Application of risk analysis to genetic issues in aquaculture

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ABSTRACT

In this review and synthesis, I explore the application of risk analysis to genetic harms posed by aquaculture, noting significant work to date and identifying areas where work is still needed. Harms posed by culture of a stock of aquatic organisms relate to chains of events occurring after an escape or release from a culture system. Direct genetic harms will flow from the cultured stock interbreeding with reproductively compatible populations in the receiving ecosystem, and could include loss of adaptation in natural populations, introgression of new genetic material into species' gene pools and, in the extreme case, loss of locally adapted populations. Risk assessment is an estimation of the likelihood of the occurrence of genetic harm becoming realized following exposure to a genetic hazard. The likelihood of harm being realized given exposure to a hazard is difficult to quantify with current knowledge, and we might often be restricted to evaluating risk qualitatively on the basis of: (1) the species at issue, (2) the effect of genetic background or improvement on the net fitness of the animal in the receiving ecosystem at issue and (3) the stability and resiliency of receiving community. Should distribution and production of a cultured stock pose unacceptable genetic harm to a population in the receiving ecosystem, the question then turns to design, selection and implementation of a programme of actions to minimize risk. Effective communication of principles and application of risk analysis is needed to organizations in both developed and developing countries.

INTRODUCTION

The development of aquaculture poses major benefits for mankind. Application of quantitative and molecular genetic principles plays an important and growing role in the development of aquaculture. Many approaches have been applied to obtain genetically superior aquaculture stocks (Tave, 1993; Dunham, 2004; Gjedrem, 2005), including use of high-performance nonindigenous stocks and species and development and use of selectively bred stocks, interspecific hybrids, triploids and transgenic lines. Genetic improvement of cultured stocks has increased production levels and production efficiency (WFC, 2003; ADB, 2005).

There is growing recognition that aquaculture can pose harms to natural aquatic systems (Pillay, 1992; Bardach, 1997; Costa-Pierce, 2003). Among them are genetic harms to natural populations in receiving ecosystems, including loss of adaptation in natural populations, introgression of new genetic material into species' gene pools and in the extreme case, loss of locally adapted populations. As I explain below, principles of risk analysis can be applied to genetic harms posed by aquaculture. The purpose of a genetic risk analysis is to identify risk pathways, estimate risk probabilities, develop procedures to manage risk and communicate the results to stakeholders, thereby minimizing harm to aquatic and human populations. Principles of risk analysis have been applied to aquaculture (Reantaso, Subasinghe and Van Anrooy, 2006), including aspects relating to use of non-indigenous species (e.g. Kohler and Courteney, 1986) and to some types of genetic manipulations, most notably to triploid oysters (Dew, Berkson and Hallerman, 2003; NRC, 2004c) and transgenic fishes (e.g. OAB, 1990; Hallerman and Kapuscinski, 1995; Kapuscinski *et al.*, 2007a), but less thoroughly or not at all to others. Here, I explore the application of risk analysis to genetic harms posed by aquaculture, noting significant work to date and identifying areas where work is still needed.

RELEVANT INTERNATIONAL POLICY

Recognition that aquaculture poses genetic harms to natural populations is relatively recent and has not received a high level of attention by governmental and intergovernmental agencies. Hence, standards, guiding principles and codes of conduct vary widely among the respective approaches used to produce cultured stocks (Table 1). Transfer and use of non-indigenous species is addressed in a number of national policies and international agreements (Welcomme, 1986; Sindermann, 1986; Thorgaard and Allen, 1992). Research and commercial use of genetically modified organisms (GMOs) is subject to the Convention on Biological Diversity (CBD, 1992), specifically the Cartegena Protocol on Biosafety under that convention (CBD, 2000) and implementation policies flowing from it, with national polices for aquatic GMOs mostly still under development. Use of non-indigenous stocks, interspecific hybrids and ploidy-manipulated aquatic species is regulated in some, but not all countries.

SCOPING A RISK ANALYSIS

Consideration of genetic harms posed by cultured fishes must be based on an understanding of key concepts underlying the science and practice of risk analysis

TABLE 1

Selected policies, codes of practice and databases relevant to genetic risk analysis for aquaculture stocks

Exotic species
Code of Conduct for Responsible Fisheries (FAO, 2007b)
Code of Practice on the Introduction and Transfer of Marine Organisms (EIFAC, 1988; ICES, 1995)
FAO Technical Paper 294 (Welcomme, 1988)
Database of Introductions of Aquatic Species (FAO, 2007a)
Non-indigenous genotypes
United States court order[1]
Genetically modified organisms
Convention on Biological Diversity (CBD, 1992)
Cartagena Protocol on Biosafety (CBD, 2000)
United States Coordinated Framework for the Regulation of Biotechnology (OSTP 1985, 1986)
United States Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish (ABRAC, 1995)
European Union Directive 2001/18/EC (EU, 2001)
Norwegian Gene Technology Act (Norwegian Ministry of Environment, 1993)

[1] In its ruling in *U.S. Public Interest Research Group vs. Atlantic Salmon of Maine*, the United States District Court in Maine on May 28, 2003 banned culture of European strain Atlantic salmon in United States waters (NRC, 2004a).

(NRC, 2002). In a genetic context, a *harm* is defined as gene pool perturbation resulting in negative impacts to a species. A *hazard* is defined as an *agent or process* that has the potential to produce harm. A *risk* is defined as the *likelihood* of harm resulting from exposure to the hazard. Risk, R, is estimated as the product of the probability of exposure, $P(E)$, and the conditional probability of harm given that exposure has occurred, $P(H|E)$. That is, $R = P(E) \times P(H|E)$. The steps in risk analysis, then, are to:

1) identify potential harms;
2) identify hazards that might lead to harms;
3) define what exposure means for an aquaculture stock and assess the likelihood of exposure, $P(E)$;
4) quantify the likelihood of harm given that exposure has occurred, $P(H|E)$; and
5) multiply the resulting probabilities to yield a quantitative estimate of risk.

Exact probabilities of risk are difficult or impossible to determine for all types of possible harm. Indeed, it is unlikely that all possible harms would be known *a priori*, particularly with respect to any indirect effects. Hence, it may be necessary – based on current knowledge of population genetics, population dynamics, receiving ecological communities and experience with cultured stocks – to classify levels of concern regarding likely genetic impacts posed by cultured stocks into *qualitative* categories ranging from low to high.

Risk assessment might best be considered as embedded in a three-stage, interactive framework involving the range of stakeholders (Figure 1). Involvement of the full range of stakeholders will bring all existing knowledge into the process, make the process transparent to stakeholders and enhance the understanding and acceptance of the outcome of risk analysis. Stage I involves identifying the problem at hand, engaging stakeholders, identifying possible technical solutions to the problem at hand and identifying potential harms, risk pathways and assessment methods. Stage II is the risk assessment itself, leading to estimating the likelihood that harm will become realized should a proposed action be taken. Upon estimation of that risk, a decision is faced as to whether the risk is acceptable. If it is acceptable, the decision may be made to go forward. If the level of risk is unacceptably high, risk management measures would be identified and residual risk quantified, and the decision of whether to go forward would again be considered. Should the proposed action be implemented, genetic, ecological and social outcomes should be monitored. Because all potential harms and associated pathways cannot be known and precisely predicted *a priori*, it will be necessary to update the risk analysis as knowledge accumulates using an adaptive management approach (NRC, 1996; Kapuscinski, Nega and Hallerman, 1999). Below, I focus on genetic harms and elaborate on each step in risk assessment.

HARM IDENTIFICATION

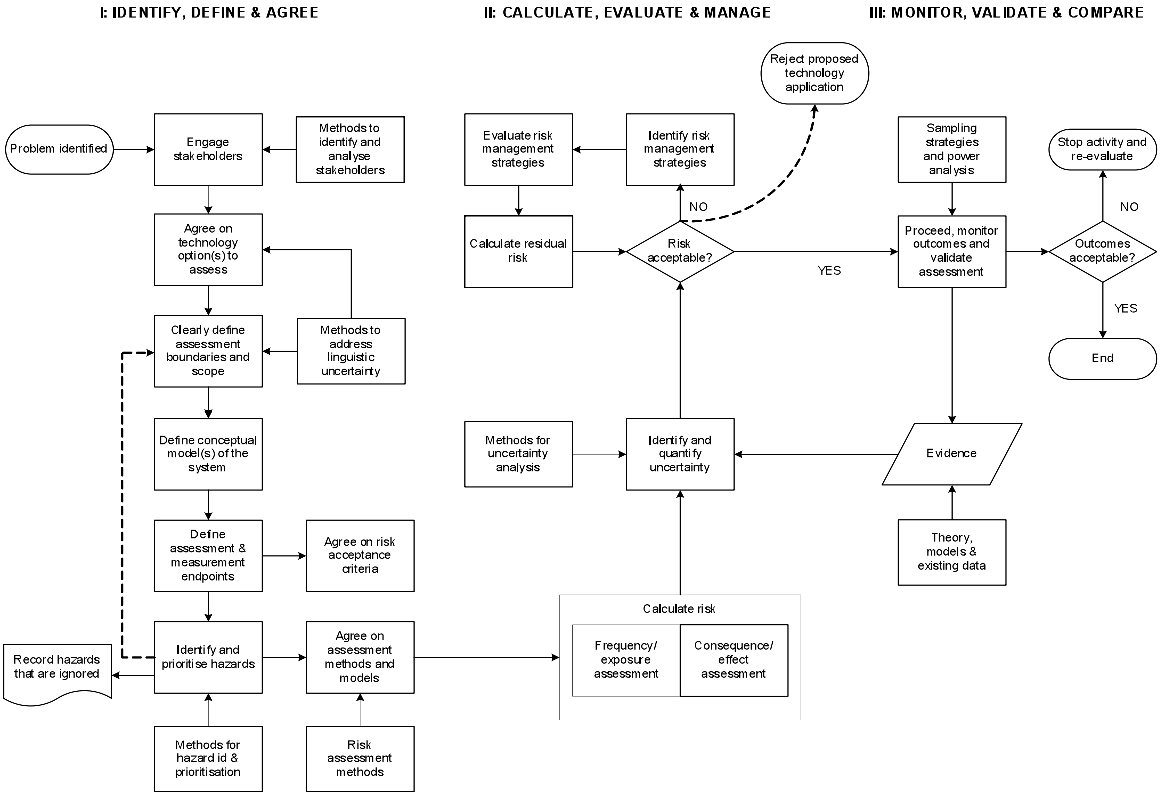
The harms posed by culture of a stock of aquatic organisms relate to chains of events occurring after an escape or release from a culture system. Potential harm must be identified on a case-by-case basis and will depend on the phenotype of the organism, and not *per se* on the genetic manipulation used to produce the stock. *Direct* genetic harms will flow from the cultured stock interbreeding with reproductively compatible populations in the receiving ecosystem. Indirect effects will flow from competition or predation by the cultured stock on other populations or species in the receiving ecosystem.

Loss of adaptation

Natural selection mediates adaptation of a population to its environment by changing allele frequencies at fitness-related genes. Allele frequencies at fitness-related genes will differ among cultured stocks and wild populations. Interbreeding with escaped cultured organisms will displace allele frequencies at fitness-related genes in wild

FIGURE 1

Overview of a highly interactive risk assessment framework (Hayes *et al.* 2007). The framework is divided into three stages. In the first stage, participants agree upon an assessment option, define the scope of the assessment, agree on a conceptual model, identify assessment and measurement endpoints and culminate with an agreed-upon list of prioritized hazard end-points. In the second stage, the risks and uncertainties associated with these hazard end-points are assessed, risks compared to predetermined acceptance criteria and, as appropriate, risk management strategies identified and evaluated. In the third stage, results of monitoring are used to test the predictions of the risk assessment, thereby generating additional data used to reexamine uncertainty in the risk assessment



populations from selective optima, posing loss of fitness. The degree of harm will be a function of the degree of differentiation among the two gene pools, the relative proportion of spawners from the respective groups and the selective pressure imposed by the receiving ecosystem. While it should be noted that not all natural populations at selective optima, the chance of improving fitness through breeding with escaped fish is remote.

For some traits, fitness depends upon expressing *combinations* of alleles across fitness-related loci. The coadapted gene complexes arise by chance and are maintained by natural selection (Hallerman, 2003). For example, anadromous salmonids must express an appropriate combination of run timing, embryonic development rate, post-hatching behaviour, migration and maturation traits in order to complete their life cycle. Interbreeding of differently coadapted populations poses outbreeding depression, or loss of fitness due to breakdown of coadapted gene complexes. The degree of harm will be a function of the degree of difference of the coadapted phenotypes and how many genes determine the traits at issue.

Although we often focus on underlying genotypes, fitness is a phenotypic trait. When selecting mates, individuals must assume that phenotype is a reliable indicator of fitness. This assumption is not always reliable. For example, size is often a fitness-related trait in fishes. However, a fish may be large because it grew in a culture system, not because it expresses genes conferring fitness in the wild. In particular, expression of an introduced growth hormone gene may confer large size upon a transgenic fish, although its offspring may exhibit decreased viability. Such unfavourable tradeoffs among fitness-related traits are termed Trojan gene effects (Muir and Howard, 2001). If the magnitude of the tradeoff is sufficiently large, under certain demographic conditions, a population may face the risk of extinction.

Cultured stocks often have lower effective population sizes (N_e) than natural populations. Escape or release of cultured stocks can decrease the effective size of a receiving population, even if the census count of individuals rises (Ryman and Laikre, 1991). Smaller effective population size implies less genetic variability and less ability to respond adaptively to changes in selection pressures. For example, resistance to pathogens and parasites is often a function of allelic or haplotypic diversity, especially at major histocompatibility complex loci affecting recognition of non-self and coordination of immune response (Hedrick, 2002). It also heightens the risk of subsequent inbreeding.

Introgressive hybridization

Escape or stocking of a non-indigenous species poses possible interbreeding with a reproductively compatible species in the receiving environment. Should the resulting interspecific hybrid prove fertile, it poses the risk of introgressive hybridization with the native species, threatening the genetic integrity of the native species (Campton, 1987; Rhymer and Simberloff, 1996). Similarly, escape or stocking of a fertile interspecific hybrid poses the harm of introgressive hybridization.

Indirect effects

Escape or release of cultured stocks may also pose *indirect* genetic harms to populations in the receiving ecosystem. Through competition or predation, by reducing the abundance of affected populations, the cultured stocks may reduce their effective population size, causing loss of genetic variability and ability to adapt in face of changing selective pressure, and also increase the likelihood of subsequent inbreeding and extinction. Should cultured fish interbreed unsuccessfully with a population in the receiving ecosystem, the loss of reproductive investment increases demographic risk. This mechanism can be realized by interbreeding of a cultured stock and a natural population resulting in a sterile hybrid. Also, triploid males of some species undergo gonadal maturation, steroidogenesis and gametogenesis, and may secure matings (Benfey *et al.*, 1989; Inada and Taniguchi, 1991; Kitamura, Ogata and Onozato, 1991). Any such matings would result in aneuploid broods (Benfey *et al.*, 1986), which would not prove viable (Inada and Taniguchi, 1991). Indirect effects also may be realized through changes in the aquatic community caused by the cultured stocks.

Case studies illustrating potential harms posed by cultured stocks are presented in Box 1.

Sources of information

Sources of information to support harm identification will vary for different classes of aquaculture stocks. There is a large literature on harms posed by non-indigenous species, including species pertinent to aquaculture, as well as policies developed to control their introduction and use. Impacts of exotic fishes in the United States are reviewed in a volume edited by Courtenay and Stauffer (1984). The American Fisheries Society featured discussion of issues posed by introduced species in a special publication of *Fisheries* (Kohler, 1986). Book-length treatments include Rosenfield and Mann (1992) and Devoe (1992). Ecological and socio-economic impacts of invasive alien species were reviewed by Ciruna, Meyerson and Gutierrez (2004). A Database of

BOX 1

Genetic harms posed by cultured organisms

Entry of cultured fish into natural populations may pose genetic harms to receiving populations (Waples, 1991; Utter, 2003; Kapuscinski and Brister, 2001). Here, I present examples of such potential harms.

Direct effects. Interbreeding of cultured stocks and natural populations poses direct genetic harms. Natural selection operates upon alleles at fitness-related loci, over time mediating adaptation of populations to their environments. Across a landscape, spatial heterogeneity of natural selection results in adaptive genetic divergence of populations. However, escape of widely cultured fish stocks and interbreeding with local populations will tend to homogenize genetic variation over time. Escapes of Atlantic salmon (*Salmo salar*) from net-pen aquaculture comprise 70 percent of the spawning stock in some Norwegian rivers, with a mean of 29 percent across rivers. Mork (1991) developed a model to assess one-generation effects of escape and interbreeding of cultured fish on genetic differentiation of natural populations. Substantial reductions in genetic differentiation – i.e. reductions of up to 80 percent in the genetic differentiation statistic, G_{ST} – were predicted. Gharrett (1994) modeled the net effects of immigration and selection on the rate of genetic change on natural populations, but concluded that without knowing the extent of genetically effective migration and the magnitude of loss of fitness, it is not possible to predict outcomes. Focusing on salmonids, Hindar, Ryman and Utter (1991) reviewed studies of the genetic effects of cultured fish on natural fish populations, finding a wide variety of effects, from no detectable effect to complete introgression to complete replacement of natural populations. They recommended measures for genetic protection of natural populations, including secure confinement, use of sterile fish and monitoring of gene flow. Case studies involving non-salmonid species are less numerous. A survey of channel catfish (*Ictalurus punctatus*) populations in Alabama, United States (Simmons *et al.,* 2006) showed no evidence of genetic impact from loss of cultured fish into natural populations, i.e. no apparent dispacement of allele frequencies of natural populations near fish farms from those of natural populations farther away.

Selective forces acting across fitness-related loci may result in combinations of alleles – termed coadapted gene complexes – that confer fitness upon their carriers. Interbreeding of a cultured stock with a locally adapted natural population may lead to outbreeding depression and loss of fitness. Cultured Atlantic salmon stocks are genetically and behaviourally differentiated from natural populations (Einum and Fleming 1997; Gross 1998, NRC 2004a). A two-generation experiment comparing fitness traits among wild, cultured, F_1, F_2 and backcross salmon showed that cultured and hybrid salmon exhibited reduced survival, but faster growth than wild fish, and that their parr displaced wild parr competitively (McGinnity *et al.,* 2003). In an independent experiment, the lifetime reproductive success of farmed salmon was 16 percent that of native salmon, and the productivity of the native population was reduced by more than 30 percent by interbreeding (Fleming *et al.,* 2000).

Fishes select mates on the basis of phenotype, which is taken as a reliable indicator of fitness. When phenotype is misleading and individuals choose mates whose offspring ultimately exhibit low fitness, this is termed the Trojan gene effect (Muir and Howard, 2001). The theory was developed in order to assess risks associated with interbreeding of escaped or released transgenic fish with a natural population. Recurrence equations predict the frequency of the transgene and population number as a function of the degree of tradeoff among, for example, heightened mating success and reduced juvenile viability. Simulations showed that fitness values determine whether the transgene persists, is purged from the gene pool by selection or a Trojan gene effect occurs, leading the population to crash. Experiments are ongoing to parameterize the model using growth hormone-transgenic medaka and Atlantic salmon. While the theory was developed for risk assessment for transgenic fish, it could be applied to any organism whose fitness is affected by genetic manipulation.

BOX 1 (continued)

Genetic harms posed by cultured organisms

Genetically effective sizes of cultured stocks typically are lower than those of natural populations. Escape or release of cultured fish into a receiving population may reduce N_e and increase the risk of inbreeding if the proportion of cultured fish is sufficiently high, an outcome termed the Ryman-Laikre (1991) effect. Wang and Ryman (1991) and Waples and Do (1994) extended the theory to multiple generations and considered the effect of population age structure. Hatchery Atlantic salmon exhibited significant changes in allele frequencies and loss of low-frequency alleles relative to the natural population from which they had been derived one generation earlier (Tessier, Bernatchez and Wright 1997). Estimates of drift and inbreeding effective population sizes showed that the risk of random genetic drift and inbreeding had doubled over the one generation of supplementation.

Introgressive hybridization. Escape or release of interspecific hybrids, if fertile, pose the harm of introgressive hybridization. For example, hybrid catfish (*Clarias macrocephalus x C. gariepinus*) escaping from farms in central Thailand interbred with native populations of *C. macrocephalus*, giving rise to introgressive hybridization with both wild and cultured stocks (Senanan *et al.,* 2004). Similarly, poor management of tilapia stocks led to unwanted hybridization of previously pure species to occur by escapes into the wild, as well as by intrusions from the wild (McAndrew and Majumdar 1983, Macaranas *et al.,* 1986). In Bangladeshi hatcheries, 8.3 percent of silver carp (*Hypophthalmichthys molotrix*) broodstock exhibited bighead carp (*Aristichthys nobilis*) alleles, while 23.3 percent of bighead carp exhibited silver carp alleles (Sattar *et al.,* 2005). While some individuals may have been F_1 hybrids, others were advanced-generation hybrids, compromising the integrity of the respective broodstocks and their performance in aquaculture.

Indirect effects. Escape or release of cultured stocks in the absence of interbreeding may pose indirect effects. To elaborate on one possible mechanism, triploidy often is used as a means of reproductively confining cultured stocks, and all-female triploid stocks may be produced to minimize demographic risks to a receiving population. However, use of triploid aquaculture stocks raises three issues (NRC 2004c). A first issue is the efficacy with which triploids are produced, which differs between the interploid cross among tetraploids and diploids (near 100 percent) and *de novo* induction (generally <100 percent) methods (Downing and Allen 1987; Guo, deBrosse and Allen 1996), but does not reach a full 100 percent. Hence, triploid verification will have to be implemented to manage risk. A second issue has to do with the stability of the triploid state. A small percentage of Pacific and Suminoe oysters have shown signs of progressive reversion to the diploid state, depending on species, individual and tissue (S.K. Allen, Jr., quoted in NRC 2004c). A third issue pertains to the functional sterility of triploid adults. Triploid males of some species may undergo gonadal maturation and steroidogenesis (Benfey *et al.* 1989). Male triploid fish have sometimes been found to produce haploid or aneuploid sperm (Lincoln and Scott 1984; Allen, Thiery and Hagstrom 1986; Benfey *et al.,* 1986; Allen 1987) Should they mate with diploid females (Inada and Taniguchi 1991; Kitamura, Ogata and Onozato 1991), the resulting broods will prove inviable, reducing the reproductive success of the receiving population. Triploid females generally show little ovary development, although there are some apparent exceptions in both fish (Benfey and Sutterlin 1984) and shellfish (Komaru and Wada, 1989, Allen and Downing, 1990). Triploid Pacific and Suminoe oysters are almost, but not completely sterile (Allen and Downing, 1990, Guo and Allen 1994). Should the non-native species escape genetic confinement in the Cheapeake Bay, it would pose competition with the already-declining native Eastern oyster (NRC 2004c, Box 2).

Introductions of Aquatic Species (DIAS) is maintained by the Food and Agriculture Organization of the United Nations (FAO) (Bartley *et al.,* 2006; FAO, 2007a). Studies identifying harms posed by non-indigenous genotypes to receiving populations notably include Hindar, Ryman and Utter (1991), Utter (2003), and Kapuscinski and Brister (2001). Campton (1987) reviews interspecific hybridization in fishes, and Schwartz (1972, 1981) provides citations to the early literature on hybridization in fishes. Harms posed by triploids have been reviewed by ABRAC (1995) and the NRC (2004b, c). Harms posed by transgenic fish and shellfish have been reviewed by ABRAC (1995), the Scientists' Working Group on Biosafety (1998), the NRC (2002, 2004a) and Kapuscinski *et al.* (2007a).

HAZARD IDENTIFICATION

In the context of genetic risk analysis, the hazardous agent is the cultured stock because it is the entity that poses genetic harm to populations in a receiving ecosystem. In the aquaculture context, the hazardous agent may be a non-indigenous species; an interspecific hybrid; or a non-indigenous, selectively bred, triploid or transgenic stock.

RISK ASSESSMENT

In the context of genetic risk analysis, risk assessment is an estimation of the likelihood of the occurrence of genetic harm becoming realized following exposure to a genetic hazard. Because realization of harm would require occurrence of a chain of events, it often is useful to consider risk assessment in terms of the components of the chain. For example, Figure 2 illustrates the sequence of events needed to assess the likelihood of direct genetic harm becoming realized from culture of a transgenic fish (Kapuscinski *et al.*, 2007b). To illustrate risk assessment for a specific case, examples of the types of data, studies and scientific expertise that would be needed to assess risks related to gene flow from transgenic fish to wild populations are presented in Table 2. Below, I elaborate upon release assessment, exposure assessment and consequence assessment, followed by risk estimation.

Likelihood of release

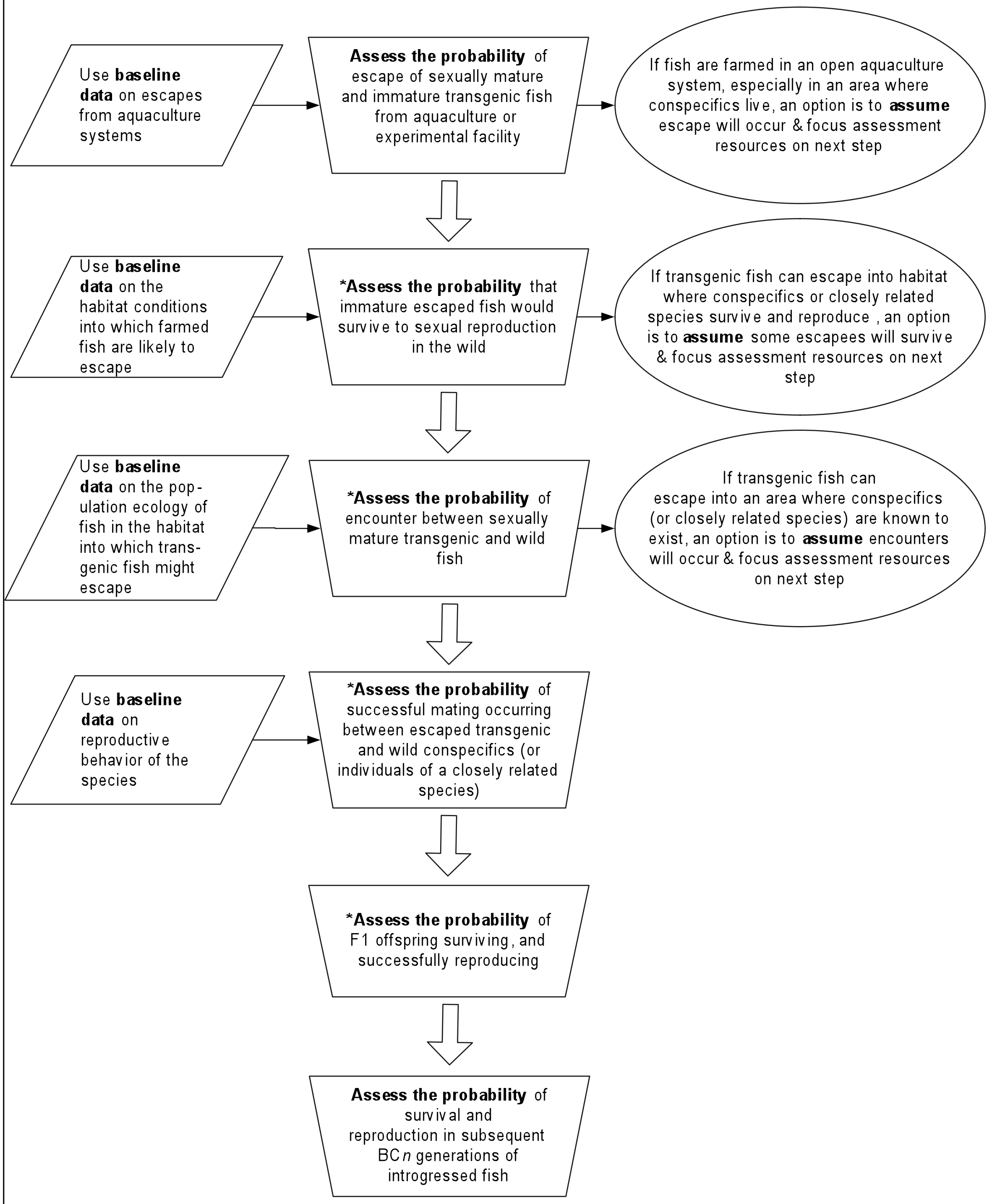
Routine aquaculture operations frequently involve the loss of small numbers of cultured fish to the natural environment, with occasional catastrophic losses of larger numbers of fish due to equipment failure, storm damage or flood (Hallerman and Kapuscinski, 1992; CEQ and OSTP, 2001). The information required for a release assessment in a particular context relates to the biological factors, commodity factors and country factors pertinent to that aquaculture system. Biological factors relate to the aquatic species at issue, as they affect the likelihood of escape. Finfishes are mobile; in particular the smallest life stages are hard to confine. Crustaceans vary, with many decapods able to escape by crawling or burrowing out of culture systems. Molluscs are easy to confine at the benthic adult stage, but harder to confine at the pelagic juvenile stages; in some cases, the earliest life stages can escape confinement in aerosols. Commodity factors relate to production methods; that is, different culture systems provide a continuum of confinement, from low to high ranging from extensive production in near-natural systems, to cages and net-pens in oceans and lakes, to intensive production in managed ponds and raceways, to indoor recirculating systems. Country factors are a consequence of policies and permit systems regulating aspects of siting, culture systems and operations management procedures, as they all affect likelihood of release. In the lack of express or enforced policies, operations of individual farms will vary widely and complicate a release assessment. Especially for developing-country contexts, such a release assessment must assume that cultured stock will escape.

Likelihood of exposure

Upon escape or release, for a cultured stock to prove a hazard, it must establish itself in the community long enough to impose harm. Hence, for risk assessment, the critical factor is the likelihood that the cultured stock will become established in the receiving

FIGURE 2

A conceptual pathway for conducting an assessment of gene flow from a ransgenicstock to a wild population (Kapuscinski *et al.*, 2007 b). Asterisks denote assessment steps that require empirical data on traits of the transgenic fish



ecosystem, which is *P(E)*. The likelihood of establishment is dependent on three factors: the species' invasiveness, fitness of the selectively bred stock and characteristics of the receiving ecosystem.

A first aspect of evaluating likelihood of genetic exposure to a cultured stock is the species' invasiveness, i.e. its ability to escape, disperse and become feral in aquatic communities. Many aquaculture species – notably including tilapias, carps and salmonids – exhibit great abilities to disperse and establish themselves in ecosystems in which they are not native.

A second aspect of ecological exposure is the fitness of the cultured stock in the receiving ecosystem. Production traits in domesticated aquaculture stocks include improved growth rate, feed conversion efficiency and disease resistance. Traits conferring fitness in culture systems may not be the same as those conferring fitness

TABLE 2

Examples of types of data, studies and scientific expertise needed to assess gene flow from transgenic fish to wild populations (Kapuscinski *et al.*, 2007 b)

Description of Data Need	Types of Studies[1] (Generally from Simplest to Most Complex):	Studies (May) Require Expertise in:
Data to estimate entry potential		
What is the rate of escape from existing aquaculture or experimental facilities ("propagule pressure")?	• Field studies to detect and quantify escapees • Mandatory self-reporting of escapes by relevant facilities (requires infrastructure for enforcement) • Mark-recapture studies • Use of molecular genetics markers • Mixed-stock analysis • Video surveillance	• Fisheries assessment methods • Molecular genetics methods, such as PCR-mediated detection of specific genes
What is the pattern of escapes from existing aquaculture facilities?	• Field studies to detect escapees • Molecular lab studies, especially when genetic markers are the only way to differentiate cultured and wild fish • Use of telemetry systems	• Fish population dynamics and field assessment methods • Life history of the species in question • Spatial (GIS) modeling
What proportion of immature transgenic escapees are likely to survive to sexual maturity in the natural environment?	• Mark-recapture field experiments • Laboratory experiments to determine survival rates relative to wild-type • Mixed-stock analysis	• Life history of the species in question • Fish population dynamics and field assessment methods • Fish ecology
Data to estimate introgression potential		
Do transgenic escapees disperse in a spatial and temporal pattern and in a phenotypic state that make them likely to find available mates?	• Field sampling for presence of escapees at critical times and places vis-à-vis the native population • Laboratory experiments and spatial modeling	• Life history of the species in question • Fisheries assessment methods • Spatial (GIS) modeling
Are transgenic escapees likely to mate with wild conspecifics (or to hybridize with closely related species) in the natural environment?	• Laboratory studies of mating behaviours of transgenic fish • Field sampling to determine what environments are suitable for reproduction	• Life history of the species in question, especially of mating behaviours and breeding in captivity • Fisheries assessment methods
Are F_1 or BC_n progeny likely to survive and reproduce successfully in the natural environment?	• Laboratory experiments in which matings between transgenic and wild fish can be controlled	• Life history of the species in question, especially of mating behaviours and breeding in captivity • Genetics and breeding programmes
What is the relative net fitness of transgenic fish, compared to a selected captive or wild population?	• Laboratory experiments in which transgenic and comparative strains of fish can be bred and measured for fitness components (fecundity, fertility, age at sexual maturity, mating advantage, juvenile viability, adult viability)	• Life history of the species in question, especially as it might guide prioritizing the most important fitness component traits to examine
What is the spatial distribution of populations of wild conspecifics, or closely related species, in the accessible ecosystem?	• Field sampling for presence of wild fish • Telemetry studies	• Fish systematics (ichthyology) for correct identification of fish species in the wild • Fish behavioural ecology • Fisheries assessment methods • Population genetics techniques and analysis
How many reproductively active wild conspecifics, or closely related species, live in the accessible ecosystem?	• Field sampling for direct estimation of abundance of wild fish • Mark-recapture studies	• Fish population dynamics and field assessment methods
Other desirable data		
How might transgenic fish's phenotype be expressed in a variable natural environment?	• Laboratory experiments in which fish can be exposed to manipulations of environmental variables contributing to survival and reproductive success in the wild (e.g. variable density, natural food or other simulations of natural habitat features)	• Fish behaviour • Fish genetics • Life history of the species in question, especially as it might guide prioritizing the most important environmental variables
What is the population genetic structure of the wild populations?	• Field sampling wild fish to collect tissue • Laboratory analysis of genetic structure of population (allozyme to DNA marker studies)	• Population genetics techniques and analysis
How will the genetic background of the transgenic and wild strains affect the probability of introgression?	• Laboratory experiments in which matings between transgenic and wild fish from different strains can be controlled	• Life history of the species in question, especially of mating behaviours and breeding in captivity • Genetics and breeding programmes

[1] Any studies using transgenic fish should be well confined to prevent the escape of transgenic fish into the wild.

in the wild. A key question, then, is how genetic improvement might indirectly affect traits determining fitness in the receiving ecosystem, perhaps affecting the likelihood that the cultured stock would become established in the receiving ecosystem. Genetic improvement that increases fitness increases the probability of establishment and results in a higher level of genetic concern. It is difficult to make predictions of the effects of genetic improvement on fitness in the wild in a general sense. For example, experience with domestic farm animals suggests that selective breeding generally does not increase the fitness of animals in natural environments, for example, because of physiologic imbalances or growth demands in excess of food availability in natural environments. However, genetic concerns posed by aquaculture stocks expressing improved production traits cannot be dismissed as non-concerns. Selective breeding has not differentiated most fish stocks dramatically from the wild type and, hence, their fitness in the wild generally is expected to remain high. It is possible for selectively bred stocks to overcome, for example, viability disadvantages if other fitness components are enhanced, such as mating success, fecundity or age at sexual maturity. The key issue is change in the *net* fitness of the selectively bred fish over the *entire* life cycle. The six net fitness components of an organism's life cycle to be considered are juvenile viability, adult viability, age at sexual maturity, female fecundity, male fertility and mating success (Muir and Howard, 2001).

The third aspect of ecological exposure is the stability and resilience of the receiving community. A community is regarded as stable if ecological structure and function indicators return to initial conditions following perturbation (Pimm, 1984). Resilience is the property of how fast the structure or function indicators return to their initial conditions following perturbation. Ecosystems that are most stable will suffer the least harm, with unstable communities suffering the greatest harm. For example, decreases in native species following introductions of tilapias occurred most frequently in aquatic ecosystems with less diversified fish faunas; decreases in native species were observed in high elevation lakes of Madagascar with few native species, but not in coastal lakes with many native species (Moreau, 1983). Characterization of community stability and resilience does not generally prove straightforward. Agreement on how to assess community resiliency likely will come only when viewpoints focusing separately on population dynamics, energetics and adaptations of individual species are reconciled (Ricklefs, 1990).

A key caveat for assessing ecological exposure is that we cannot limit the spread of an escaped aquaculture stock to a particular receiving ecosystem. Thus, we must consider whether a cultured stock can become established in all possible ecosystems to which it can gain access. If any of these communities is vulnerable, ecological concern would be high. For this reason, precaution suggests that risk should be assessed and managed for the most vulnerable ecosystem into which the escaped or released aquaculture stock is likely to gain access.

CONSEQUENCE ASSESSMENT

Because of the uniqueness of each cultured stock, culture system and receiving ecosystem, evaluating ecological risk will have to be conducted on a case-by-case basis. The likelihood of harm being realized given exposure to a hazard is difficult to quantify, especially with a lack of empirical data for the many kinds of genetic stocks at issue. This linkage is the weakest aspect of current understanding for genetic risk analysis. As a consequence, we might often be restricted to evaluating risk *qualitatively* on the basis of: (1) the species at issue, (2) the effect of genetic background or improvement on the net fitness of the animal in the receiving ecosystem at issue and (3) the stability and resiliency of receiving community. The outcome of such an analysis is likely to be a predication that likelihood of harm given exposure to a genetic hazard is "high", "medium", "low" or "near-zero".

Estimation of risk

Rating an overall level of genetic risk posed by a given action then would be based on the product of the three factors, likelihood of release, likelihood of exposure and likelihood of harm given exposure. Because the overall level of genetic risk is a product, if one is negligible, then the overall level of concern would be low. In contrast, genetic improvement that increases fitness of a highly invasive species for introduction into a vulnerable community raises a high level of concern. The estimate of risk might then be compared to a previously set acceptable level of risk (ALOR) to determine whether to go ahead, whether to reconsider the action under conditions of risk management or whether to reject the action at issue.

RISK MANAGEMENT

Should an oversight body determine that distribution and production of a cultured stock poses genetic harm to a population in the receiving ecosystem, the question then turns to how to manage the associated risk. Risk management is the design, selection and implementation of a programme of actions to minimize risk. Considering genetic harms in the context of formal risk analysis, it becomes clear that the best approach for minimizing the likelihood of harm being realized is to minimize exposure to the hazard (Mair, Nam and Solar, in press). Four non-mutually exclusive approaches include: (1) geographic location, (2) physically confining the cultured stock on aquaculture facilities, (3) reproductively confining cultured stocks and (4) operations management.

Geographic location. Context is key; the ease or difficulty of managing risk will depend greatly on the geographic location of an aquaculture facility. Sites subject to flooding, violent storms or wave action are poorly suited for confinement of production stocks.

Physical confinement. Physical confinement of cultured aquatic organisms will require a combination of measures in order to prove effective (ABRAC, 1995). Virtually all physical confinement systems will include barriers to escape of cultured organisms from the culture site, including mechanical or physical/chemical barriers. Mechanical barriers are structures that physically hold back cultured organisms from escaping the project site. Examples include stationary or moving screens (e.g. floor drains, standpipe screens), tank covers, filters (e.g. gravel traps), grinders or pumps and French drains. A French drain is a filter for screening effluent from an aquaculture facility that contains gravel and geotextiles through which even small lifestages cannot pass. Physical or chemical barriers use manipulation of physical (e.g. temperature) or chemical (e.g. pH) attributes of effluent water to induce 100 percent mortality of any escaped organisms before they can reach the accessible ecosystem. The set of barriers must prevent escape of the hardest-to-retain lifestage held at the aquaculture operation, usually the smallest lifestage. Because no barrier is 100 percent effective at all times, for effective physical confinement, each possible escape path from the aquaculture facility would have redundant barriers to escape of cultured organisms. Barriers also must prevent access of predators that can carry cultured organisms off-site (e.g. avian predators) or damage ponds (e.g. muskrats), allowing escape of cultured organisms.

Reproductive confinement. A key element of many risk management strategies is reproductive confinement, especially for cases where physical confinement alone is unlikely to prove effective. Two approaches, culture of monosex or sterile stocks, might be applied singly or in combination. All-triploid stocks can be produced most reliably by the crossing of diploid and tetraploid broodstock, although lack of tetraploid broodstock precludes the approach for many species. Alternatively, triploid stocks can be produced by *de novo* induction. *De novo* triploidy induction is not always 100 percent effective and, hence, triploid broods will have to be screened to determine whether they are indeed all-triploid (NRC, 2004b). This extra handling and screening adds to the cost of seed-stock production. Other approaches for reproductive confinement may become available in the future (Devlin and Donaldson, 1992), including the possibility of reversible sterility through transgenesis (Uzbekova *et al.,* 2000).

Operations management. Operations management is a key, though often overlooked, aspect of a confinement system. Measures are needed to: (1) ensure that normal activities of workers at the aquaculture operation are consistent with the goal of effective confinement, (2) prevent unauthorized human access to the site and (3) ensure regular inspection and maintenance of physical confinement systems. Effective supervision of project personnel is critical for operations management. Materials transfer agreements may prove important for limiting ill-considered distribution of aquaculture stocks.

Operations management must consider biosecurity after cultured organisms are removed purposefully from the culture site, that is, through the marketing process. For biosecurity purposes, it would be best if only dead fish were sent to market. This is counter to marketing practices in many countries, where live sales prevail. Live sale is a known route for introductions of non-indigenous species, and evidenced by recent introductions of snakeheads (Perciformes: Channidae) and swamp eels (Synbranchiformes: Synbranchidae) in the United States (Collins *et al.*, 2002; Orrell and Weight, 2005).

Effective risk management calls for combinations of confinements. Combinations of risk management measures are advisable so that failure of any one measure will not necessarily lead to escape of confined stocks. It is infeasible to anticipate the best combination of risk management measures for every possible case. Differences in species, production traits, receiving ecosystems and culture systems will affect the case-by-case determination of appropriate risk management measures. The issue of what combination of risk management measures proves practical for a programme where the goal is to provide poor farmers with access to high-performance stocks requires further discussion.

Adaptive management. Many critical unknowns complicate risk assessment and risk management for aquaculture stocks. The adaptive management approach is based on recognition that knowledge of the environmental and social systems into which the aquaculture stocks would enter is *always* incomplete. Management should evolve as knowledge of these systems increases. Management cannot adapt if realized by a single passage through breeding, decision of whether and how to distribute the stocks and implementation of the distribution programme. Instead, adaptive management would include risk assessment for candidate areas for distribution, incorporation of risk management in the distribution programme and capacity building as appropriate to meet programme goals. Once the aquaculture stocks are distributed, culture operations and receiving ecosystems would be monitored for indicators of ecological and social conditions. Should monitoring indicate that benefits are being realized without harms occurring, then few if any adjustments to programme implementation are required. However, should monitoring indicate that production of cultured stocks is *not* contributing to nutritional and economic well-being of farmers or that the stocks are escaping and impacting receiving ecosystems, then it will prove necessary to redefine goals, revise implementation and continue monitoring. Kapuscinski, Nega and Hallerman (1999) discuss adaptive management regarding biotechnologically modified organisms; the general approach is readily adaptable to all classes of aquaculture stocks.

RISK COMMUNICATION

Genetic risk communication is the transmission of the ongoing process and ultimate results of genetic risk analysis to stakeholders and the general public. In particular, pre-agreed contingency plans, which are part of the FAO (1995) precautionary approach, as a useful form of risk communication and for achieving agreement on what to do if things go wrong, or well. Genetic risk assessment and risk management are emerging areas in aquaculture science. While genetic hazards are well known, the associated risks are not well quantified. Genetic risk management, while widely applied at the research scale, is not widely applied at commercial aquaculture operations. Hence, we do not yet have a body of case studies to exemplify effective communication of genetic risk management.

Development and implementation of communication strategies for genetic risk analysis will involve crafting the message appropriate to the case at hand and its effective delivery to target audiences. Two sorts of message are at issue – general explanation of risk analysis as applied to genetic harms and information about applications of risk analysis to specific genetic issues facing the aquaculture community. Results of risk analysis should be communicated to all stakeholders, including agency officials (in national, regional and international agencies, including the FAO, the aquaculture sector, the nongovernmental organization (NGO) sector, the academic sector and the general public. Different groups of stakeholders will be reached most effectively by different means. Written materials will include FAO publications, such as the proceedings of this workshop, and technical manuals (e.g. ABRAC, 1995; Scientists' Working Group on Biosafety, 1998; Kapuscinski *et al.*, 2007a). Electronic media will include interactive websites (e.g. ABRAC, 1995). Risk communication through direct interpersonal contact will prove effective and should include discussions of aquaculture extension agents with small farmers and workshops at regional aquaculture meetings targeting the commercial sector. Instructional materials should be developed that integrate genetic risk analysis into fisheries and aquaculture curricula.

CONCLUSIONS

Aquaculture operations pose genetic harms to natural populations in the receiving environment. The risk analysis framework is useful for identifying, evaluating and addressing genetic harms posed by escape or release of aquaculture stocks. Direct genetic harms include loss of adaptation, introgressive hybridization and reduction of effective population size, community-level changes; indirect effects upon other species might be mediated by predation or competition. The likelihood of release from an aquaculture operation depends upon the species, culture system and operations management practices at issue. The likelihood of exposure due to establishment of an aquaculture stock in the receiving ecosystem depends upon its invasiveness and net fitness, and upon the stability and resilience of the receiving ecosystem. The likelihood of harm becoming realized given exposure to the hazard is difficult to quantify given present knowledge, and in the immediate term, may be best considered qualitatively. Risk is estimated by multiplying the likelihoods of release, exposure and harm given exposure to the hazard. In the aquaculture context, risk management focuses on minimizing exposure to the hazard by means of physical confinement, reproductive confinement and operations management procedures. Effective risk communication will require explanation of how risk analysis is applied to genetic issues, as well as discussion of case studies relevant to aquaculture.

FUTURE CHALLENGES

A number of technical issues face genetic risk analysis for aquaculture stocks. Regarding genetic risk assessment, more baseline data and case studies are needed. Opportunities for many informative case studies were effectively lost for the lack of baseline data or because we did not monitor a population until after a genetic harm was realized. Background information useful as case study material is scattered across the scientific and grey literature and is not as well developed for aquaculture as for fisheries management. Understanding of some key issues – e.g. likelihood of outbreeding depression and fitness of transgenic fishes – is still emerging. Other future challenges include lack of knowledge of: long-term impacts of genetic changes, levels of variation needed to maintain viable populations over the long term and relative risks of different classes of genetically modified aquaculture stocks. Hence, development of quantitative genetic risk analysis is very incomplete, especially with regard to estimating the likelihood of harm becoming realized given exposure to a hazardous agent. There are but a handful of definitive case studies of formal genetic risk analysis in the aquaculture literature – notable examples include the finding of no significant impact for the Auburn University field test of transgenic common carp (OAB, 1990) and the risk analysis for introduction of triploid Asian oysters into Chesapeake Bay (Dew, Berkson and Hallerman, 2003; NRC, 2004c; Box 2). Taken together, all these observations suggest the need for more genetic risk analysis studies, especially for nonsalmonid systems. Regarding risk management, while reliable confinement can be achieved for capital-intensive systems, more effort must be directed to developing and demonstrating cost-effective confinement systems for small aquaculture operations.

Regarding oversight of aquaculture by governments and non-governmental organizations, while the theory of risk analysis is established, we as a profession need to apply it, drawing upon definitive case studies for guidance. As experience is gained, an adaptive approach to management of aquaculture systems would be appropriate, not only for genetic risks, but also more generally for other types of risks. Effective communication of principles and application of risk analysis is needed to organizations in both developed and developing countries. There is a need for capacity-building in oversight bodies, especially in the public sector.

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