

Interface of biotechnology and ecology for environmental risk assessments of transgenic fish

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Genetically engineered fish with enhanced phenotypic traits have yet to be implemented into commercial applications. This is partly because of the difficulties in reliably predicting the ecological risk of transgenic fish should they escape into the wild. The ecological consequences of the phenotypic differences between transgenic and wild-type fish, as determined in the laboratory, can be uncertain because of genotype-by-environment effects (GXE). Additionally, we are limited in our ability to extrapolate simple phenotypes to the complex ecological interactions that occur in nature. Genetic background can also shape the phenotypic effects of transgenes, which, over time and among different wild populations, can make risk assessments a continuously evolving target. These uncertainties suggest that assessments of transgenic fish in contained facilities need to be conducted under as wide a range of conditions as possible, and that efficacious physical and biological containment strategies remain as crucial approaches to ensure the safe application of transgenic fish technology.

This year marks the twentieth anniversary of the first research on transgenic fish [1]. During this time, more than 30 species of fish have been genetically engineered, including many of the major world aquaculture species (e.g. carps, tilapia, catfish and salmonids). Because demand for foods from aquatic sources continues to grow in response to human population growth and diminishing wild fish stocks, transgenic technologies are being explored for their potential to increase aquaculture production efficiency and yield. Although several commercially important traits are being modified (Table 1), to date, most effort has been targeted to enhancing growth and feed conversion efficiency through the transfer of growth hormone (GH) gene constructs (Figure 1).

Despite initial achievements, commercial implementation of transgenic fish technology for food production has remained elusive. The lack of implementation has been, in part, due to the technical challenges and regulatory requirements in addition to the significant opposition to transgenic fish within the scientific, public and

aquaculture-producer sectors [2,3]. Such concerns are derived largely from legitimate (and far-fetched) speculation about food safety issues and the potential ecological harm that might arise if transgenic fish were to escape into nature [4]. Food safety issues from the view of the National Research Council (NRC) [4] are generally minimal and, in most cases, appear to be well managed under the existing regulatory frameworks present in many global jurisdictions.

For environmental risk assessments and mitigation of transgenic fish, conceptual and theoretical bases for assessment have been elaborated [4–9]. However, for most transgenic fish, insufficient publicly accessible data are available to resolve the complex issues that are necessary both for risk assessments and to develop consumer and commercial confidence. For transgenic fish technology to move forward, empirical risk assessment research needs to be undertaken and presented in parallel with strain development, enabling this maturing technology to have the essential information available to support regulatory and social requirements. This article discusses the current status and uncertainties associated with empirical risk assessment research on transgenic fish, including evaluation of potential impacts, estimation of fitness and approaches for biological containment.

Phenotypic effects of GH transgenesis in fish

The GH gene constructs used in fish have generally followed the designs used in other vertebrate systems [10], with piscine DNA constructs now most commonly used. GH overexpression in fish can cause significant enhancement of growth rate, which can result in large differences in size at a particular age in several species (e.g. typically 2–10-fold, and up to 35- and 37-fold weight gain in loach and salmonids, respectively; Table 1, Figure 1) and a compression of the life history of the species [11,12]. Fast-growing GH-transgenic fish can mature to have body sizes larger than those seen in nature [13–15], but in salmonid species, which die after sexual maturity, GH-transgenic individuals reach the size of a normal wild-type adult in a shorter period of time [12,16]. The distinct effects observed among species and strains reveals the need for risk assessments to be performed on a case-by-case basis.

Because GH acts on many processes in addition to growth, transgenic fish overexpressing this hormone can

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Table 1. Examples of transgenes introduced into fish that cause significant phenotypic effects

Phenotype targeted	Species	Transgene	Reference
Growth (> twofold)	Atlantic salmon	Growth hormone	[61]
	Tilapia		[62,63]
	Rainbow trout		[11,15,64]
	Coho salmon		[12,65]
	Chinook salmon		[11]
	Rohu		[66]
	Loach		[14,67]
Freeze tolerance	Atlantic salmon	Antifreeze protein	[68]
Disease resistance	Catfish	Cecropin	[69]
	Carp	Lactoferrin	[70]
	Medaka	Cecropin	[71]
Carbohydrate metabolism	Rainbow trout	Glucose transporter	[72]
	Rainbow trout	Hexokinase	[72]
Reproduction	Rainbow trout	Antisense GnRH	[57]
Lipid metabolism	Zebrafish	D6-desaturase	[73]
Phosphorus metabolism	Zebrafish	Phytase	[74]
Vitamin C metabolism	Rainbow trout	L-gulonono-gamma-lactone oxidase	[75,76]

show a broad range of pleiotropic effects on morphology, physiology, metabolism, immunology and behaviour. For example, GH-transgenic salmonids can display altered skeletal structure [17], reduced disease resistance [18], impaired swimming ability [19], altered oxygen utilization and metabolism [20,21], altered gonadal development [16], altered gut, gill, muscle, heart and liver structures [22–25] and modified regulation of GH, thyroid, and insulin-like growth factor I (IGF-I) hormones [26–29]. Major effects are also observed in the behaviour of GH-transgenic fish, including significantly enhanced feeding motivation and reduced discrimination of prey choice, reduced schooling tendency and increased predation mortality [30–35]. GH-transgenic fish have been observed to display altered breeding behaviours under contained laboratory conditions, with reduced mating success observed for coho salmon and enhanced success for medaka [13,16]; however, for salmon, these observations have been difficult to interpret in view of large culture effects on phenotype. Many of the phenotypic effects observed in transgenic fish are anticipated to alter production characteristics (e.g. growth rate, feed conversion efficiency and disease resistance), directly and indirectly. Similarly, such phenotypic changes are expected to influence the survival and reproductive capabilities of the organism, in nature, in complex and interacting ways (Figure 2).

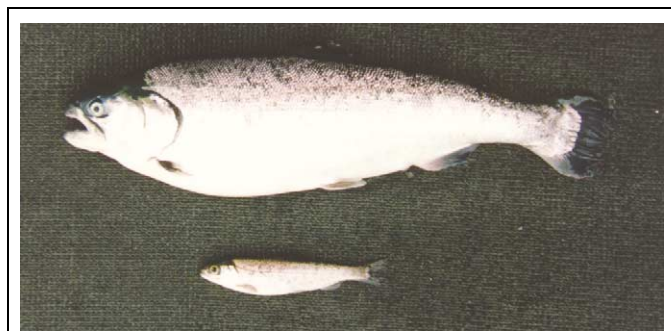


Figure 1. Effect of growth hormone (GH) transgenesis on growth in fish in culture conditions. Non-transgenic (bottom) and GH-transgenic (top) coho salmon siblings at 14 months of age. Photo: R Devlin, Fisheries and Oceans Canada.

Risk assessment framework

Formal risk assessment terminology, and methods, for transgenic animals have been documented [4]. There are three crucial components to risk assessment: risk is the probability of harm (e.g. a negative effect on a prey population) resulting from exposure to a hazard (e.g. elevated feeding by transgenic fish). In the case of a transgenic fish, exposure to hazards results from a two-step process: escape from culture facilities followed by possible spread of the transgenic fish into the environment. If a transgene cannot sustain itself in populations, any possible harms arising from the phenotypic attributes of the animal are anticipated to be short term and, in most cases, manageable.

Potential ecological effects of GH transgenesis

To assess potential harms to the ecosystem, a series of issues require examination. These can be organized into simple logical models as shown in Figure 3. Steps in this process include assessment of the probabilities of introduction, survival and establishment, and the consequences if such events occur. Such evaluations can become comprehensive, and comprise interacting hierarchies of questions that provide useful insight and direction into the requirements for risk assessments [7].

The aquaculture of many species involves rearing large numbers of fish in net pens or open earthen ponds, making cultured fish vulnerable to accidental escape into the wild, from which full recovery is virtually impossible. Environmental effects might (or might not) be interpreted as harms depending on various scientific and social criteria but are anticipated to arise from escaped transgenic fish if they are phenotypically distinct from conspecifics or are completely novel to the ecosystem. Evolutionary theory would suggest that wild fish should have achieved a local fitness maximum through selection, and that anthropogenically induced genetic changes from transgenesis should not often result in enhanced fitness. This has been interpreted to mean that the risk of harm from escaped transgenic fish is low [36]. Nevertheless, it remains possible that transgenic fish possess novel phenotypes and a genetic capacity not previously available to the species, which could

environmental impact. Norwegian Institute for Nature publication, 215; and [37]), and it is the introduction of novel phenotypic traits, such as high fecundity, growth rate, environmental tolerance and longevity, that most probably make a species successfully invasive [38]. Such traits have been modified in transgenic fish, for example, by transgenes conferring growth, disease resistance or extensions to the biophysical range (Table 1).

Requirements for fitness assessments

If transgenic fish do escape into nature, the likelihood of genetic spread of a transgene is dependent on total fitness and natural selection. Assessing the true natural fitness of transgenic fish is a momentous task because many hundreds, if not thousands, of factors are anticipated to influence the survival and reproduction of transgenic fish in the wild (Figure 2). For example, we know that juvenile viability in salmonids is affected by many factors (such as embryonic survival, tolerance of stress and physical variables, disease resistance, predator avoidance and foraging success), each of which is, in turn, influenced by many other variables. Similar complexity exists for all life-history stages. To simplify this complexity, a net fitness approach for risk assessments has been developed that categorizes the plethora of variables into six major survival and reproductive fitness parameters (juvenile viability, adult viability, ages of sexual maturity and at each mature age, female fecundity, male fertility and mating advantage), which can be used for modelling population effects of transgenes [8,39,40]. Determination of these net fitness values from fish in nature could enable estimation of lifetime fitness to define the spread of a transgene through a population. An important finding of this theoretical work is that combinations of fitness changes in transgenic organisms might result in unanticipated population effects, including extinctions. For example, growth-enhancing transgenes, conferring both a mating advantage and survival impairment (within certain ranges), might drive the transgene into a population at high frequencies and cause population extinction [40]. This is described as a Trojan gene effect.

Clearly, the best environment from which to acquire fitness data would be from nature, an approach that has been used to estimate the fitness and invasiveness of transgenic crops [41]. However, at present, non-confined releases of fertile transgenic fish to nature for direct assessment of fitness parameters and invasiveness are not viewed as prudent because of risk uncertainty. At the other extreme, the use of simple aquarium environments for estimates of fitness parameters are unlikely to provide definitive data for risk assessments because they are not generated in the presence of the myriad of interacting forces that operate in nature (e.g. lifetime viabilities of salmon in an aquarium facility can exceed 80% compared with the average survival to maturity in nature of ~0.1%). Because of these difficulties indirect methods of assessment are used.

Indirect methods of fitness assessment

Three main approaches have been used to generate empirical information for risk assessments of transgenic fish:

- (1) Individual physiological and behavioural characteristics have been evaluated in various laboratory apparatus (e.g. behaviour chambers, disease challenge facilities and swim tunnels) [18,19,31,32,34,35]. Studies under such controlled conditions can reveal information on mechanisms (e.g. physiological and behavioural traits) and developmental potential of the transgenic fish and assists the interpretation of results from experiments that are carried out in more complex environments.
- (2) Use of semi-natural systems [32,33,42] enables laboratory effects to be reduced and physical complexity and natural behavioural interactions to be introduced into experimental designs in microcosms and mesocosms (e.g. enable foraging, predation, swimming, metabolic and energetic effects, in addition to carry-over effects from earlier life-history stages, to all operate simultaneously). For species from small or relatively simple environments (e.g. some tropical fish species), or for certain life history stages of larger fish, this approach might enable an adequate simulation of nature.
- (3) Release to nature of surrogate non-transgenic fish strains that have similar phenotypes as transgenic strains. In the case of growth-enhanced salmon, fast-growing domesticated strains or GH-implanted fish might provide useful fitness information without the risk of introducing transgenes into the wild [43–46].

Each of these approaches also has limitations, which will be discussed in the following section.

Current limitations of empirical indirect fitness assessments

From information gained from these three indirect approaches, estimates of net fitness could, in theory, be applied in modelling exercises to predict the potential spread and impact of transgenic fish in nature. However, although considerable progress has been achieved for GH-transgenic coho salmon, rainbow trout and medaka, we have not yet been able to generate sufficient reliable data, using any method, to enable accurate prediction of transgene spread or estimate the impacts on nature. This inability is due, in part, to several limitations associated with empirical fitness assessments for species that must be reared in contained aquarium facilities.

Difficulty in extrapolating individual phenotypes to the whole organism

Our ability to extrapolate individual physiological and behavioural phenotypes to consequences in nature is limited. Identification of the effects on individual traits has been achieved in many cases but their magnitude and consequences in nature remain largely speculative (e.g. reduced swimming speed [19,47] would certainly affect predator avoidance, but to what degree?) Similarly, it is difficult to quantify tradeoffs and synergistic or antagonistic interactions arising from the pleiotropic effects of a transgene (Figure 2). For example, enhanced feeding motivation and development rate result in enhanced ability to acquire resources but this occurs at the cost of

a reduced response to predators and lower survival rates [30,31,33–35,42].

Genotype-by-environment interactions affecting phenotype

A complication arises from the use of laboratory environments, or even semi-natural systems, to rear transgenic fish because these conditions can, themselves, significantly alter phenotype and, thus, experimental data. The differential response of two genotypes to natural and culture environments arising from genotype-by-environment interactions (GXE) can present a large obstacle to our current ability to generate useful information for natural fitness estimates from laboratory-reared transgenic fish [9,16,48]. For example, non-transgenic coho salmon raised in culture conditions show altered growth, morphology, colouration, egg size, fecundity, and spawning ability relative to wild fish with the same genetic background [16]. However, in the absence of GH-transgenic salmon from nature (Figure 4a) we cannot know whether the relative phenotypic differences of fish in culture are directly proportional to those that would be found between GH-transgenic and wild-type fish. Indeed,

strong environmental effects have been observed within and between studies for fitness-related traits, including competitive growth and survival, adult morphology and spawning ability, and for foraging behaviour in GH-transgenic salmon [16,30,31,42,49,50]. Thus, transgenic and non-transgenic genotypes respond to changing environments in different ways, which cannot be predicted (Figure 4b). Similarly, the absence of an effect in the laboratory does not demonstrate that effects would not occur in nature, for example, a disease resistance transgene, which has no effect on viability under pathogen-free laboratory conditions, might confer an enormous fitness advantage in nature. Thus, specific laboratory conditions can dramatically influence experimental results. Consequently, assessments should be performed under as wide a range of experimental conditions as possible to determine the limits of important parameter values.

To be confident that GXE effects are understood sufficiently to enable estimation of natural fitness of transgenic fish, an important benchmark to achieve would be to produce cultured non-transgenics with the same morphological, physiological and behavioural phenotypes as wild fish derived from the multiple environments that are found in nature. This would require the use of highly naturalized mesocosms to provide the same level of complex interactions that are seen in nature. In such facilities, the phenotype of transgenic fish should more closely approximate that found in nature; however, in the absence of wild transgenic animals (Figure 4), we can never be completely certain that such mesocosm culture-conditions would also be sufficient to naturalize transgenic animals. For those species from large, complex ecosystems, it remains a daunting task to develop laboratory facilities that will adequately simulate nature at all life history stages, for example, for coho salmon, some aspects of natal stream environments can be approximated, whereas simulating main stem rivers, estuaries and the North Pacific Ocean is extremely difficult.

There might be some special circumstances where phenotypic assessments performed under ideal laboratory conditions could provide useful estimates of fitness. The simplest case is when characteristics of the host species (transgenic or otherwise) do not enable survival in a novel environment (e.g. thermal tolerance). Similarly, if a transgene causes large phenotypic effects (e.g. conferring major effects on swimming performance), the fitness differences observed between transgenic and non-transgenic fish in the laboratory might be extended to those anticipated from nature. It might also be possible to establish an upper limit to transgene risk in cases where the function of the transgene is understood sufficiently to establish laboratory conditions that provide the most favourable conditions for transgene survival. If, under such conditions, the net fitness for transgenic fish was less than that for non-transgenic fish, then it would take an unanticipated GXE interaction to confer enhanced fitness in a native environment. Thus, an important question for the future is to determine to what degree GXE effects can be predicted to enable definition of the best laboratory conditions – a task expected to be most difficult for

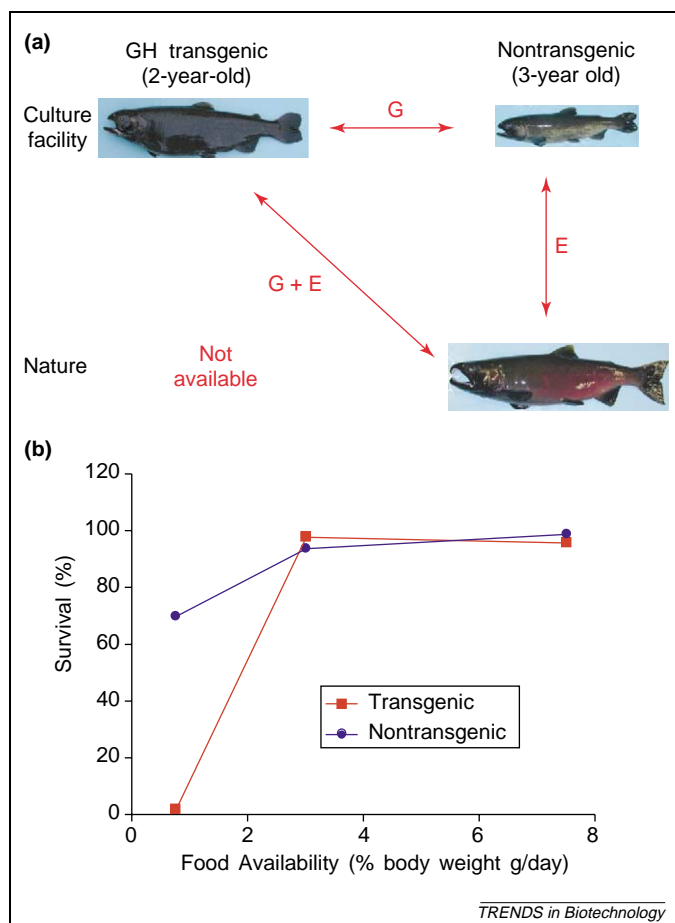


Figure 4. Examples of genotype-by-environment (GXE) effects influencing risk assessment data. (a) Genotype and Environmental effects on growth, morphology and colour phenotypes in sexually maturing coho salmon arising from rearing in culture and wild environments [16]. Phenotype of transgenic fish from nature is not known, preventing elucidation of GXE interactions affecting phenotype. (b) Nonparallel reaction norms revealing GXE effects for % survival of transgenic and non-transgenic GH coho salmon at high (7.5% body weight/day), non-transgenic satiating (3% body weight/day) and low (0.75% body weight/day) food supplies [49].

transgenes conferring complex pleiotropic traits or those with poorly defined functions.

Difficulty of using surrogate fish in natural environments, with extrapolation to transgenic fish

Surrogate fish (e.g. sterile triploid transgenic fish, GH-implanted fish or domesticated strains) can provide useful insights into the potential effects of transgenic strains. However, surrogate fish might possess a range of differences in phenotype from transgenic fish, which could, in some cases, result in differences in fitness between surrogate and transgenic fish. For example, domesticated coho salmon do not show the high level of growth stimulation achieved by GH transgenesis [15].

Importance of genetic background

Background genotype strongly influences the phenotypic effects of GH transgenes in salmonids [15], with a slow-growing wild strain showing a greater response to GH transgenesis than a fast-growing domesticated strain (Figure 5). From a risk assessment perspective, most of the examinations of transgene effects are conducted in well-characterized strains but, in nature, a 'fit' transgene will encounter many different genetic backgrounds as it spreads through a population. Each background genotype will confer a distinct phenotype and thus improve or decrease fitness potential; as such, the magnitude of fitness estimates made in one genetic background might not apply to others. Hence, transgene effects, and transgene fitness, are anticipated to change during time and space through encounters with, and natural selection of, novel genetic backgrounds, therefore making risk assessments a complicated evolutionary moving-target. A potential secondary consequence of these effects to wild populations is that the background genetics selected for optimum fitness in the presence of the transgene is unlikely to be that which affords maximum fitness to non-transgenic wild fish.

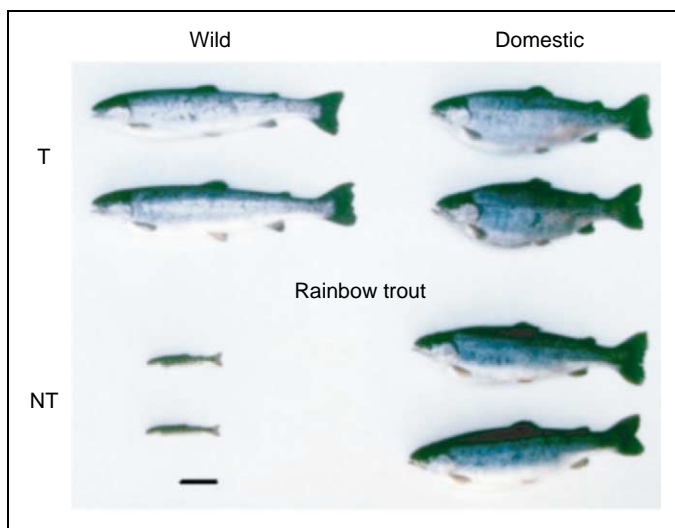


Figure 5. Effects of genetic background on phenotypic assessments of transgenic fish. A GH transgene stimulates growth more in a slow growing (wild) than a fast growing (domesticated) strain of rainbow trout [15]. T: GH transgenic; NT: non-transgenic. Reprinted from [15] with permission.

Containment strategies are key to future use of transgenic fish

In those cases where natural fitness cannot be estimated with reliability, containment approaches coupled with conditional impairment of viability or fertility could, in theory, minimize the ecological effects of transgenic strains [9,41]. All transgenic fish are currently reared in land-based facilities and, worldwide, we are unaware of any escapees entering nature. However, such confinement measures become more difficult, but not impossible, at the large scales necessary for aquaculture production.

Biotechnological approaches to reproductive containment are also being explored as methods for minimizing the effects of escaped production fish. Perhaps the simplest approach is to rear transgenic fish in climatic zones that cannot support survival or reproduction in the event of escape. The application of mono-sex technology, which enables the culture of large numbers of fish of a single sex [9], could also provide a high level of containment against the multigenerational impacts of escaped transgenic fish in cases where breeding partners (conspecifics or otherwise) do not exist in nature (e.g. Atlantic salmon in the South Pacific Ocean). In cases where conspecifics are present, triploidy technologies can be used to induce sterility [9,41]: these cause gonadal dysgenesis in females and infertility in males [51]. Coupling triploidization with mono-sex female technologies can produce sterility in large numbers of production fish and could be an attractive approach despite the impairment of growth and reduced disease-resistance observed in triploid GH-transgenic fish [12,18,52].

From a risk assessment perspective, the efficacy of sterility techniques becomes a crucial variable: the release of even a single fertile animal could initiate a process with ecological impacts. Currently, induction of triploidy is highly effective (up to 99.8% in laboratory experiments, R.H. Devlin, unpublished data), which would indeed provide a significant level of containment. However, the escape of fish from aquaculture facilities can involve large numbers of animals (greater than 500 000 in some cases), which, based on the previous estimate, could result in the release of ~1000 diploid transgenic animals. Stochastic effects on survival, which act on individuals in natural populations, might delay but do not eliminate the introduction of transgenic individuals into breeding populations if escapes occur regularly. Thus, assuming that screening every production animal to verify triploidy would be prohibitive, further research is required to improve this technique as a sterilization method. One alternative is the use of tetraploid fish, which, when crossed to wild (diploid) fish, produce triploid progeny [53]. The large-scale efficacy of the tetraploidy approach has not been examined, but reversions of the tetraploid strains to diploidy have been noted in salmonids and loach in laboratory studies [54,55].

Transgenic approaches to containment are also being explored to permit the survival and breeding of broodstock and production fish in culture but not if they have escaped to the wild. Conditional control of these effects can be achieved by repressible or inducible promoter systems and dietary or hormonal complementation [56,57].

Sterilization gene-constructs have been considered, to repress expression of gonadotropin-releasing hormone or sex steroid biosynthesis by antisense expression, as has the ablation of germ line tissue [41,57,58]. These methods have, to date, not been completely effective in aquaculture species and have not been examined for their large-scale efficacy. Conditional viability or fertility impairment might also be feasible methods. These methods use a combination of lethal and rescue gene constructs that would cause the demise of transgene containing animals when separated (as would occur by outbreeding in nature) [59]. Transgenes can also be designed to induce non-viability in only the few diploids present in female triploid mono-sex populations (e.g. vitellogenin promoter driving thiaminase to induce thiamine deficiency). Where transgenic fish are introduced into zones that lack other breeding partners, the sterile-feral or non-viable-feral and daughterless approaches can also be applied to limit transgenic population growth [60]. Containment constructs should ideally be in tight linkage with the transgene conferring the beneficial trait, and the use of containment strategies based on recessive genetic functions are anticipated to be less subject to reversion by mutation than those based on novel dominant functions. Containment constructs should also have minimal potential to cause environmental consequences themselves: it has been noted that constructs designed to affect viability conditionally, for example, requiring a supplement in feed to enable survival, could induce viability impacts (Hindar, K. 1993 *Genetically engineered fish and their possible environmental impact*. Norwegian Institute for Nature publication, 215). Thus, considerable care must be taken when anticipating the potential effects of containment constructs to ensure that they only occur in escaped cultured fish.

The level of containment required for a particular transgene will ultimately depend on the potential harm that it might cause in nature in addition to the level to which such harm is acceptable: these are determined by public policy in decision analyses. The use of combinations of containment approaches (physical and multiple biological) are anticipated to deliver the best opportunity to prevent sustained ecological impacts arising from escaped transgenic fish.

Conclusions

Transgenic fish technology provides a key biotechnological opportunity to enhance global production and quality of aquatic foods, particularly at the local level in developing nations where aquaculture has an important role in producing animal protein for human consumption. The issues raised above regarding the estimation of natural fitness present significant obstacles but also raise challenges that require resolution if transgenic fish technology is to reach its potential in the coming decades. Although improved facilities are needed to enable the culture of transgenic strains with natural phenotypes for risk assessments, this approach will only partially solve the problems associated with fitness assessments. As such, it is clear that biotechnological solutions are urgently required for containment,

to prevent interactions between transgenic fish and wild fish populations. In response to the needs of a burgeoning human population, food production activities have destroyed many forest and prairie ecosystems, globally. The future application of safe biotechnological solutions to enhance the efficiency of food production, including transgenic fish, might stem the need for continued growth of conventional agricultural production systems to the benefit of natural ecosystems.

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