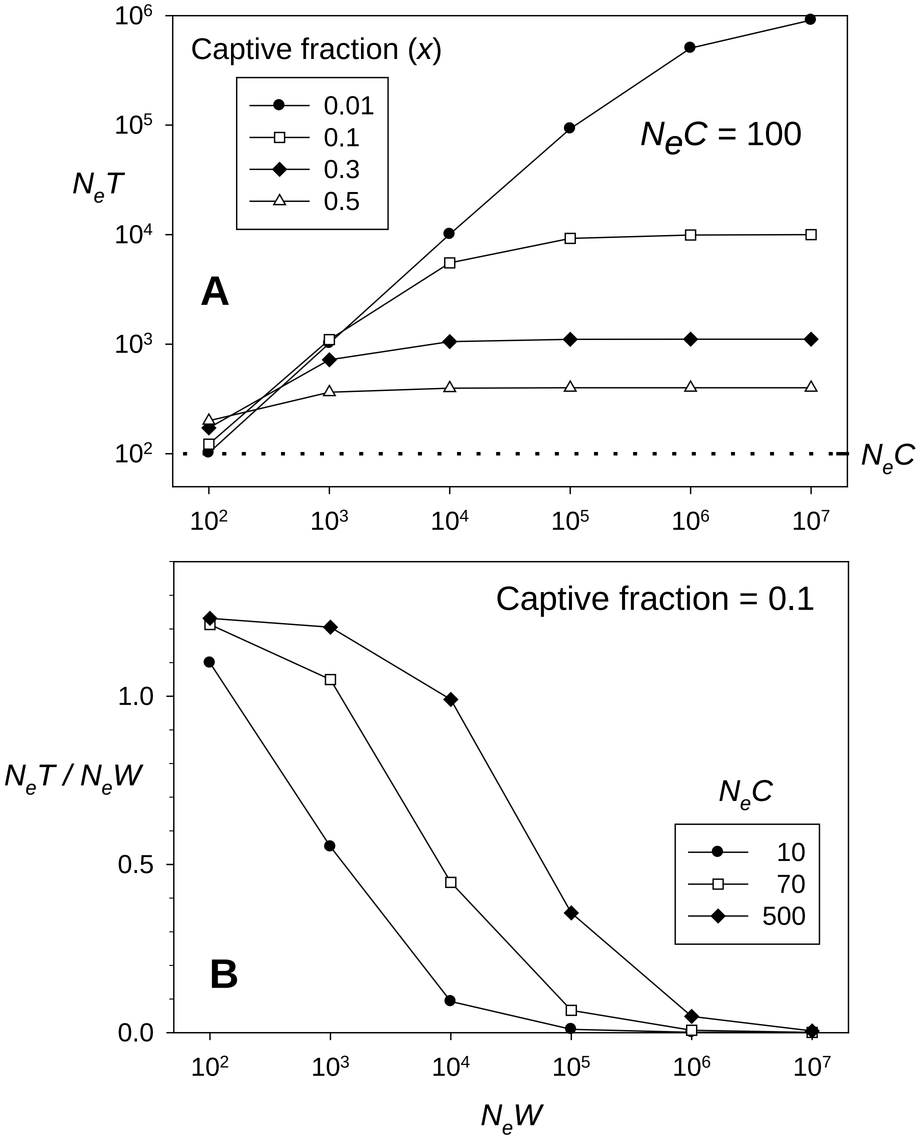

Figure A-9. Illustration of the Ryman-Laikre effect and its consequences for effective population size (N_e). In panel A, unless x is small, N_eT will not be much larger than N_eC, regardless how large N_eW is. In this example, it is assumed that N_eC is 100. In panel B, unless N_eW is relatively small and N_eC is unusually large or x is very small, N_eT generally will be a small fraction of N_eW. This example assumes that the captive fraction (x) is 0.1; that is, progeny of captive individuals make up 10% of the overall natural spawners.

The magnitude of the N_e / N ratio in marine species remains controversial. Hedgecock (1994) proposed that the ratio could be very low in marine species because of highly variable, "sweepstakes" recruitment. According to this hypothesis, most females produce no surviving offspring at all, while progeny of a rare few individuals happen to end up in the right place at the right time to find sufficient food and escape predators. This scenario predicts family correlated survival and could lead to very low N_e / N ratios. Hauser and Carvalho (2008) reviewed empirical estimates for marine species (which include several in the range 10^{-3}–10^{-5}) and

Table A-3. Examples of the Ryman-Laikre effect for some scenarios that involve relatively small fractional contributions from escapes ($x \leq 0.1$). N is the initial wild population size. It is assumed that captive individuals are removed from the wild population and this reduces effective population size in the wild (N_eW) by the quantity N_eC.

	Aquaculture fraction (*x*)									
	0.001	**0.001**	**0.001**	**0.01**	**0.01**	**0.01**	**0.01**	**0.1**	**0.1**	**0.1**
$N = 1{,}000$										
N_eW	500	930	990	500	930	990	500	930	990	—
N_eC	500	70	10	500	70	10	500	70	10	—
N_eT	501	932	992	510	948	1,000	610	986	550	—
N_eT / N_eW	—	0.50	0.93	0.99	0.51	0.95	1.00	0.61	0.99	0.55
$N = 10^6$										
N_eW	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	$\approx 10^6$	—
N_eC	500	70	10	500	70	10	500	70	10	—
N_eT	$\approx 10^6$	9.9×10^5	9.1×10^5	8.5×10^5	4.2×10^5	91,074	48,053	6,961	999	—
N_eT / N_eW	—	1.00	0.99	0.91	0.85	0.42	0.09	0.05	0.007	0.001

suggested more detailed mechanisms that could explain such tiny ratios. However, all of the tiny estimates are from indirect genetic methods, which can be subject to various biases. Additional discussion of these issues can be found in Hedrick (2005), Palstra and Ruzzante (2011), and Hare et al. (2011).

Under some circumstances, it is possible for the Ryman-Laikre effect to increase overall effective size. This can only happen if the ratio of effective size to census size is higher in captivity than it is in the wild, that is, when $N_eC / N_C > N_eW / N_W$. Empirical data show that in captive populations, a relatively few individuals generally contribute disproportionally to offspring production and this reduces N_eC / N_C. However, N_eW / N_W can also be quite low in natural marine populations, and if the difference between N_eC / N_C and N_eW / N_W is large enough, it can more than compensate for differential productivity between captive and natural spawners. Table A-4 provides an example.

Table A-4. Hypothetical examples showing how the Ryman-Laikre effect can result in an increase in N_eT compared to what it would have been without a captive program (N_eW^*), provided the N_e / N ratio is substantially higher in captivity than in the wild.

	Scenario A	**Scenario B**
x	0.50	0.50
N_w	500.00	500.00
N_c	50.00	50.00
N_eW / N_w	0.10	0.10
N_eC / N_c	0.20	0.50
N_eW^*	50.00	50.00
N_eW	45.00	45.00
N_eC	10.00	25.00
N_eT	33.00	64.00
N_eT / N_eW^*	0.66	1.29

In this hypothetical example, the census size in the wild is N_w = 500 and N_eW / N_w is 0.1, so in the absence of a captive program, wild effective size is N_eW^* = 50, and this is useful as a point of reference. N_C = 50 adults are collected from the wild for broodstock, leaving 450 in the wild with effective size N_eW = 45. If we assume that N_eC / N_C = 0.2 (scenario A), then N_eC = 10, and N_eT = 33, assuming the captive fish produce half of the offspring the next generation (x = 0.5). The overall effective size (33) is only 2/3 of the N_eW^* = 50 that would have occurred without a captive program, so overall N_e declined in spite of a higher effective size:census size in captivity. However, if this differential is stronger (N_eC / N_C = 0.5, Scenario B), the result can be an increase in overall N_e (to 64 in this case). Hedrick et al. (1995; 2000) showed that, in a captive propagation program for the endangered Sacramento River winter-run Chinook salmon (*Oncorhynchus tshawytscha*), the relatively modest contribution of program fish (small x), combined with relatively high N_b / N ratio in the hatchery, meant that the juvenile release program did not appreciably reduce N_eW compared to N_W and might have increased it slightly.

Figure 3 and Table 4 (body of this report) assume a single generation of cultured-wild genetic interactions. Several authors (Waples and Do 1994, Wang and Ryman 2001, Duchesne and Bernatchez 2002) have considered the more realistic scenario that involves multiple generations of genetic interactions. In such analyses, an important factor is whether the natural population increases in abundance as a result of supplementation with cultured individuals; if so, the reduced levels of genetic drift in the larger overall population can help compensate for increased levels of inbreeding attributable to using a relatively few breeders in captivity. As increasing natural population abundance is not a likely consequence (nor a general objective) of commercial aquaculture, the relevant scenarios involve populations that do not increase in size but might, over time, receive continual inputs of genes from cultured individuals derived from relatively few parents. Under these conditions, the Ryman-Laikre effects are cumulative across generations, in which case regularly incorporating fresh spawners into the broodstock is important to help reduce long-term erosion of diversity.

The above material and that in the main text also assumes a single closed population. An alternative way to think about the problem involves a system of subpopulations connected by migration. Under these conditions, the global (metapopulation) effective size can be either larger or smaller than the sum of the subpopulation N_es, depending on various factors related to patterns of migration, local extinction, and spatial and temporal variation in productivity (see Whitlock and Barton 1997 and Wang and Caballero 1999 for discussion). Tufto and Hindar (2003) considered a variation of this type of model that allowed consideration of source-sink dynamics (e.g., unidirectional migration from one population into another, as might occur with unintentional escapes from an aquaculture program). Results of this model showed that the total effective size can be reduced by several orders of magnitude if the captive component of a population is much smaller than the wild component—a result similar to the conclusions reached using the Ryman-Laikre model. Migration can greatly speed up restoration of genetic variation in depleted populations, as demonstrated by Duchesne and Bernatchez (2002).

Loss of diversity among populations

A pervasive theme in ecology is that biological diversity contributes to the stability of ecosystem processes and the services they provide, a concept that has become a major argument for conservation of biodiversity. In ecology, conservation of biological diversity has focused primarily on the effects of species diversity on ecosystem stability, but evidence is growing that diversity among populations within species may also be important to ecosystem dynamics. The population-level diversity of some species may act like a diversified portfolio of investments, buffering fisheries and incomes from the ups and downs of particular populations. For general reading about the statistical and ecological basis of this portfolio effect, see Markowitz (1952), May (1974), Tilman and Downing (1994), and Doak et al. (1998).

Perhaps the best empirical example of the benefits of the portfolio effect is the fishery for sockeye salmon in Bristol Bay, Alaska (Hilborn et al. 2003, Schindler et al. 2010). Harvest has been maintained at roughly 10 million fish annually for a century, in spite of large annual fluctuations in abundance of individual stocks and stock complexes. Notably, the stock complex that currently produces more than half of the total harvest was a minor contributor (<10%) to the fishery a half-century ago. Other examples involving aquatic species include Worm et al. (2006), Lindley et al. (2007), Levin and Lubchenco (2008), and Greene et al. (2010).

Genetic diversity is an important component of the diversity among populations. The F statistics developed by Wright have been enormously useful for characterizing genetic variation among natural populations. Wright (1931) showed that, under an island model at equilibrium, the expected value of F_{ST}, which measures genetic divergence among populations, is given approximately by

$$F_{ST} = 1 / (1 + 4mN_e) \tag{3}$$

Although the numerous assumptions underlying this simplistic model are rarely if ever completely satisfied in nature (Waples 1998, Whitlock and McCauly 1999), this basic relationship has been widely used to provide insights into levels of migration consistent with molecular genetic data.

In recent years, a great deal of attention has focused on trying to understand the relationship between the parameters m and mN_e and what they mean for marine species. The migration rate, m (the fraction of individuals exchanged each generation), is key to estimating demographic connectivity and independence among local populations, and this has sparked considerable interest in estimating marine dispersal, especially at planktonic larval stages (Cowen et al. 2000, Shanks et al. 2003, Levin 2006, Bradbury et al. 2008). In contrast, molecular genetic data primarily provide information about mN_e (the product of migration rate and effective size; see above equation), the units of which are individuals per generation. This produces a disconnect between the saturation points for genetic versus demographic dispersal. In large marine populations, tiny migration rates (m) that have little influence on demographic connectivity could produce a large enough number of migrants (mN_e) to essentially homogenize indices of neutral genetic diversity. A number of authors have discussed the challenges this poses to drawing inferences about demographic processes in marine species from genetic data (Hauser and Carvalho 1994, Waples 1998, Palsboll et al. 2007, Waples et al. 2008, Lowe and Allendorf 2010).

Patterns of genetic variation in aquatic species have been reviewed by Gyllensten (1985), Ward (1994), and Hauser and Carvalho (2008). Ward et al. (1994) reviewed molecular genetic data (primarily allozymes) for fishes and found that mean values for F_{ST} were highest for freshwater species, moderate for anadromous species, and lowest for marine species. Conversely, Dewoody and Avise (2000) found that levels of microsatellite diversity within populations (average numbers of alleles per locus and average heterozygosity) showed the opposite trend: marine fishes had the highest levels of within-population diversity and freshwater fishes had the lowest (comparable to values found in nonpiscine animals). Because F_{ST} is inversely related to the combined parameter mN_e, a low F_{ST} can be due to a high migration rate (m), a large effective size (N_e), or both. Thus large marine populations with low F_{ST} still might experience low migration rates, which facilitate local adaptation.

The relationship between F_{ST} and mN_e can take a long time to reach equilibrium. If a population system is distorted from historical migration-drift equilibrium, then original migration patterns (at rate m per generation) are restored, the number of generations required for F_{ST} to move half the distance toward the new equilibrium (T_{50}) is given by (Whitlock 1992):

$$T_{50} = \frac{\ln(0.5)}{\ln[(1-m)^2(1-1/(2N_e))]} \tag{4}$$

Equation 4 can be informative for evaluating anthropogenic and “natural” disturbances. For example, to consider the example shown in Figure 5 (body of this report), where human activities have completely homogenized diversity among populations ($F_{ST} = 0$), assuming local $N_e = 1{,}000$ and $m = 0.004$ (so that $mN_e = 4$), this equation predicts about 81 generations are required to move halfway toward the new equilibrium, in good agreement with simulation results (e.g., after 81 generations following complete homogenization, F_{ST} had risen to a bit over 0.02, about halfway to the new equilibrium of about 0.045). Alternatively, consider a scenario where a marine population restricted to a glacial refugium during the Pleistocene epoch subsequently dispersed into different areas, where it maintained moderately large population sizes ($N_e = 10^4$) connected by weak gene flow ($mN_e = 1$, so $m = 10^{-4}$).

Equation 4 indicates that under these conditions, it would take more than 2,700 generations for F_{ST} to reach even half its new equilibrium value of 0.2. It is thus clear that many large marine populations whose distribution was affected by Pleistocene or more recent events could have F_{ST} values that are well below equilibrium for current levels of gene flow. In these cases, use of equilibrium models based on empirical F_{ST} values would overestimate current levels of gene flow, which would tend to underestimate potential for local adaptation. This would be particularly true for species with long generation times.

Figure A-10 illustrates these points by contrasting time to reach equilibrium for two groups of 10 populations: one with $N_e = 100$ in each subpopulation and one with $N_e = 1{,}000$. In this example, both groups had the same level of original population subdivision ($F_{ST} \approx 0.04$) and both were completely homogenized ($F_{ST} = 0$) by anthropogenic activities. If quasi-natural conditions and original levels of connectivity are restored, the system with relatively small ($N_e = 100$) subpopulations will approach its original F_{ST} level within about 10 generations—a time that is very rapid on evolutionary time scales but still could represent a considerable number of years for long-lived species. In contrast, in the system of larger subpopulations, restoration of the original level of population subdivision would require 200–300 generations.

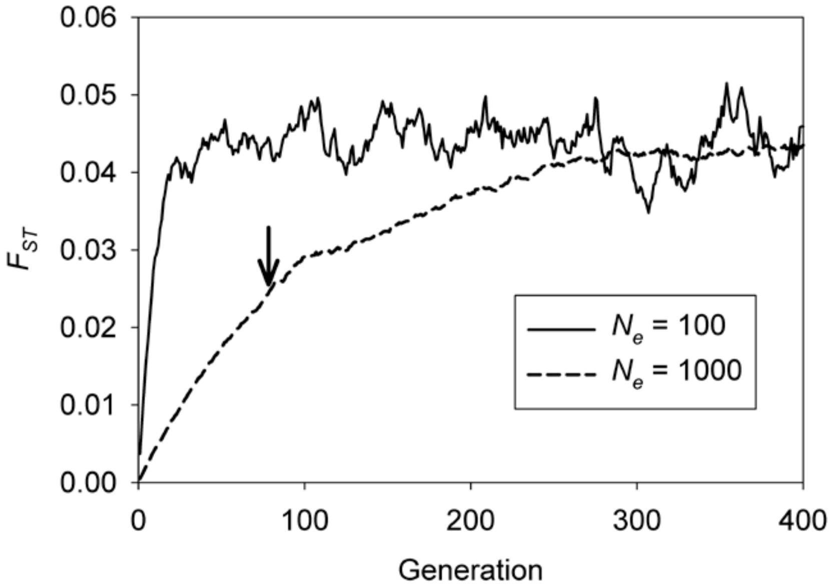

Figure A-10. Time to reach migration-drift equilibrium (indicated by stable F_{ST} over time) in two groups of 10 subpopulations with different effective sizes (N_e = 100 and N_e = 1,000). It is assumed that both metapopulations originally were characterized by a level of migration such that $mN_e = 4$, which produces an equilibrium F_{ST} of about 0.045, and that these natural migration rates were restored after an event that completely homogenized population subdivision. Results are based on simulated data using 20 microsatellite loci, each with a maximum of 10 alleles. The arrow indicates 81 generations, the theoretical time to move 50% of the way to a new equilibrium based on Equation 4 and assuming N_e = 1,000, m = 0.004.

Ryman et al. (1995b) proposed that F_{ST} might be used as a metric to gauge the effects of loss of diversity among populations. One possible criterion might be that anthropogenic activities should not be allowed to reduce F_{ST} appreciably from the value that occurs under "natural" conditions.

This example of inherent risk trade-offs emphasizes the point that once humans intervene directly in survival and reproduction of natural populations, we become managers of these processes. Over evolutionary time scales, natural populations sort out among themselves the levels of connectivity that are adaptive. When humans intervene and decide what levels of connectivity to manage for, errors are possible in both directions. Many (perhaps most) species that are prospective targets of marine aquaculture can disperse widely, either as adults, eggs/larvae, or both. Furthermore, aquaculture escapes might disperse more widely than typical natural fish because they don't have an identifiable home range or territory. This effect is well documented in some hatchery and aquaculture programs for Pacific and Atlantic salmon; the extent to which it applies to typical marine species largely remains to be determined.

Loss of fitness

Frankham (2008) reviewed empirical evidence for genetic adaptation to captivity in species conservation programs. He found predictable correlations between degree of domestication and selection strength, N_e, and number of generations in captivity and discussed some factors that could help alleviate deleterious effects on wild populations when captive populations were reintroduced to the wild. Christie et al. (2012) showed that adaptation of wild steelhead to a hatchery environment can be detected within a single generation, with potentially serious consequences for fitness in the wild. An analysis by Doyle (1983) of domestication in aquaculture settings suggested that inadvertent domestication selection can be as strong as deliberate artificial selection. Hauser and Carvalho (2008) reviewed evidence that is beginning to accumulate to suggest that even marine species with high dispersal capability can exhibit local adaptations.

Inbreeding depression and outbreeding depression can be thought of as phenomena that occur at either extreme of a continuum that measures genetic differences among individuals that interbreed (Waples 1995). Reduced fitness can occur if the individuals are either too similar genetically (as in close relatives) or too genetically divergent. Lynch (1991) presented a uniform framework for considering inbreeding depression and outbreeding depression and showed that it is possible for both phenomena to occur at the same time.

Outbreeding depression can occur through two related mechanisms: a) loss of local adaptation (simple dilution of locally adapted alleles by nonlocal alleles) or b) breakdown of coadapted gene complexes (groups of genes that function effectively as a unit). If individuals from two divergent populations interbreed, loss of local adaptation is generally found in the F_1 generation, but breakdown of coadapted gene complexes typically is seen only in the F_2 or later generations (unless the fitness consequences resulting from this breakdown are very strong). This is because F_1 hybrids contain one complete chromosomal complement from each population, and it is not until they interbreed to produce the F_2 generation that genetic shuffling occurs among the parental sets of chromosomes (Tallmon et al. 2004). Recently, Edmands (2007) and Frankham et al. (2011) reviewed evidence for both inbreeding depression and outbreeding depression and reached somewhat different conclusions about their relative importance: Edmands (2007) concluded that the risks were more or less comparable and Frankham et al. (2011) concluded that the risks of outbreeding depression were generally rather small. They agreed, however, that empirical studies of inbreeding depression are much more common than those of outbreeding depression. Nevertheless, the direct consequences of inbreeding have seldom been examined in fish in the wild (Wang et al. 2002). Thrower and Hard (2009) showed that these consequences can be serious for survival of steelhead released to the wild, even if inbreeding depression is weak or undetectable in captivity.

Tufto (2001) used a simple quantitative genetic model to evaluate the demographic consequences of releasing individuals that are maladapted to local conditions (as might occur unintentionally with aquaculture escapes). He found that reductions in local abundance can occur if the fraction of released individuals is sufficiently large and they are sufficiently maladapted to local conditions.

Lorenzen (2005) presented a detailed theoretical analysis of the potential for marine stock enhancement to provide societal benefits over and above those that can be obtained from optimal exploitation of wild stocks alone. His analysis focused on population dynamics, recruitment, and density dependence and accounted for biological differences between hatchery and wild fish. Although the latter did not involve an explicit genetic model, some of his results are relevant to the question of whether it is better to pursue strategy 1, keep the cultured population as similar as possible to the wild population, or strategy 2, maximize divergence between cultured and wild population.

Lorenzen concluded that, assuming gene flow from the cultured population into the wild continues indefinitely at a constant rate, the worst-case scenario is when the hatchery population is only moderately maladapted compared to the wild population. This implies that either extreme strategy (if attainable) might be preferable to an intermediate one. If this is the case, then the optimal strategy will depend on a) the shape of the relationship between the degree of hatchery-wild difference and wild population fitness and b) the practical limits to how “similar” and “different” the hatchery and wild populations can be (Figure 6, body of this report; see also Lorenzen et al. 2012, Huisman and Tufto 2012).

This conclusion is supported by a more recent study by Baskett and Waples (in press), who used a model that includes quantitative genetics and population dynamics to tackle the key question regarding the merits of strategy 1 versus strategy 2. Important results include the following:

- Although the quantitative genetic model developed by Lande (1976) and adopted by Ford (2002) has been shown to be relatively robust to simplifying assumptions, extreme forms of the “different” strategy strongly violate two key assumptions: selection is weak and phenotypic distributions are normal and unimodal. As a consequence, it is necessary to consider the full phenotypic distributions to properly evaluate the “different” strategy.
- In their extreme forms, either strategy could potentially be consistent with high wild population fitness. Whether the necessary extremes can be achieved is an open question. For either strategy, it will be important to evaluate the consequences for wild populations if things do not go as planned.
- The “different” strategy is only viable if there are opportunities for strong purifying selection to eliminate maladapted genotypes after escape but before reproduction.
- Effects on fitness can be sensitive to the relative life cycle timing of escape/release, density dependence, and natural selection.

In its most common formulation, Dollo’s Law, named after the late nineteenth and early twentieth century Belgian paleontologist Louis Dollo, states that complex characters that have been lost during the course of evolution cannot be regained (Simpson 1953). It is something less than a law, as some exceptions have been noted. A broader view of the underlying principle is that evolution is generally irreversible for either of two reasons: 1) evolution of even simple structures is exceedingly complex and it is statistically improbable that the exact same course of evolution would be followed more than once, forwards or backwards; 2) the process of evolution is constrained in many ways, and many transitions might only be permissible in one direction. If novel selective pressures are relaxed, the rate at which fitness increases will not necessarily be as rapid, because the forces acting to increase fitness might not be as strong (Allendorf and Hard 2009). For a recent review of ideas related to Dollo’s Law, see Collin and Miglietta (2008).

In a recent summary of 266 peer-reviewed papers, Araki and Schmid (2010) asked whether hatchery stocks and wild stocks differed in fitness and in genetic variation, and whether stocking affected population abundance. A variety of species were included in their review—

salmonid and flatfish species were most studied (17.3% and 15.1%, respectively), followed by bream, drum, and cod species with 8.1%, 4.4%, and 4.0%. Seventy studies compared hatchery and wild stocks, of which 23 showed significantly negative effects of hatchery rearing on the fitness of stocked fish and 28 showed reduced genetic variation in hatchery populations. None of these studies showed a positive effect of hatchery rearing on the fitness of released fish.

Araki and Schmid (2010) found a few cases where no obvious effect of hatchery rearing was observed and a positive contribution to population abundance was indicated. Studies on black sea bream (*Acanthopagrus schlegelii*) in Hiroshima Bay, Japan, did not reveal a fitness effect of hatchery rearing and also provided the only example of a potentially positive contribution of hatchery stocking on population abundance (Jeong et al. 2007, Blanco Gonzalez et al. 2008a, 2008b). Araki and Schmid (2010) noted that this case study also provided an example of reduced size-at-age over time, potentially due to the intensive stocking and the likely increased competition for natural resources (Blanco Gonzalez et al. 2009). A recent study from Korea suggested loss of alleles in farmed black sea bream relative to wild populations, but no significant reduction in heterozygosity (An et al. 2010).

The last decade has seen increasing recognition that most problems in conservation are not strictly ecological or evolutionary, but eco-evolutionary in the sense that they involve the interaction of ecological and evolutionary processes (Stockwell et al. 2003, Kinnison and Hairston 2007, Ezard et al. 2009, Carlson et al. 2011). This perspective is particularly important in evaluating the consequences of fitness reductions in wild populations caused by genetic interactions with cultured individuals. Although natural selection can help restore fitness if the flow of maladapted genes stops, this comes at a demographic cost to the population.

Risks to Natural Populations from other Anthropogenic Activities

This is a very large topic and we make no attempt to be comprehensive. To provide some context for evaluating effects of marine aquaculture, we briefly discuss three anthropogenic factors that have potentially large effects on natural populations of marine species.

Fishing

In the United States, the Magnuson-Stevens Fishery Conservation and Management Act (MSA, Public Law 94-265, revised 1996 and 2006) directs conservation and management actions for marine species under federal jurisdiction. National Standard One of the revised statute stipulates that, “Conservation and management measures shall prevent overfishing while achieving, on a continuing basis, the optimum yield from each fishery for the United States fishing industry.”

The term overfishing has been defined as a level of fishing mortality that jeopardizes the capacity of a fishery to produce the maximum sustainable yield on a continuing basis, while overfished describes a stock whose size is sufficiently small that a management change is required to achieve rebuilding. Fixed biological references points are used to determine whether stocks are overfished; if so, the MSA automatically triggers aggressive management actions that are projected to rebuild the stock within a short time period (10 years or less in most cases).

These provisions make MSA one of the strongest conservation laws in the world. By way of comparison, for example, Canada has no federal fishery management system with fixed biological reference points comparable to MSA, and COSEWIC (Committee on the Status of Endangered Wildlife in Canada, the scientific body charged with assessing status under Canada's Species at Risk Act) has concluded that a number of marine fish populations in Canada are at significant extinction risk (a list of marine species that have been evaluated is at http://www.cosewic.gc.ca/eng/sct5/index_e.cfm).

However, in spite of some successful rebuilding efforts, the latest assessment shows that at least 46 U.S. stocks remain overfished, according to MSA guidelines (NMFS 2009). Even though overfished stocks might still have relatively large numbers of adults, reductions in allelic diversity can be substantial (Ryman et al. 1995b). Furthermore, MSA does little to address increasing concerns by some that fishery selectivity (in particular, preferential harvest of older and larger individuals) exerts strong evolutionary pressure that can change genetically based life history traits of natural populations, potentially compromising their long-term viability (Walsh et al. 2006, Law 2007). Under many plausible scenarios, harvest rates that are consistent with long-term sustainability could be much lower than those that would be predicted to produce maximum sustainable yield using current models.

Unfortunately, our understanding of the genetic underpinnings of life history traits susceptible to fishery selectivity is too limited to permit robust predictions about long-term consequences of selective harvest. In some respects, therefore, uncertainties about the evolutionary consequences of selective harvest parallel uncertainties associated with long-term fitness consequences of interbreeding between captive and wild fish. Although Hard et al. (2008) concluded that no single study has irrefutably demonstrated fishery-induced evolution in a wild population, considerable circumstantial evidence for its occurrence exists. In addition, empirical evidence is accumulating that evolutionary changes in life histories of some heavily exploited fish populations might already be widespread (Swain et al. 2007). High and selective exploitation has the potential to substantially decrease genetic diversity and reduce the capacity of a population to respond to natural evolutionary forces, even if exploitation and the intensity of selection are subsequently reduced (Allendorf and Hard 2009). Indeed, the failure of many collapsed populations to recover following substantial reductions in exploitation (Hutchings 2000) provided early indications that fishery-induced evolution might result from overexploitation.

Figure A-11 illustrates some of the tradeoffs inherent in implementation of harvest management. Low harvest rates (0 ≈ 0.2) promote diversity and ecosystem functions (high biomass, large individuals, few collapsed species); moderate harvest rates (0.4 ≈ 0.6) can maximize catch but have noticeable ecosystem effects; higher harvest rates generally produce greater employment opportunities but at substantial biodiversity costs (see also Botsford et al. 1997).

Loss and degradation of habitat

For terrestrial species around the world, loss and degradation of habitat is perhaps the most pervasive threat to biodiversity. In contrast, the role that offshore marine habitats play in population dynamics of marine species is poorly understood, in part because studies are difficult

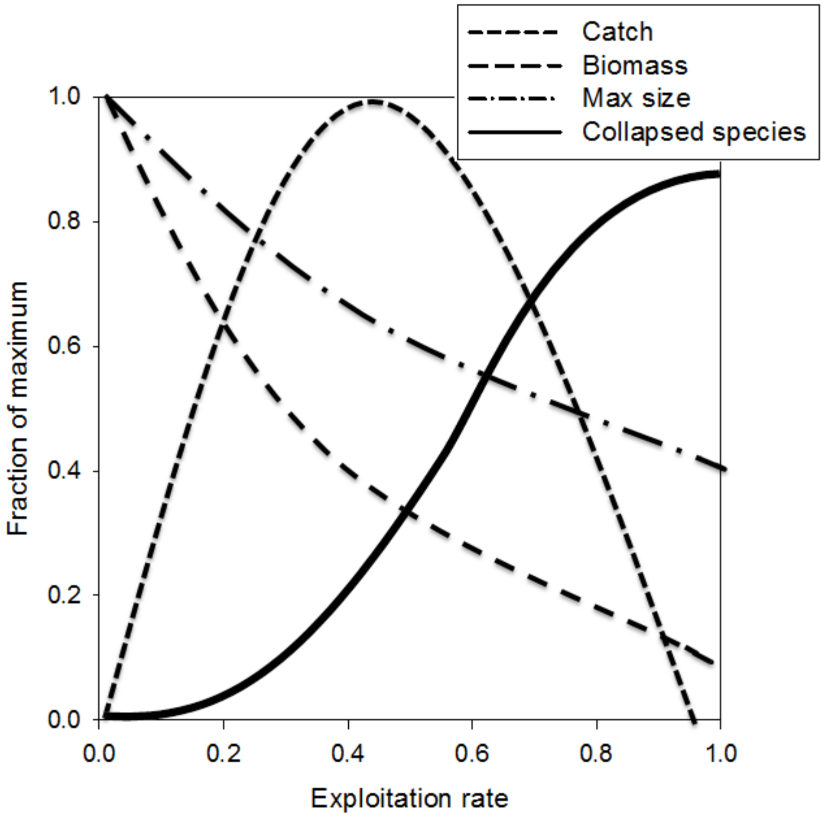

Figure A-11. Schematic representation of expected effects of increasing exploitation rate on several biological indicators of population and ecosystem health. (Adapted from an analysis by Worm et al. 2009 of the Georges Bank marine fish community.)

to conduct. Bottom trawls can dramatically affect the sea floor as well as structure-forming invertebrates that play primary roles in supporting marine ecosystems (de Marignac et al. 2008). Until the 1980s, these activities were largely restricted to soft bottom habitats, but in recent years technological advances have allowed trawlers to fish nearly all benthic habitats on the continental shelves and deeper slopes (CEEF 2002).

Effects of anthropogenic changes to nearshore habitats are better documented than their offshore counterparts. A large number of marine fish species with offshore distributions as adults use nearshore and estuarine habitats for juvenile rearing (Beck et al. 2001). This is typically the most sensitive life stage for marine species. Nearshore habitats are also indirectly affected by land-based activities that alter patterns of biogenic and inorganic material delivery to the ocean. For example, a series of large, mainstem dams and reservoirs in the Columbia River basin has dramatically altered physical and biological characteristics of the river's plume (Bottom et al. 2005), which can play an important ecological and energetic role for planktivores such as larval and juvenile fish (Peterson and Peterson 2008).

Global climate change poses many complex challenges for fishery management (Hollowed and Bailey 2009), but two in particular are directly relevant to habitats provided by reef-building corals. On the one hand, warmer sea temperatures can exceed the thermal tolerance for corals and their photosynthetic symbionts, leading to mass bleaching events. On the other hand, higher atmospheric CO_2 levels reduce pH in the ocean, making it more difficult for calcifying organisms to build their skeletons. In the decades to come, these two factors are expected to create extremely stressful conditions for corals and the organisms that depend on them for food and habitat (Hoegh-Guldberg et al. 2007).

Pollution

To date, efforts to assess pollution effects have primarily focused on pollution that is both inexpensive to monitor and overtly harmful to coastal and blue-water ecosystems. Much less is known about the impacts of toxic chemical contaminants that require expensive analytical methodologies to track in the marine environment. Toxics also pose a complex research challenge because they have nuanced (and often nonlethal) but important effects on physiology and performance of individual fish. Understanding linkages between toxic compounds in the marine environment and the fitness of marine organisms requires research expertise in immunology, reproductive biology, endocrinology, neurobiology, developmental biology, and other specialized physiological disciplines. Moreover, evaluating the significance of pollution relative to other human-induced impacts requires quantitative extrapolation of biological responses for specific organ systems in individual fish up to the scale of populations and communities. This is thus a very challenging area of research, but work by NOAA scientists and others is beginning to connect the dots between toxic chemicals and the health, viability, and sustainable management of wild fish stocks. Key factors to consider in assessing overall risks from pollution include the following:

1. Marine fish are still at risk from pollution discharges that took place in the twentieth century (Peterson et al. 2003). Many toxics are pervasive and persistent in the marine environment, and they can be difficult or impossible to mitigate once released.
2. Society is manufacturing and releasing chemicals into the ocean at a rate that exceeds the capacity of scientific institutions to study and understand their ecological impacts. More than 75,000 potentially toxic substances are currently manufactured in the United States. Of these, the chemicals that pose a substantive risk to marine fish populations probably number in the thousands. Classes of chemicals of emerging concern include nanomaterials, pharmaceuticals, and plasticizers.
3. Pollution inputs to the ocean are highly heterogeneous in space and time. Although persistent and bioaccumulative chemicals can now be found in every corner of the globe, the geographical severity of the pollution problem tends to scale with human population density. All socioeconomic indicators point to an expanding pollution problem in the decades ahead, in tandem with human population growth and development along the coastal margins of the United States. For example, the future risk of oil spills will likely increase in proportion to increasing maritime commerce and transport.
4. Diffuse sources of pollution (e.g., atmospheric deposition and terrestrial stormwater runoff) have overtaken conventional end-of-pipe discharges in terms of total loadings to the ocean. These nonpoint sources are much more difficult to control.
5. The extent to which toxics will interact with other large-scale ecological forcing pressures (e.g., climate change and ocean acidification) is largely unknown.

6. Existing coastal monitoring programs throughout the United States are inadequate to accurately profile pollution exposure for many marine fish stocks.

Discussion

Although these three risks from other anthropogenic activities were considered above under separate subheadings, they often have overlapping and synergistic effects on natural populations. For example, in the broad sense pollution can be considered a component of habitat, and many physical effects on marine habitats would co-occur with pollution, especially in coastal areas. Furthermore, pollution is one of the ecological impacts of marine aquaculture that has caused the greatest concern and the motives for initiating artificial propagation programs can be tied to habitat loss. Finally, an unfortunate and long-lasting habitat consequence of some fishing activities is the persistence of derelict fishing nets that pose ongoing risks to marine species (Good et al. 2010). Therefore, these anthropogenic impacts are not independent and their cumulative impacts can be synergistic (Crain et al. 2008, Hutchings and Fraser 2008).

Managing Risk

Monitoring

The primary events that aquaculturists, public regulatory agencies, or both should monitor and quantify where feasible include: the magnitude and frequency of escapes, the frequency of reproductive encounters, occurrence of F_1 hybrid offspring between cultured and wild fish, and the occurrence of backcross descendants in the wild, indicative of genetic introgression (Kapuscinski et al. 2007). These events can help in estimating the likelihood of adverse genetic effects. This likelihood is a function of a number of probabilities, acting in sequence, including the probability of escape, survival of escaped fish to maturity, probability of encounter, probability of mating, probability of successful reproduction, and the probability of postreproduction survival of hybrid offspring in the wild.

Key considerations

The consequences of genetic interactions between cultured and wild marine fish for natural population structure and viability depend on a number of key factors. The parameters that tend to determine the consequences of genetic interactions between these groups, once they occur, depend primarily on the genetic divergence between the groups, particularly for traits important to survival and fitness in the wild. These parameters include the difference in genetic background between the cultured and wild individuals, and the degree to which plasticity influences the expression of the phenotype in the wild versus the protective culture environment.

Careful monitoring must be a priority

Determining whether such consequences have occurred requires constant vigilance and consistent, careful monitoring. Aquaculture monitoring programs should be designed and implemented before the initiation of a culture program or commercial operation. Monitoring design is critical; it must identify specific, measurable end points that are closely linked to the presence and impact of escaped cultured fish (Senanan et al. 2007). These end points include

(Figure 9, body of this report): the pattern and frequency of escaped cultured fish at all life stages (their presence, abundance, and distribution), detecting F_1 progeny (their presence, abundance, and distribution), detecting backcross progeny (their presence, abundance, and distribution), detecting changes in both cultured and wild populations (their abundances and phenotypic differences), and detecting changes in local fish species and their relative abundance. Some important issues to keep in mind are the power to detect such effects—which depends on the size of the program, the differences between the groups, and the efficacy of monitoring—and identifying clear remedial responses when effects are detected. The use of genetic markers, external marks, and other tools can greatly facilitate detection.

Use of Models in Research

Reliable predictions of the fate of a wild population receiving immigration of cultured fish would require that all of the population parameters were known; this is hardly true of any population and particularly not for wild fish populations (Ryman et al. 1995a). Ecologically and genetically realistic experiments, such as lifetime fitness studies (and lifetime effects studies), are time consuming and costly to undertake and nonetheless only describe a particular combination of wild and cultured fish for a particular set of environmental conditions. The only available solution to this problem is to use the results of empirical and experimental studies to develop computer-based simulation models. A handful of such models are presented below.

Modeling how interbreeding can change the composition of wild populations

Hutchings (1991) modeled the threat to wild salmon populations experiencing various intrusion rates of escaped farmed salmon and small or large fitness differences among the offspring. Based on data from common garden experiments of wild and farm Atlantic salmon, Hindar et al. (2006) developed a more detailed model to predict the future of wild salmon populations experiencing invasions of escaped farm salmon. Simulations with a fixed intrusion rate of 20% escaped farm salmon at spawning (a long-term average for Norwegian populations) suggest that substantial changes take place in wild salmon populations within 10 salmon generations (≈40 years). Low-invasion scenarios suggest that farm offspring are unlikely to establish in the population, whereas high-invasion scenarios suggest that populations are eventually composed of hybrid and farm descendants. Recovery of the wild population was not likely under all circumstances, even after many decades of no further intrusions. The model also suggested that managers of wild salmon will have difficulty finding broodstock of the original wild population after a few generations of high intrusion rates.

Quantitative genetic model for immigration of maladapted individuals

A model incorporating density-dependent effects of escaped farmed fish on wild populations was developed by Tufto (2001). He used a quantitative genetic model that included immigration of maladapted individuals into wild populations where the outcome was determined by density-dependent regulation and local stabilizing selection. One result was a reduction in total equilibrium size (carrying capacity) when immigrants deviated more than 2.8 genetic SDs from the local optimum and immigration was high relative to the strength of stabilizing selection. Comparison of the selected strains of farmed Atlantic salmon and wild populations (e.g., in growth rate) suggests that the above conditions are typical of those that occur presently in many rivers (Tufto 2001).

Modeling changes in effective population size (N_e) caused by interbreeding

Any reduction in absolute population size is expected to lead to a reduction in N_e, unless the species has some behavioral pattern that equalizes reproductive success or family size when numbers decline. Changes in migration patterns between local populations also have the potential to change the N_e of the total population. Whereas low, symmetric gene flow between populations increases the total N_e, asymmetric gene flow will decrease it (Tufto and Hindar 2003). In the extreme case of one-way gene flow, the total N_e will eventually approximate that of the donors. As an example, it has been estimated that the major strains of farm Atlantic salmon in Norway have an average N_e of about 80 individuals. If we ignore genetic differences between each of the four major strains, the total N_e of farm salmon is roughly 320 individuals. The total N_e of the wild Atlantic salmon is not known, but it is probably on the order of 10^4 or 10^5 per generation. The total N_e of the farm plus wild salmon, assuming one-way gene flow from escaped farmed to wild populations, is then N_e = 320 individuals (Tufto and Hindar 2003).

The Trojan Gene hypothesis for genetically modified fish

Muir and Howard (1999) developed the Trojan Gene hypothesis to study a situation where genetically modified fish are developed that attain a large size at sexual maturity and thereby totally dominate spawning, should they escape to the wild. The Trojan Gene hypothesis has been used to model introduction(s) of cultured fish (genotypes) that have superior fitness at breeding but leave offspring with very poor survival capabilities, potentially leading to a population extinction vortex. A net fitness model to help quantify trade-offs between mating advantages of genetically modified organisms in the wild and reduced offspring viability was subsequently developed by Muir and Howard (2001, 2002). This model can be useful in evaluating relative invasion and extinction risks (Kapuscinski et al. 2007).

Appendix B: Aquaculture Genetic Management Plan (AGMP)

Note: This is a draft attempt to adapt the Hatchery Genetic Management Plan template that is used in the Pacific Northwest for salmon hatcheries to finfish aquaculture programs.

1. General Program Description

1.1. Name of aquaculture program.

1.2. Species and population (or stock) under culture.

Give common and scientific names.

1.3. Responsible organization and individuals.

Indicate lead contact and on-site operations staff lead.
Name (and title):
Institution or Company:
Address:
Telephone:
Fax:
Email:

Other institutions, companies, co-operators, or organizations involved, including contractors and extent of involvement in the program:

1.4. Location(s) of culture program and associated facilities.

Include location and state.

1.5. Type of program.

The default assumption is “closed culture” (natural populations not regularly incorporated into broodstock); explain if different.

1.6. Purpose (goal) of program.

The default assumption is commercial production; explain if different. Example: “The goal of this program is the production of red drum for commercial food use.”

1.7. List of program Performance Standards.

Performance standards are designed to achieve the program goal or purpose, and are generally measurable, realistic, and time specific. Examples of these standards, their corresponding indicators, and how they are incorporated into a monitoring and evaluation plan are shown in Table B-1.

Table B-1. Example of performance standards.

Performance standards	Performance indicators	Monitoring and evaluation plan
Meet program production goals	Number of adults collected and individuals reared by life stage	Monthly program production records
Minimize opportunities for interaction with wild fish through proper broodstock management and marking	Numbers (type) of marked fish	Monthly program production records
Maintain stock integrity and genetic diversity	Number of broodstock collected, phenotypic characteristics (sex, age, size, etc.), N_e	Spawning guidelines, monthly program production records
Minimize interactions with wild fish through effective containment methods	Records of documented escapes and losses unaccounted for	Monthly program production records, marking data
Maximize in-culture survival of broodstock and their progeny	Fish culturists and pathologists will monitor the health of cultured stocks on a monthly basis and recommend preventative actions to maintain fish health	Monthly program production records Fish health monitoring records
Limit the impact of pathogens associated with culture program on wild fish	Fish pathologists will diagnose fish health problems and minimize their impact Vaccines will be administered when appropriate to protect fish health	Fish health monitoring records
	A fish health database will be maintained to identify trends in fish health and disease and implement fish health management plans based on findings	
Ensure culture operations comply with state and federal water quality standards through proper environmental monitoring	National Pollutant Discharge Elimination System (NPDES) compliance requirements	Monthly NPDES report records

1.8. List of program performance indicators, designated by benefits and risks.

Performance indicators provide information about the degree to which program standards have been achieved. Monitoring and evaluation of performance indicators is important to determine the success of efforts to maximize benefits and minimize risks. The list of performance indicators should be separated into two categories: benefits that the aquaculture program will provide to the industry, or in meeting culture objectives while minimizing adverse impacts on natural species and the environment; and risks to natural fish that can be posed by the aquaculture program, including indicators that respond to uncertainties regarding program effects associated with a lack of data.

1.8.1. Performance indicators addressing benefits.

Example: “Quantify survival and production rates for aquaculture program fish to market.”

1.8.2. Performance indicators addressing risks.

Example: “Quantify escapes of aquaculture fish and their genetic impacts on natural fish populations.”

1.9. Expected size of operation or program.

In responding to the two elements below, take into account the potential for effects on natural populations from escapes of cultured fish or from collection of natural fish for broodstock.

1.9.1. Proposed annual broodstock collection level, if any (maximum number of adult fish).

1.9.2. Expected annual level of escapes (maximum number) by life stage and location.

Use standardized life stage definitions by species: embryo, larva, juvenile, subadult, mature adult, spawning adult.

1.10. Current program performance, including estimated survival rates, production levels, and escape levels. Indicate the source of the data.

Provide estimated survival rate, total production number, and escape number data available for the most recent 12 years, or for the number of years of available and dependable information. Indicate program goals for these parameters.

1.11. Date program started (years in operation), or is expected to start.

1.12. Expected program duration.

2. Relationship of Program to other Management Objectives

2.1. Describe alignment of the aquaculture program with any regional or national aquaculture plan or policy or other regionally accepted policies. Explain any proposed deviations from the plan or policies.

Example: "The aquaculture program will be operated consistent with the____________, with the exception of ____________."

2.2. List all existing cooperative agreements, memoranda of understanding, memoranda of agreement, or other management plans or court orders under which program operates.

Indicate whether this AGMP is consistent with these plans and commitments, and explain any discrepancies.

3. Facilities

Provide descriptions of the aquaculture facilities that are to be included in this plan, including dimensions of collection, holding, incubation, and rearing facilities. Indicate the fish life stage held or reared in each. Also describe any instance where operation of the culture facilities, or new construction, results in destruction or adverse modification of critical habitat designated for natural populations.

3.1. Broodstock collection facilities (or methods).

3.2. Fish transportation equipment (description of pen, tank truck, or container used).

3.3. Broodstock holding and spawning facilities.

3.4. Incubation facilities.

3.5. Rearing facilities.

3.6. Describe operational difficulties or disasters that have led to significant fish mortality.

3.7. Indicate available backup systems, and risk aversion measures that will be applied, that minimize the likelihood for the take of natural fish that can result from equipment failure, water loss, flooding, disease transmission, or other events that could lead to injury or mortality.

Example: "The facility will be staffed full-time, and equipped with a low-water alarm system to help prevent catastrophic fish loss resulting from system failure" or "The program will use only commercially available broodstock and will not involve direct take of wild fish."

4. Broodstock Origin and Identity

Describe the origin and identity of broodstock used in the program, its status, annual collection goals, and relationship to wild fish of the same species and population.

4.1. Source.

List all historical sources of broodstock for the program. Be specific (e.g., natural spawners from location X; commercial broodstock from Corporation Y).

4.2. Supporting information.

4.2.1. History.

Provide a brief narrative history of the broodstock sources. For natural populations, specify their status relative to critical and viable population thresholds. For existing aquaculture stocks, include information on how and when they were founded, sources of broodstock since founding, and any purposeful or inadvertent selection applied that changed characteristics of the founding broodstock.

4.2.2. Program size.

Specify number of breeders of each sex to be used each year or the total number and sex ratio. Specify plans for rotating or replacing broodstock and how long (number of years) individual breeders will be used. For broodstocks originating from natural populations, provide estimates of the proportion of the natural population that will be collected for broodstock and explain how broodstock collection will affect the natural population status relative to critical and viable thresholds.

4.2.3. Past and proposed level of natural fish in broodstock.

If using an existing broodstock, include information on how many natural fish were incorporated annually into the broodstock.

4.2.4. Genetic or ecological differences.

Describe any known genotypic, phenotypic, or behavioral differences between current or proposed aquaculture stocks and natural stocks in the target area. What is the source of this information?

4.2.5. Reasons for choosing.

Describe any special traits or characteristics for which broodstock was selected.

4.3. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse genetic or ecological effects to listed natural fish.

5. Broodstock Collection

Include information on the location, time, and method of capture (e.g., net, trap, beach seine, etc.) Describe capture efficiency and measures to reduce sources of bias that could lead to a nonrepresentative sample of the desired broodstock source.

5.1. Source.

Indicate whether commercial broodstock will be used or wild fish will be collected. If the latter, describe the method for a) identifying the target population, if more than one population might be present, and b) distinguishing aquaculture-origin fish from natural fish.

5.2. Sampling design.

Identify the target number and life stage to be collected. Provide data for previous years if applicable.

5.3. Disposition of aquaculture-origin fish collected in surplus of broodstock needs.

Describe procedures for remaining within programmed broodstock collection or allowable cultured fish levels, including culling.

5.4. Fish transportation and holding methods.

Describe procedures for the transportation (if necessary) and holding of fish, especially if captured unripe or as juveniles. Include length of time in transit and care before and during transit and holding, including application of anesthetics, salves, and antibiotics.

5.5. Describe fish health maintenance and sanitation procedures applied.

5.6. Disposition of carcasses.

Include information for spawned and unspawned carcasses, sale or other disposal methods, and use.

5.7. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse genetic or ecological effects to listed natural fish resulting from the broodstock collection program.

Example: “This program will use commercial broodstock and will not involve direct take of wild fish” or “The risk of fish disease amplification will be minimized by following Fish Health Policy sanitation and fish health maintenance and monitoring guidelines.”

6. Mating

Describe fish mating procedures that will be used, including those applied to meet performance indicators identified previously.

6.1. Selection method.

Specify how spawners are chosen (e.g., randomly over natural breeding season, randomly from mature fish on a certain day, selectively chosen, or prioritized based on aquaculture or natural origin).

6.2. Males.

Specify expected use of backup males, and repeat spawners.

6.3. Fertilization.

Describe spawning protocols applied, including the fertilization scheme used (such as equal sex ratios and 1:1 individual matings, equal sex ratios and pooled gametes, or factorial matings). Explain any fish health and sanitation procedures used for disease prevention.

6.4. Cryopreserved gametes.

If used, describe number of donors, year of collection, number of times donors were used in the past, and expected and observed viability.

6.5. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse genetic or ecological effects to listed natural fish resulting from the mating scheme.

7. Incubation and Rearing

Specify any management goals (e.g., increase juvenile survival) under which the aquaculture program is currently operating for the stock in the appropriate sections below. Provide data on the success of meeting the desired hatchery goals.

7.1. Incubation.

7.1.1. Number of eggs taken and survival rates to hatching.

Provide data for the most recent 12 years (1998–2009) or for years dependable data are available.

7.1.2. Cause for, and disposition of, surplus egg takes.

Describe circumstances where extra eggs can be taken (e.g., as a safeguard against potential incubation losses), and the disposition of surplus fish safely carried through to the egg or larval stage to prevent exceeding programmed levels.

7.1.3. Loading densities applied during incubation.

Provide egg size data, standard incubator flows, standard loading, and other physical conditions (or other incubation density parameters).

7.1.4. Incubation conditions.

Describe monitoring methods, temperature regimes, minimum dissolved oxygen criteria (influent/effluent), silt management procedures (if applicable), and any other parameters monitored.

7.1.5. Fish health maintenance and monitoring.

Describe fungus control methods, disease monitoring and treatment procedures, incidence of yolk-sac malformation, and egg mortality removal methods.

7.1.6. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse genetic and ecological effects to fish during incubation.

Example: “Eggs will be incubated using __________ to minimize risk of catastrophic loss due to __________.”

7.2. Rearing.

7.2.1. Provide survival rate data (average program performance) by life stage for the most recent 12 years (1998–2009) or for years dependable data are available.

7.2.2. Density and loading criteria (goals and actual levels).

Include density targets (lbs fish/gpm flow, lbs fish/ft^3 rearing volume, etc.).

7.2.3. Fish rearing conditions.

Describe monitoring methods, temperature regimes, minimum dissolved oxygen, CO_2, total gas pressure criteria (influent/effluent if available), and standard management procedures applied to rear fish.

7.2.4. Indicate biweekly or monthly fish growth information (average program performance), including length, weight, and condition factor data collected during rearing, if available.

7.2.5. Indicate monthly fish growth rate and energy reserve data (average program performance), if available.

7.2.6. Indicate food type used, daily application schedule, feeding rate range (e.g., % body weight/day and lb/gpm inflow).

7.2.7. Fish health monitoring, disease treatment, and sanitation procedures.

7.2.8. Indicate the use of “natural” rearing methods as applied in the program.

7.2.9. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse genetic and ecological effects to fish under propagation.

8. Program Effects on Natural Populations

8.1. List all permits or authorizations in hand for the aquaculture operation or program.

8.2. Provide descriptions, status, and projected actions that could affect natural populations in the target area.

Note: This is a feature of the Hatchery Genetics Management Plan process for salmon in the Pacific Northwest. In other applications, it will be important to determine whether it is more appropriate for this section to be completed by the applicant or the regulatory agency.

8.2.1. Description of natural population(s) potentially affected by the program.

Include information describing: adult age class structure, sex ratio, size range, migration timing, spawning range, spawn timing, and juvenile life history. Emphasize spatial and temporal distribution relative to cultured fish release locations.

8.2.2. Status of natural population(s) potentially affected by the program.

Describe the status of the natural population(s) relative to "critical" and "viable" population thresholds (see definitions in Attachment 1).

Provide the most recent 12-year (e.g., 2000-present) abundance data, survival data by life-stage, or other measures of productivity for the natural population, if available. Indicate the source of these data.

8.2.3. Describe aquaculture activities, including associated monitoring and evaluation and research programs that can lead to genetic effects on natural fish in the target area, and provide estimated annual levels of take (see Attachment 1 for definition of take).

Describe aquaculture activities that can lead to the take of natural populations in the target area, including how, where, and when the takes might occur; the risk potential for their occurrence; and the likely effects of the take. Example: "Broodstock collection directed at red drum has a "high" potential to take natural red drum adults, through capture, and handling at _______ between _______ (date) and _______ (date). Trapping and handling devices and methods can lead to injury to natural fish through descaling, delayed migration and spawning, or delayed mortality as a result of injury or increased susceptibility to predation."

Provide information regarding past takes associated with the program (if known), including numbers taken and observed injury or mortality levels for natural fish.

Provide projected annual take levels for natural fish by life stage (juvenile and adult) quantified (to the extent feasible) by the type of take resulting from the aquaculture program (e.g., capture, handling, tagging, injury, or lethal take). Provide projected annual take levels for natural fish.

Indicate contingency plans for addressing situations where take levels within a given year have exceeded, or are projected to exceed, take levels described in this plan for the program. Example: "The number of days that red drum are collected at _______ will be reduced if the total mortality of handled fish is projected in-season to exceed the 1998–2009 maximum observed level of _______ fish."

8.2.4. Ecological interactions.

Describe fishes or other species that could 1) be negatively impacted by the program, 2) be positively impacted by program. Give most attention to interactions between natural and program fish.

9. Monitoring and Evaluation of Performance Indicators

This section describes how performance indicators listed in Section 1.10 will be monitored. Results of performance indicator monitoring will be evaluated annually and used to adaptively manage the aquaculture program, as needed, to meet performance standards.

9.1. Monitoring and evaluation of performance indicators presented in Section 1.10.

9.1.1. Describe plans and methods proposed to collect data necessary to respond to each performance indicator identified for the program.

9.1.2. Indicate whether funding, staffing, and other support logistics are available or committed to allow implementation of the monitoring and evaluation program.

9.2. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse genetic and ecological effects to natural fish resulting from monitoring and evaluation activities.

10. Research

Provide the following information for any research programs conducted in direct association with the aquaculture operation or program described in this AGMP. Provide sufficient detail to allow for the independent assessment of the effects of the research program on natural fish. If applicable, correlate with research indicated as needed in any propagation plan approved by NOAA. Attach a copy of any formal research proposal addressing activities covered in this section. Include estimated take levels for the research program with take levels provided for the associated aquaculture program in Table B-2.

10.1. Objective or purpose.

Indicate why the research is needed, its benefit or effect on natural fish populations, and broad significance of the proposed project.

10.2. Cooperating and funding agencies.

10.3. Principal investigator or project supervisor and staff.

10.4. Status of stock, particularly the group affected by project, if different than the stock(s) described in Section 2.

10.5. Techniques: include capture methods, drugs, samples collected, tags applied.

Table B-2. Estimated natural fish take levels by aquaculture activity. Instructions: 1) an entry for a fish to be taken should be in the take category that describes the greatest impact; 2) each take entered in the table should be in one take category only (there should not be more than one entry for the same sampling event); and 3) if an individual fish is taken more than once on separate occasions, each take must be entered in this take table.

Species affected:____________________ Population:__________________ Activity:_________________

Location:____________________ Dates:__________________ Program operator:___________________

	Annual take of natural fish by life stage (number of fish)			
Type of take	Eggs	Larvae	Juveniles	Adults
Observe or harass[a]				
Collect for transport[b]				
Capture, handle, and release[c]				
Capture, handle, tag/mark/tissue sample, release[d]				
Removal (e.g., broodstock)[e]				
Intentional lethal take[f]				
Unintentional lethal take[g]				
Other take (specify)[h]				

[a] Contact with natural fish through surveys, collection, or other effects.
[b] Take associated with fishing or trapping operations where natural fish are captured and transported.
[c] Take associated with fishing or trapping operations where natural fish are captured or handled.
[d] Take occurring due to tagging or biosampling of fish collected through fishing or trapping operations.
[e] Natural fish removed from the wild and collected for use as broodstock.
[f] Intentional mortality of natural fish, usually as a result of spawning as broodstock.
[g] Unintentional mortality of natural fish, including loss of fish during transport or holding prior to spawning or prior to transfer.
[h] Other takes not identified above as a category.

10.6. Dates or time period in which research activity occurs.

10.7. Care and maintenance of live fish or eggs, holding duration, transport methods.

10.8. Expected type and effects of take and potential for injury or mortality.

10.9. Level of take of natural fish: number or range of fish handled, injured, or killed by sex, age, or size, if not already indicated in Section 2 and the attached "take table" (Table B-2).

10.10. Alternative methods to achieve project objectives.

10.11. List species similar or related to the cultured species; provide number and causes of mortality related to this research project.

10.12. Indicate risk aversion measures that will be applied to minimize the likelihood for adverse ecological effects, injury, or mortality to natural fish as a result of the proposed research activities.

11. Attachments and Citations

Include all references cited in the AGMP. In particular, indicate databases used to provide data for each section. Include electronic links to the databases used (if feasible) or to the staff person responsible for maintaining the database referenced (indicate e-mail address). Attach or cite (where commonly available) relevant reports that describe the facility operation and impacts on natural species or their critical habitat. Include any Environmental Impact Statements, Environmental Assessments, Biological Assessments, benefit/risk assessments, or other analysis or plans that provide pertinent background information to facilitate evaluation of the AGMP.

12. Certification Language and Signature of Responsible Party

"I hereby certify that the information provided is complete, true, and correct to the best of my knowledge and belief. I understand that the information provided in this AGMP is submitted for the purpose of _________________________, and that any false statement may subject me to the criminal penalties of _________________________.

Name, title, and signature of applicant:

Certified by ______________________________ Date: _____________

Attachment 1: Definition of Terms Referenced in the AGMP Template

Cultured fish: A fish that has spent some part of its life cycle in an artificial environment and whose parents were spawned in an artificial environment.

Cultured population: A population that depends on spawning, incubation, hatching, or rearing in a hatchery or other artificial propagation facility.

Hazard: Undesirable events that an aquaculture program is attempting to avoid.

Natural fish: A fish that has spent essentially all of its life cycle in the wild and whose parents spawned in the wild. Synonymous with *natural origin recruit (NOR).*

Natural origin recruit (NOR): See **natural fish**.

Natural population: A population that is sustained by natural spawning and rearing in the natural habitat.

Population: A group of historically interbreeding fish of the same species of hatchery, natural, or unknown parentage that have developed a unique gene pool, that breed in approximately the same place and time, and whose progeny tend to return and breed in approximately the same place and time. They often, but not always, can be separated from another population by genotypic or demographic characteristics. This term is synonymous with stock.

Preservation (conservation): The use of artificial propagation to conserve genetic resources of a fish population at extremely low population abundance and high potential for extinction, using methods such as captive propagation and cryopreservation.

Research: The study of critical uncertainties regarding the application and effectiveness of artificial propagation for augmentation, mitigation, conservation, and restoration purposes, and identification of how to effectively use artificial propagation to address those purposes.

Stock: See *population*.

Take: As defined under the U.S. Endangered Species Act, Section 3(19), to take is to “harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct.”

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NOAA Technical Memorandum NMFS-NWFSC-

118 **Stout, H.A., P.W. Lawson, D.L. Bottom, T.D. Cooney, M.J. Ford, C.E. Jordan, R.G. Kope, L.M. Kruzic, G.R. Pess, G.H. Reeves, M.D. Scheuerell, T.C. Wainwright, R.S. Waples, E. Ward, L.A. Weitkamp, J.G. Williams, and T.H. Williams. 2012.** Scientific conclusions of the status review for Oregon coast coho salmon (*Oncorhynchus kisutch*). U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-118, 242 p. NTIS number PB2012-113458.

117 **Maynard, D.J., T.A. Flagg, W.C. McAuley, D.A. Frost, B. Kluver, M.R. Wastel, J.E. Colt, and W.W. Dickhoff. 2012.** Fish culture technology and practices for captive broodstock rearing of ESA-listed salmon stocks. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-117, 65 p. NTIS number PB2012-110667.

116 **Lian, C.E. 2012.** West Coast open access groundfish and salmon troller survey: Protocol and results for 2005 and 2006. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-116, 52 p. NTIS number PB2012-107486.

115 **Plummer, M.L., W. Morrison, and E. Steiner. 2012.** Allocation of fishery harvests under the Magnuson-Stevens Fishery Conservation and Management Act: Principles and practice. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-115, 84 p. NTIS number PB2012-107485.

114 **Bradburn, M.J., A.A. Keller, and B.H. Horness. 2011.** The 2003 to 2008 U.S. West Coast bottom trawl surveys of groundfish resources off Washington, Oregon, and California: Estimates of distribution, abundance, length, and age composition. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-114, 323 p. NTIS number PB2012-105507.

113 **Ford, M.J. (ed.). 2011.** Status review update for Pacific salmon and steelhead listed under the Endangered Species Act: Pacific Northwest. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-113, 281 p. NTIS number PB2012-104687.

112 **Skidmore, P.B., C.R. Thorne, B.L. Cluer, G.R. Pess, J.M. Castro, T.J. Beechie, and C.C. Shea. 2011.** Science base and tools for evaluating stream engineering, management, and restoration proposals. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-112, 255 p. NTIS number PB2012-104660.

111 **Leonard, J., and P. Watson. 2011.** Description of the input-output model for Pacific Coast fisheries. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-111, 64 p. NTIS number PB2011-113405.

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