African Scientist
Vol. 16, No. 4, December 31, 2015
Printed in Nigeria

1595-6881/2015 $5.00 + 0.00

http://www.niseb.org/afs

AFS 16403

Environmental Concerns

National Research Council (US) Committee on Defining Science-Based Concerns Associated with Products of Animal Biotechnology, Washington DC, U.S.A.

Potential impacts on the environment from the escape or release of genetically engineered organisms was the committee's greatest science-based concerns associated with animal biotechnology, in large part due to the uncertainty inherent in identifying environmental problems early on and the difficulty of remediation once a problem has been identified. The intent of this chapter is to identify the risks to the environment posed by GE animals, prioritize those risks, and explain the criteria used for selecting them. The committee based its assessment on principles of risk analysis that are general in their application and not limited to currently developed biotechnology. Where possible, examples from the scientific literature are used, while in others hypothetical examples are used to illustrate risks that exist in theory but thus far have not been observed.

The committee explicitly recognized that along with potential risks, there might be many benefits of biotechnology for alleviating human suffering and for addressing problems with growing food demands. The ultimate decision of when or where to use biotechnology will be evaluated not only in relation to these benefits, but also to those of alternative technologies. However, the charge to this committee was not to examine the benefits of biotechnology, or of the technical alternatives, but rather to "develop a consensus listing of risk issues in the food safety, animal safety, and environmental safety areas for various animal biotechnology product categories." The committee also was asked "to provide criteria for selection of those risk issues considered most important that need to be addressed or managed for the various product categories." By using definitions of risk and hazard established in previous National Research Council reports, the committee attempted to rank those concerns. In these two ways, the committee attempted to put those concerns in perspective and to provide a balanced viewpoint.

Any analysis of GE organisms and their potential impact on the environment needs to distinguish between organisms engineered for deliberate release and those that are engineered with the intention of confinement but escape or are inadvertently released. The discussion in this report focuses primarily on the latter category, but the committee recognized the possibility of intentional release of GE organisms into the environment and expressed a high level of concern about it. This chapter also focuses primarily on risks as a result of genetically engineered (GE) animals entering natural environments and transgene spread through vertical gene transmission (the sexual transfer of genetic information between genomes) followed by natural selection. The risk of horizontal gene transfer (the nonsexual transfer of genetic information between genomes; Kidwell, 1993) is discussed primarily in another article.

This articleiu, therefore, is organized into a discussion of: (1) general principles of risk analysis, (2) general aspects of the organism, transgene, or transgene function that can be used *a priori* to prioritize GE animals for level of environmental concern, (3) risks posed by key classes of GE animals, and (4) the need for further research directed at improving our understanding of hazards and estimating risks posed by genetically engineered animals.

GENERAL PRINCIPLES OF RISK ANALYSIS

Consideration of environmental concerns posed by GE animals must be based on an understanding of key concepts underlying the science and practice of ecologic risk assessment. A seminal review of risk assessment methodology (NRC, 1983) states, "Regulatory actions are based on two distinct elements, risk assessment, and risk management. Risk assessment is the use of the factual base to define the health effect of exposure of individuals or populations to hazardous material and situations." Risk management is "the process of weighing policy alternatives and selecting the most appropriate regulatory action, integrating the results of risk assessment with engineering data and with social, economic, and political concerns to reach a decision." Clearly, risk management is beyond the purview of this committee, while elements of risk assessment are needed to prioritize concerns.

Understanding Risk: Informing Decisions in a Democratic Society (NRC, 1996) updated the 1983 NRC study and provided two important definitions: *Hazard*: an act or phenomenon that has the potential to produce harm, and *Risk:* the likelihood of harm resulting from exposure to the hazard. While the earlier study describes risk assessment as containing some or all of the following steps: (1) hazard identification, (2) dose-response assessment, (3) exposure assessment, and (4) risk characterization. These steps do not apply well to GE organisms in the environment because dose-response and exposure assessments are intended to apply to substances that can be quantified in discrete amounts and that cannot reproduce themselves. Adapting principles from both studies (NRC, 1983; 1996) to the current problem, the committee used the definitions of risk and hazard to develop a set of working steps.

Defining Risk

Risk, as defined, is a probability that can be quantified and expressed in an equation, thereby providing a method to prioritize concerns. However, exact probabilities of risk might be difficult or impossible to determine for all categories of possible harm. Indeed, all possible harms might not be known or knowable *a priori*, particularly with respect to secondary effects. On the other hand, based on current knowledge of population genetics and receiving ecosystems, and experience with domesticated species, it is possible to classify GE organisms into categories of high to low probabilities of spread into the environment. Risk of possible harms (known and unknown) can then be inferred from the probability of spread (i.e., risk of harm to a healthy natural population is low), if the transgene is purged from the population. This method is used only to prioritize the likelihood of a GE organism to destabilize a natural community; it does not address possible harms to humans, direct or indirect.

Because risk is the joint result of exposure and harm, it is the product of two probabilities: the probability of exposure, $P(E)$, and the conditional probability of harm given that exposure has occurred, $P(H|E)$, that is, Risk, $R = P(E) \times P(H|E)$. In this context, the steps in risk analysis are: (1) to identify the potential harms regardless of likelihood, (2) to identify the potential hazards that might produce those harms, (3) to define what exposure means for a GE organism and the likelihood of exposure, $P(E)$, (4) to quantify the likelihood of harm given that exposure has occurred, $P(H|E)$, and (5) to multiply the resulting probabilities to prioritize risk. Because all potential harms might not be known or cannot be known, it will be necessary to update this procedure continually as knowledge accumulates, using an adaptive management approach (NRC, 1996; Kapuscinski, 2002).

PRIORITIZING GE ANIMALS FOR LEVEL OF ENVIRONMENTAL CONCERN

Steps in Ecologic Risk Assessment

Identifying Potential Harms and Hazards

In an ecologic context, harm is defined as gene pool, species, or community perturbation resulting in negative impacts to community stability. These include displacement or reduction in the number of species that exist in a community or numbers within each species. This definition is all-encompassing and broad, but can be further refined once a particular GE organism is identified and the environment into which it might escape or be released is known. The hazard is the GE organism itself because it is the agent that might cause negative impacts to community stability. These negative impacts might be either direct (e.g., resulting from direct competition for limited food or resources)—or indirect, caused by changes in other biotic factors utilized or needed by the ecologic community (Scientists' Working Group on Biosafety, 1998).

The process of prioritizing concerns will vary from case to case because of the uniqueness of each GE construct, transgenic founder individual from which a line is derived, and receiving ecosystem (USDA, 1995). However, based on the principles of risk assessment, the committee attempted to prioritize environmental concerns posed by GE animals by considering the following variables: (1) the effect of the transgene on the "fitness" of the animal within the ecosystem into which it is released, (2) the ability of the GE animal to escape and disperse into diverse communities, and (3) the stability and resiliency of the receiving community. These three variables determine the likelihood that a GE organism will become established in a receiving community—a critical factor in risk assessment.

Defining What Exposure Means for a GE Organism and the Likelihood of Exposure: P(E)

Exposure is a threshold phenomenon because an initial escape or release of a GE organism might not have a measurable effect on the receiving community; the organism might not be able to establish itself in the community, and might be lost rapidly due to natural selection. Thus, provided the natural population is not already endangered, exposure must be more than just release or escape for a GE organism to prove a hazard. The GE organism must spread into the community. The committee, therefore, defines exposure as the establishment of a GE organism in the community, and in the following text, establishment will be substituted for exposure. For risk assessment, the critical factor is the likelihood the GE organism will become established in a community, which is $P(E)$. This conclusion does not mean that risk cannot occur without establishment. As discussed later, if a transgene causes local species extinctions, either because the population size is critical or because the transgene produces a Trojan gene effect, considerable harm might result. However, these are special cases that can be addressed as such. The likelihood of establishment is dependent on an organism's fitness and ability to escape and disperse in diverse communities (Scientists' Working Group on Biosafety, 1998), and the qualities of the receiving community.

Fitness

Once a transgene is introduced into a community, whether by vertical or horizontal gene transfer, natural selection for fitness will determine the ultimate fate of the transgene if the population is large enough to withstand the initial perturbations (Muir and Howard, 2001). Fitness is quantified relative to that of other individuals in the population and is simply the genetic contribution by an individual's descendants to future generations of a population (Ricklefs, 1990). Fitness in this context refers not only to its survival component, but also its reproductive component, that is, to *all* aspects of the organism's phenotype that affect spread of the transgene. Muir and Howard, in modeling the potential spread of a transgene (2001; 2002a,b), reduced these aspects to six net fitness components: juvenile and adult viability, age at sexual maturity, female fecundity, male fertility, and mating success. The model is based on the assumption that natural selection acting through these components will determine the ultimate fate of the transgene.

The last component, mating success, often is overlooked because it generally is not a factor in artificial breeding programs; it often is, however, the strongest factor driving natural selection (Hoekstra *et al*., 2001). For example, increased adult size in most species of fish is positively correlated with mating success (as, for example, in many salmonid species: Jones, 1959; Schroder, 1982; Jarvi, 1990; Groot and Margolis, 1991). With Japanese medaka (*Oryzias latipes*), males 25 percent above average in size realized a 400 percent increase in mating success (Howard *et al*., 1998). Such increases in mating success could result in the spread of a transgene even if the transgene reduces survival rate (Muir and Howard, 1999).

From a population genetics perspective, if a GE organism is more fit than its wild relatives in the receiving population, the GE organism eventually will replace its relatives or become established in that community. If it is less fit, the engineered trait eventually will be removed from the receiving population. If the fitness of transgenic and nontransgenic individuals is similar, the likely outcome is persistence of both transgenic and nontransgenic genotypes (Hedrick, 2001; Muir and Howard, 2001).

The effect of genetic engineering on fitness can be determined either prospectively or retrospectively. Appendix A of the Scientists' Working Group on Biosafety (1998) provides a prospective assessment of factors that would affect an organism's ability to become established in the environment, while Muir and Howard (2001a,b; 2002) provide a retrospective method based on measurement of net fitness components.

From a prospective view, the key factor affecting fitness is transgene functionality within the GE organism. Functionality can be divided into four broad categories: those that increase adaptability of the GE organism to a wider range of environmental conditions, usually through new functionality; those that alter existing traits for improved performance within standard production agriculture; those that produce new or novel products; and those that produce animals or animal products for human medical benefit.

Increased Adaptability

A transgene might increase an organism's adaptation to a wider range of environmental conditions, for example, by increasing freeze tolerance (Fletcher *et al*., 1992) or removing a limiting growth factor, perhaps allowing the organism to synthesize an amino acid that was previously limiting, or to digest previously indigestible carbon sources such as cellulose, or to obtain phosphorous from previously inaccessible sources, such as phytic acid (Golovan *et al*., 2001a,b). Finally, a transgene can be used to increase disease resistance by, for example, disabling retroviruses, producing coat proteins that activate the immune system against certain viruses or that bind to receptor molecules by which viruses enter cells, or by producing antibiotics to protect against bacterial infections (Dunham *et al*., 2002; Jia *et al*., 2000; Sarmasik *et al*., 2002).

Such adaptations also could allow GE animals to invade or persist in ecosystems where they otherwise could not, such as salt or brackish water, while maintaining populations in communities where they normally occur, such as freshwater lakes and streams. Such a combination could result in a sustained invasion of the new community from the species' original or introduced range until complete colonization results. Hence, a transgene that increases fitness or adaptation increases the probability of establishment and results in the highest level of concern for establishment.

Enhanced Existing Traits

Selective breeding as well as genetic engineering have enhanced the productivity and growth of many domesticated farm animals. Many transgenic animals have been engineered for enhanced growth rates (Hammer *et al*., 1985; Pursel *et al*., 1987; Devlin *et al*., 1994; 1995a; 1995b; Rahman and Maclean, 1999). Production traits in domesticated farm animals include, for example, growth rate, feed efficiency, egg number, milk yield, litter size, and fiber yield (e.g., wool). Experience with conventional selection for such traits in domesticated farm animals suggests that such modifications do not increase the fitness of animals in natural environments, often because of physiologic imbalances or growth demands in excess of the food available in natural environments. Transgenic animals designed to meet these objectives might be even less fit than those developed using selective breeding.

Selective breeding is based on manipulation of polygenic inheritance, in which the resulting phenotype results from the cumulative effect of changes in allele frequencies of many genes with a distribution of effects from small to large (Lynch and Walsh, 1998) and which are selected over multiple generations. In contrast, transgenesis involves one or few genes with relatively large effects, introduced in a single founder generation. In the selective breeding process, the correlated traits needed to support enhanced growth and reproduction, such as skeletal and vascular systems, also are selected for indirectly; this is not always the case with transgenics (Farrell *et al*., 1997; Muir and Howard, 2001; 2002b; see https://www.ncbi.nlm.nih.gov/books/n/nap10418/ddd00097/ regarding animal wellbeing concerns). Because of these homeostatic imbalances, domesticated animals transgenic for enhanced production traits might exhibit a greater reduction in fitness than their selectively bred counterparts. Experience with GE animals developed to date tends to support this contention/notion. For example, swine transgenic for growth hormone displayed a number of fitness problems (see https://www.ncbi.nlm.nih.gov/books/n/nap10418/ddd00069/). Similarly, fish transgenic for growth hormone have a reduced juvenile viability (Dunham, 1994; 1996; Muir and Howard, 2001; Devlin *et al*., 2001). Collectively, these findings seem to indicate that GE organisms developed for production traits have a low probability of establishment.

However, environmental concerns posed by animals expressing these types of transgenes cannot be dismissed. First, it is possible for GE organisms to overcome viability disadvantages if other fitness components are enhanced, such as mating success, fecundity, or age at sexual maturity (Muir and Howard, 2002b). Second, the introgression of genes decreasing fitness poses a near-term demographic risk to small receiving populations (i.e., small populations might not remain viable until the transgene is selected out, which poses a risk if a threatened or endangered or otherwise valued population is at issue). Finally, the magnitude of phenotypic change that is possible with transgenesis could exceed that of conventional breeding or natural mutations. Transgenic organisms can be produced with changes in physiologic traits far beyond what is possible with naturally occurring mutations such as dwarfism or gigantism in mammals and poultry. These naturally occurring mutations are limited to approximately four times the size of a normal organism, while, for example, transgenic salmonids have been reported to grow to a mean size-at-age of four to eleven times normal (e.g., Devlin *et al*., 1994; 2001).

At the heart of the issue is how species evolve. Domestication is widely believed to be the consequence of small incremental changes in trait value, and the ecologic niche of the animal is not changed if the phenotype of a mutant individual is only slightly changed. Expression of transgenes, however, could cause mega-mutations that instantaneously and substantially change the phenotype of the transgenic organism. In terms of evolutionary theory, such a mega-mutation could give rise to a switch from the currently-occupied adaptive peak to another peak on the adaptive topography of Sewall Wright's (1969; 1982) shifting balance theory. If such a shift were to occur, the GE organism might be able to establish itself in a new community or to shift its niche within the current community. An illustrative example of a natural major mutation causing a shift in evolutionary trajectory was a major mutation for mimicry that occurred in the evolution of butterflies (Lande, 1983). The primary predator avoidance attributes in butterflies are to remain concealed (crypsis) or to resemble closely another species that is distasteful to predators (mimicry). Intermediate individuals that are neither effectively cryptic nor good mimics are likely to be eaten, thus selection acting by small steps cannot account for such evolutionary adaptations. Therefore, natural mutations followed by selection can and do result in new evolutionary lines. Similarly, the expression of a growth hormone transgene producing up to 17.3-fold greater difference in weight by 14 months of age in trout (Devlin *et al*., 2001) acts as a mega-mutation that, for example, could change an organism from being a prey of one species to being a predator upon it.

Establishment of domesticated animals in the environment as a result of adaptive peak shifts, either through conventional or transgenic technology, has not been documented. Hence, the concern for this mode of transgene establishment in natural populations is moderate to low based on currently available evidence. However, it is theoretically possible for organisms engineered for production traits to become established in communities as a result of adaptive peak shifts; any such establishment would pose a high level of concern.

Production of New or Novel Products

Animals that are genetically engineered to produce new or novel products are yet another example of transgene functionality that could influence fitness. Milk, egg white, blood, urine, seminal plasma, and silkworm cocoons from transgenic animals are candidates to produce recombinant proteins on an industrial scale (Houdebine, 2000). Animals also can be used to produce pharmaceuticals in eggs (Harvey *et al*., 2002) or milk (Wright *et al*., 1991), or fibers such as spider silk in milk (Kaplan, 2002). Such alterations in physiology will result in additional energy demands without conferring any obvious fitness advantage. Such transgenic animals might have little chance of establishment in the environment (excepting silkworms), and hence raise the lowest levels of environmental concern. However, other indirect aspects of expressing such products are still a concern, and will be discussed in a following section.

Production of Animals or Animal Products for Human Health and Medical Benefits

Three categories of animals are genetically engineered for human health and medical benefits: pets altered to reduce allergens, animals altered for xenotransplantation purposes (Tearle *et al*., 1996; Lai *et al*., 2002), and insects altered to control the spread of pests and diseases (Braig and Yan, 2002; Spielman *et al*., 2002). The first two categories most likely will either not change fitness or will result in a decline in fitness and, like animals engineered to produce new or novel products, raise the lowest levels of concern with respect to the animal's ability to establish itself in natural communities. The last category—insects altered to control the spread of pests and diseases—has mostly involved the modification of mosquitoes not to carry parasites, and has unknown effects on the fitness of the mosquito. Some reports indicate that the parasite load reduces the fitness of mosquitoes carrying it (Braig and Yan, 2002; Spielman *et al*., 2002), suggesting that transgenes decreasing the parasite load might increase fitness. In addition, changes in the insect's driver mechanisms (meiotic drive and incompatibility systems) are being proposed as a way of establishing the GE mosquito in the community. Because establishment is the objective and is critical for biocontrol using these techniques, this category of genetic engineering raises the highest probability for establishment.

Ability to Escape, Disperse, and Become Feral

Another aspect of evaluating the probability of establishment of a GE animal in a community is the organism's ability to escape, disperse, and become feral in diverse ecologic communities. This mainly is a function of the animal being transformed, though the receiving ecosystem also might be a factor (USDA, 1995).

The dispersal ability of GE animals is not known, but reasonably can be assessed from knowledge of similar domesticated species (Scientists' Working Group on Biosafety, 1998). Table 1 summarizes these characteristics for commonly farmed and laboratory species. Some communities in Australia and New Zealand have been affected dramatically, particularly by the rabbit, while in the United States and Europe, pigs, cats, mice and rats, and fish and shellfish have caused the greatest disruptions.

The more domesticated a species, the less likely it is to survive in natural environments. Highly domesticated species such as poultry or dairy cattle are not well adapted to natural conditions and might not be able to survive and reproduce in a natural setting. However, if wild or feral populations exist locally, the escaped transgenic organisms could breed with those and spread the transgene into populations that otherwise are well adapted to the local environment. If the GE animal is released into an area where a native wild or feral population of the same species exists, mates might be readily available, and the transgene could spread via mating. Even in areas where the GE species does not exist, it might breed with members of a closely related species with which it is reproductively compatible (e.g., transgenic rainbow trout, *Oncorhynchus mykiss*, with native cutthroat trout, *O. clarki*; see reviews of hybridization, e.g., Dangel, 1973; Schwartz, 1981; Campton, 1987).

In the North American agricultural system, certain agricultural animals are well confined. However, cattle and sheep roam open ranges in the West, feral pigs exist in Arkansas, Hawaii, Florida, and California, and range chickens and turkeys exist in many states. Extensive damage has been reported for feral insects imported to improve agricultural production, such as the gypsy moth (Lepidoptera: Lymantriidae), a species imported for use as a silkworm (Gerardi and Grimm, 1979), and the Africanized honeybee (*Apis mellifera scutellata*), a species imported to improve the foraging ability of European honeybees (Caron, 2002).

The committee concluded that animals that become feral easily, are highly mobile, and have caused extensive community damage pose the greatest concern. These include mice and rats, fish and shellfish, and insects. Animals that become feral easily, have moderate mobility, and have caused extensive damage to ecologic communities are next. These include cats, pigs, and goats. Animals that are less mobile, but have been known to become feral with moderate community impact, pose the next level of concern. These include dogs, horses, and rabbits. Finally, less mobile and highly domesticated animals that do not become feral easily, such as domestic chickens, cattle, and sheep, present the least concern.

Table 1: Factors contributing to level of concern for species transformed

	Factor Contributing to Concern					
Animal	**Number of Citations**[1]	**Ability to Become Feral**[2]	**Likelihood of Escape Captivity**[3]	**Mobility**[4]	**Community Disruptions Reported**[5]	**Level of Concern**[6]
Insects[8]	1804	High	High	High	Many	High
Fish[7]	186	High	High	High	Many	↓
Mice/Rats	53	High	High	High	Many	
Cat	160	High	High	Moderate	Many	
Pig	155	High	Moderate	Low	Many	
Goat	88	High	Moderate	Moderate	Some	
Horse	93	High	Moderate	High	Few	
Rabbit	8	High	Moderate	Moderate	Few	
Mink	16	High	High	Moderate	None	
Dog	11	Moderate	Moderate	Moderate	Few	
Chicken	11	Low	Moderate	Moderate	None	
Sheep	27	Low	Low	Low	Few	Low
Cattle	16	Low	Low	Low	None	

[1] Number of scientific papers dealing with feral animals of this species.
[2] Based on number of feral populations reported.
[3] Based on ability of organism to evade confinement measures by flying, digging, swimming, or jumping ability for any of the life stages.
[4] Relative dispersal distance by walking, running, flying, swimming, or hitchhiking in trucks, trains, boats, etc.
[5] Based on worldwide citations reporting community damage and extent of damage.
[6] A ranking based on the four contributing factors.
[7] Did not include shellfish, some of which (such as zebra mussel and asiatic clam) have proven highly invasive.
[8] Limited to gypsy moth and Africanized honeybee.

The Likelihood of Harm Given that Exposure has Occurred: P(H/E)

The stability and resilience of the receiving community is another factor that influences whether transitory or long-term harm results from the introduction of GE animals. Colonization by GE animals might result in local displacement of a conspecific population, which could have a disruptive effect on other species in a community, for example, by releasing competing species from resource competition or prey species from predation (Kapuscinski and Hallerman, 1990); additionally, the survival of predatory species that depend on the eliminated species could be threatened. This concern is best exemplified by the classic experiment of Paine (1966) in the rocky intertidal zone. By experimentally removing the top predator, a starfish (*Piaster* sp.), the number of species in the plot was reduced from 15 to eight. Another example is the impact of pigs on plant species diversity reported by Hone (2002). Ground rooting of feral pigs in Namadgi National Park, Australia, decreased the number of plant species, which declined to zero with intensive pig rooting. Thus, expansion of a species into new ecosystems can have a cascading impact on other species in the community with unpredictable harms (see https://www.ncbi.nlm.nih.gov/books/n/nap10418/ddd00113/ for further discussion).

Transgenes that increase fitness or adaptability also could have negative ecologic impacts if they spread into pest populations. For example, phosphorous is an element essential for growth of all life forms. Securing this vital nutrient from the environment is critical for population growth. Phosphorous is contained within all seeds in the form of phytic acid. However, phytic acid is not digestible by non-ruminants (Golovan *et al*, 2001a). The addition of a phytase gene would allow GE non-ruminants such as pigs (Golovan *et al*, 2001b) or mice (Golovan *et al*, 2001a) to obtain needed phosphorus from seeds and grains, which would increase their ability to grow and produce more offspring, thereby resulting in a greater pest potential for feral pigs (Vtorov, 1993; Hone, 2002) and mice (Krebs *et al*., 1995; King *et al*., 1996).

Pleiotropic effects of transgenes that have antagonistic effects on different net fitness components can result in unexpected harms, ranging up to local extinction of the species into which the transgene is introduced (Muir and Howard, 1999; Hedrick, 2000). For example, the transgene might increase one component of fitness, such as juvenile or adult viability, but reduce another, such as fertility (Kempthorne and Pollak, 1970; Hedrick, 2000; Muir and Howard, 2002b). The effect of a transgene in this category parallels the use of sterile males to eradicate screwworms, except that in the case of sterile males they must be released continually to achieve control; a transgene that increases the viability component of fitness will spread on its own, while the reduced fertility brings about extinction, albeit over a longer time period. Fish transgenic for production of cecropins might represent a class of GE organisms that fit into this category. Survival among channel catfish increased from 14.8% in the nontransgenic control to 40.7% fish expressing cecropins (Dunham et. al. 2002). However, pleiotropic effects on fertility were not measured. Cecropins, like some other antimicrobial products, might negatively impact survival of sperm and reduce fertility (Anderson *et al*, 2002; Zaneveld *et al* 2002). Similarly, if a transgene enhances mating success while reducing juvenile viability, less fit individuals obtain the majority of the matings, while the resulting transgenic offspring do not survive as well as nontransgenic genotypes. The result is a gradual spiraling down of population size until eventually both wild-type and transgenic genotypes become locally extinct (Muir and Howard, 1999; Hedrick, 2000). This is an example of harm as a result of a transgene that spreads into the receiving community but fails to become established because the population becomes extinct. Results of Devlin *et al.* (2001) suggest that transgenic fish might have this potential. They showed that rainbow trout transgenic for growth hormone were both larger at sexual maturity and lower in viability than their wild-type siblings. Although the mating success of transgenic males relative to wild-type males is presently unknown in rainbow trout, large body size is known to enhance male mating success in many salmonid species (Jones, 1959; Schroder, 1982; Jarvi, 1990; Groot and Margolis, 1991).

The conclusion that natural selection will determine the ultimate fate of a transgene assumes that population sizes of the native and/or competing populations are large enough to be able to rebound from a temporary inflow of possibly maladapted genes or competitors, thereby allowing time for natural selection to operate. Escape of domesticated animals, whether or not transgenic, into wild or feral populations also might affect wild-type populations adversely by introducing alleles or allele combinations that are poorly adapted to natural environments (Hindar *et al.*, 1991; Lynch and O'Hely, 2001; Utter, 2002). If the wild population is sufficiently large, these alleles eventually should be eliminated by natural selection, although it might take many generations to reach selective equilibrium. Stochastic events could fix the alleles in small populations and result in extinction of those populations (Lynch and O'Hely, 2001)

Released animals also could introduce diseases or compete with native species for limited resources, causing population declines. If introduced males are sterile, but still mate with wild females, the reproductive efforts of those females are wasted, also contributing to population decline. In these regards, escaped transgenic organisms raise many of the same concerns as newly introduced species (Regal, 1986; Tiedje *et al.*, 1989).

Finally, use of genetically engineered animals could harm the environment indirectly by changing demand for feed, number of animals used, or amount of resulting waste, and by the effects of wastes containing novel gene products on microbial and insect ecologies. Most biopharmed animals will be highly valuable and most likely will be carefully confined, but there is some likelihood that the gene products themselves would pose environmental harms. Should the milk from transgenic livestock be spilled, most novel proteins would degrade rapidly along with other milk proteins. However, not all novel proteins will degrade quickly, such as spider silk—a protein that could be expressed in milk (Kaplan, 2002). The possibility that novel proteins are present in significant amounts in the meat, stools, urine, or other secretions of the animal would need to be evaluated. Risk assessment of these products can follow traditional methods.

Long-term and transitory environmental harms are dependent on the stability and resilience of the receiving community. A community is deemed stable if and only if ecologic structure and function variables return to the initial equilibrium following perturbation from it. The community is deemed to have local stability if such a return applies for small perturbations, and global stability if it bounces back from all possible perturbations (Pimm, 1984). Resilience is the property of how fast the structures or function variables return to their equilibrium following a perturbation (Pimm, 1984). The quantitative stability of many systems has been investigated by Jefferies (1974), and mathematical methods to quantify stability were summarized by Ricklefs (1990).

These definitions potentially allow a prioritization of potential harms from GE animals based in part on the receiving community's stability and resilience. Those that are most stable will result in the least harm, with the greatest harm occurring to unstable (fragile) communities. The committee recognizes that characterization of community stability and resilience might not prove straightforward. Ricklefs (1990) states that ecologists disagree on exactly how to parameterize models used to simulate risks and predict outcomes, and that “we are far from resolving some of these questions, and the ultimate resolution, if it is possible, will likely come from reconciling a combination of viewpoints that, at present, focus separately on dynamical control, energetics, and adaptations of individual species.”

Another limitation of this approach is that one cannot necessarily limit spread of a GE organism to a particular community. Thus, based on the principles of risk, one must assume the GE animal will become established in all possible communities for which it can gain access. If any one of these communities is fragile, concern for this ecosystem would be high. For this reason, the precautionary principle suggests that risk always should be assessed and managed for the most vulnerable ecosystem into which the escaped or released GE animal is likely to gain access following a given application.

Ranking the overall concerns then can be based on the product of the three variables cited above: fitness of the GE organism, its ability to escape and disperse, and the stability of the receiving community. Because the overall concern is a product of these three variables (and not the sum), if the risk associated with any one of the variables is negligible, the overall concern would be low (but not negligible). A transgene that increases the fitness of a highly mobile species that becomes feral easily raises the greatest level of concern, (e.g., a transgene conferring salt tolerance on catfish or the phytase gene in mice). A transgene that does not increase fitness in a low-mobility species that does not become feral easily raises the least concern (e.g., a gene for spider silk in cows; Kaplan, 2002). The committee stressed that these are *a priori* listings of concerns. When an actual transgenic organism is produced, for any GE animal that has the potential to become feral, those concerns can be assessed more directly by use of the net fitness approach, as suggested by Muir and Howard (2002a,b).

RISKS POSED BY KEY CLASSES OF GE ANIMALS

Examination of the Current State of Understanding, Regulatory Issues, and Key Findings Related to Hazard Assessment

Against the background of the discussion of principles of hazards, associated risks, and potential harms posed by genetically engineered animals generally, this section examines risks posed by key classes of genetically engineered animals: terrestrial vertebrates (laboratory and domesticated animals), terrestrial invertebrates (insects, mites, and other arthropods), and aquatic animals (fish and shellfish).

Terrestrial Vertebrates

The dangers of some terrestrial animals escaping and establishing themselves in the environment are considerable. Escaped cats, rabbits, mice, rats, pigs, dogs, fox, pigs, and goats have become feral and resulted in environmental disruptions in Australia, New Zealand, parts of Europe, and the western and southern United States. Any of these animals transgenic for functions that allow greater or wider adaptation to environmental conditions can pose significant ecologic harm. Such functions include, for example, increased nutrient utilization, or new metabolic pathways allowing nutrient synthesis ability, viral or bacterial resistance in any species, and heat or cold tolerance. Few GE terrestrial vertebrates have been produced that fit this category; the best examples to date are the phytase mouse and pig (Golovan *et al.*, 2001a,b). Further studies will be needed to examine environmental implications of these and other GE terrestrial animals should they be produced.

Terrestrial Invertebrates

Insects can be genetically engineered to control the spread of pests and diseases and for other beneficial purposes. However, a number of scientific uncertainties regarding environmental harms and associated risks need to be resolved before the release of GE arthropods can be undertaken purposefully.

One of the primary alternatives to the use of insecticides for control of insects is the use of agriculturally beneficial insects, such as predators and parasitoids. Unfortunately, such beneficial insects often are destroyed by insecticide applications, yet if one waits for the beneficial insects to multiply in order to control the pest, unacceptable levels of damage to the crops already would have occurred. To address this problem, insects used for biocontrol could be genetically engineered for resistance to insecticides, thereby allowing simultaneous use of both biologic control mechanisms (Braig and Yan, 2002).

Another means of biocontrol is the release of sterile males. Unfortunately, such programs are expensive and might require the release of sterile females where the insects cannot be sexed before release. Techniques used to induce sterility, such as irradiation, often render the insect noncompetitive as a potential mate. A possible solution to these problems is to genetically engineer the insect to allow either genetic sexing, for example, through a female lethal gene, or through direct production of sterile males. Finally, GE insects can be developed to produce visual markers, such as green fluorescent protein (GFP), to determine the effectiveness of sterile release programs (Braig and Yan, 2002).

Another application of transgenesis is to control transmission of diseases by such vector organisms as mosquitoes. With GE technology, it might be possible to disrupt an insect's ability to carry and transmit diseases such as *Plasmodium*, the malaria parasite (Braig and Yan, 2002; Spielman *et al*., 2002; Ito *et al*., 2002). An environmental concern is presented because the parasite has a negative effect on the fitness of the mosquito (Braig and Yan, 2002; Spielman *et al*., 2002). Elimination of the parasite could result in the release of mosquitoes from a form of biocontrol, with a possible associated increase in mosquito populations. An increase in mosquitoes also could lead to increased spread of other mosquito-borne diseases to both animals and humans.

The development of molecular methods for genetic engineering of terrestrial arthropods (reviewed by Atkinson *et al*., 2001; Handler, 2001) has not been matched by advances in understanding how to deploy GE arthropods in practical pest management, or of how to evaluate potential harms associated with their release into the environment (Spielman, 1994; Hoy, 1995; 2000; Ashburner *et al*., 1998). Key issues pertaining to environmental risk (Hoy, 2000) include the possibility that transgenic insects released into the environment would pose unknown ecologic impacts, and that gene constructs inserted into insects could be transferred horizontally through known or unknown mechanisms to other species, thereby creating new pests.

If a genetically engineered arthropod is to be released within a practical pest management program, any potential ecologic risks associated with its release into the environment must be assessed, although guidelines for conducting such an assessment do not yet exist (Hoy, 1992a; 1992b; 1995). Anticipation of ecologic risks will depend upon predictions of the impact of changed abundance or dynamics of the engineered species upon resources or species with which the organism interacts in the environment, including predators, prey, competitors, and hosts.

Further, the methods by which horizontal gene transfer could occur should be investigated so that it can be determined whether and how to assess this particular hazard (Hoy, 2000). Should horizontal transfer of a transgene be demonstrated, it poses significant effects for the evolution of a species, introducing otherwise unavailable genetic material to the genome of a species (Droge *et al*., 1998). Horizontal gene transfer would pose no harm if the gene that is moved were lost, inactivated, or benign. However, if horizontal gene transfer confers increased fitness, perhaps by establishing the dominant, selectable antibiotic or pesticide resistance trait used in the production of the transgenic arthropod, then harm could be realized. Risk posed is not dependent solely on the frequency of transfer. Even rare events might cause ecologic impacts if the transferred gene increases the fitness of the recipient (Droge *et al*., 1998).

Considerable progress has been made in the development of methods for genetic engineering of the mosquito germ line and in identification of parasite-inhibiting molecules (Beernsten *et al*., 2000; Blair *et al*., 2000). Despite the technical progress, there remain important scientific questions that must be addressed prior to a program releasing GE mosquitoes (Braig and Yan, 2002; Spielman *et al*., 2002). Can parasite-inhibiting gene constructs indeed spread and become fixed in wild mosquito populations? In order to do so, a driver mechanism will have to be developed that would cause a disproportionate frequency of offspring of the released mosquitoes to carry the introduced construct (Braig and Yan, 2002; Spielman *et al*., 2002). Such driver mechanisms might include competitive displacement, meiotic drive (Sandler and Novitski, 1957), biased gene conversion, and others (Braig and Yan, 2002). The fate of parasite-inhibiting genes would be determined not only by the mechanism used to drive the fixation of the genes, but also by the magnitude of any loss of fitness in the host, and also by a range of ecologic and abiotic environmental factors. Possible human health effects posed by genetic engineering of disease vector insects are discussed elsewhere (https://www.ncbi.nlm.nih.gov/books/n/nap10418/ddd00061/)

In the context of environmental concerns posed by GE arthropods, it is clear that purposeful release of transgenic arthropods will depend upon prior risk assessment and risk management. Hoy (1997) called for effective containment of transgenic arthropods in the laboratory and thorough peer review by scientists and regulatory agencies prior to any field release. However, there are no U.S. or international guidelines for containment of transgenic arthropods. Additionally, there are no proven techniques for retrieving transgenic insects after environmental release should they perform in unexpected ways.

Fish and Shellfish

Considerable research effort has been devoted to development of GE fish and shellfish stocks, as they pose considerable benefits to producers. Production of some GE fish or shellfish could result in environmental benefits. For example, expression of growth hormone transgenes has been shown to increase feed conversion efficiency (Cook *et al*., 2000; Fletcher *et al*., 2000), decreasing the amount of feed needed to bring a fish to market size, while reducing wastes per unit of mass produced. Production of fish expressing a phytase transgene might allow use of less fish meal in feeds while decreasing phosphorus in effluent from aquaculture operations. However, transgenic fish and shellfish might pose environmental hazards (Kapuscinski and Hallerman, 1990; 1991; Hallerman and Kapuscinski, 1992a,b; 1993; Muir and Howard, 1999; 2001; 2002a,b). Below, the committee briefly reviews a series of empirical studies to examine potential ecologic risks posed by escaped or released transgenic fish and shellfish.

As indicated in Table 1, there are a number of important factors that contribute to risk. The risk factors for establishment in a community were high for all categories because: (1) cultured fish and shellfish stocks are not far removed from the wild type, (2) aquaculture production systems frequently are located in ecosystems containing wild or feral populations of conspecifics, (3) aquatic organisms exhibit great dispersal ability, and (4) aquacultured organisms often are marketed live.

Transgenic Atlantic salmon pose a near-term regulatory issue. A brief review of the hazards they pose provides a useful illustration of the environmental hazards posed by GE aquatic species more generally. Cultivated salmon escape from fish farms in large numbers (Carr *et al.*, 1997; Youngson *et al.*, 1997; Fisk and Lund, 1999; Volpe *et al.*, 2000), posing ecologic and genetic risks to native salmon stocks (Hansen *et al.*, 1991; Hindar *et al.*, 1991). Several studies that have focused on Atlantic salmon (*Salmo salar*) expressing a growth hormone (GH) gene construct suggest that transgenesis might affect fitness, but do not provide net fitness estimations needed for parameterizing fitness models predicting outcomes should such fish enter natural systems. GH transgenic salmon consumed food and oxygen at more rapid rates than control salmon (Stevens *et al.*, 1998); although gill surface area was 1.24 times that in control salmon, it did not compensate for the 1.6-time elevation in oxygen uptake, and the metabolic cost of swimming was 1.4 times that for control salmon (Stevens and Sutterlin, 1999). Growth-enhanced transgenic fish were significantly more willing to risk exposure to a predator in order to gain access to food (Abrahams and Sutterlin, 1999), but reduced their exposure to predators when risk was heightened further, suggesting that they might not be significantly more susceptible to predation. Transgenic salmon lost their juvenile parr markings sooner than nontransgenics, suggesting early readiness for adaptation to seawater. Thus, findings to date are fragmentary, and it is difficult to assess the likely ecologic or genetic outcome should transgenic Atlantic salmon escape captivity and invade wild populations.

Pacific salmonids include a number of aquaculturally important species that have been the subject of a large number of transgenesis experiments and a small number of risk assessment experiments. These studies collectively show results similar to those obtained with Atlantic salmon, but also show that the outcomes of introgression of a transgene might differ among receiving populations. Coho salmon (*Oncorhynchus kisutch*) expressing a growth hormone construct exhibited extraordinary growth (Devlin *et al*., 1994), underwent parr-smolt transformation approximately six months before nontransgenic siblings, and some males matured at just two years of age (Devlin *et al*., 1995b). However, swimming performance of transgenics was poor (Farrell *et al*., 1997), perhaps because of a developmental delay or from disruption of locomotor muscles or associated support systems, such as the respiratory, circulatory, or nervous systems. Some growth-enhanced fish exhibited abnormalities of opercular (gill cover) morphology that might disrupt respiration and contribute to poor swimming performance. In competitive feeding trials, Devlin *et al*. (1999) showed that GH transgenesis increases the ability to compete for food, suggesting that transgenic fish might compete successfully with native fish in the wild. Devlin *et al*. (2001) noted that the greatest response to expression of the transgene was in Coho hybrids of a wild and domesticated strain; hence, the effects of an introduced growth hormone gene might differ among stocks.

In a study posing implications for introgression of transgenes into wild populations, Devlin *et al*. (2001) examined the fitness effects of expression of a GH construct in both wild and selectively bred commercial rainbow trout (*O. mykiss*) strains. Transgenic wild-strain rainbow trout retained the slender body morphology of the wild-type strain, but their final size at maturity was much larger than that of their nontransgenic ancestors. Both domestic and wild-strain trout exhibited reduced viability; in the domestic strain, all transgenic individuals died before sexual maturation. The tradeoff of size (and likely mating success) and decreased viability parallels the case modeled by Muir and Howard (1999), and suggests that the viability of a receiving population might be compromised. Devlin *et al*. (2001) noted that the greatest response to expression of the transgene was in hybrids of a wild and domesticated strain; hence, the effects of an introduced growth hormone gene might differ among stocks. The importance of genetic background on expression of growth hormone was demonstrated also by Siewerdt *et al*. (2000a,b) and Parks *et al*. (2000a,b).

While indicative that risk issues must be regarded with seriousness, the growing collection of empirical risk assessment studies of transgenic salmonids does not yet provide a body of data useful for parameterizing a model useful for predicting the likelihood that transgenes would become permanently introgressed into wild or feral salmon populations.

However, many of the same physiologic and behavioral differences seen in GE salmon can be induced by using growth hormone implants (Johnsson *et al*., 1999). As such, implanted fish can model the effects of the transgene and allow the fish to be safely tested in native habitats—an experiment that would be hazardous with GE fish. Working with brown trout (*Salmo trutta*), Johnsson *et al*. (1999) showed that survival of GH-implanted trout did not differ from that of controls under field conditions with natural predation levels. They concluded that GH-manipulated fish might compete successfully with wild fish despite behavioral differences observed in the laboratory for characteristics such as predator avoidance, foraging ability, and over-winter survival (Johnsson *et al*., 2000). These results emphasize the need to measure all components of fitness under conditions similar to those found in nature—a task that might not be possible for some species.

Possible environmental hazard pathways posed by the escape of transgenic crustaceans and mollusks into natural ecosystems have not yet been thoroughly considered. Research has not yet assessed ecologic risks posed by production of these organisms. Many freshwater crustaceans, such as crayfishes, are capable of overland dispersal; further, they are produced in extensive systems, where confinement is difficult. Many marine crustaceans have planktonic larvae, thus complicating confinement. Confinement of mollusks can prove difficult at the larval stage (USDA, 1995). Further, because the larval stages drift in the water column before settlement and metamorphosis to the sessile juvenile form, they have great dispersal capability.

The committee's review of ecologic principles and empirical data suggests a considerable risk of ecologic hazards being realized should transgenic fish or shellfish enter natural ecosystems. In particular, greater empirical knowledge is needed to predict the outcome should transgenes become introgressed into natural populations of aquatic organisms.

NEED FOR MORE INFORMATION CONCERNING RISK ASSESSMENT AND RISK MANAGEMENT

Many critical unknowns complicate risk assessment and risk management of genetically engineered animals. Greater knowledge in these areas would support an informed judgment of whether and how to go forward with approval for marketing particular genetically engineered animals. For example, results of well-designed, interdisciplinary studies could prove useful for parameterizing net fitness-based models used for predicting whether transgenic genotypes would persist in natural populations. Should GE animals be approved, post-commercialization monitoring would provide a check on the utility of predictive models, suggest improved means of risk management, and support adaptive management of GE animals (Kapuscinski *et al*., 1999; Kapuscinski, 2002). More information supporting risk assessment and risk management also would support regulatory decision-making, and it would promote public confidence in the environmental safety of genetically engineered animals.

References

Abrahams, M. V., and A. Sutterlin. 1999. The foraging and antipredator behavior of growth-enhanced transgenic Atlantic salmon. Animal Behavior 58:933–942.

Anderson, R. A., K. A. Feathergill, X. H. Diao, M. D. Cooper, R. Kirkpatrick, B. C. Herold, G. F. Doncel, C. J. Chany, D. P. Waller, W. F. Rencher, and L. J. D. Zaneveld. 2002. Preclinical evaluation of sodium cellulose sulfate (Ushercell) as a contraceptive antimicrobial agent. Journal of Andrology 23:426–438.

Ashburner, M., M. A. Hoy, and J. Peloquin. 1998. Transformation of arthropods—Research needs and long term prospects. Insect Molecular Biology 7:201–213.

Atkinson, P. W., A. C. Pinkerton, and D. A. O'Brochta. 2001. Genetic transforamtion systems in insects. Annual Review of Entomology 46:317–346.

Beernsten, B. T., A. A. James, and B. M. Christensen. 2000. Genetics of mosquito vector competence. Microbiology and Molecular Biology Reviews 64:115.

Blair, C. D., Z. N. Adelman, and K. E. Olsen. 2000. Molecular strategies for interrupting arthropod-borne virus transmission by mosquitos. Clinical Microbiology Reviews 13:651.

Braig, H. R., and G. Yan. 2002. The spread of genetic constructs in natural insect populations. Pp. 251–314 in Genetically Engineered Organisms: Assessing Environmental and Human Health Effects, D. K. Letourneau, editor; and B. E. Burrows, editor. , eds. Washington, D.C.: CRC Press.

Campton, D. E. 1987. Natural hybridization and introgression in fishes: Methods of detection and genetic interpretations. Pp. 161–192 in Population Genetics and Fishery Management, N. Ryman, editor; and F. Utter, editor. , eds. Seattle: University of Washington Press.

Caron, D. M. 2002. Africanized honey bees in the Americas. American Bee Journal 142:327– 328.

Carr, J. W., J. M. Anderson, F. G. Whoriskey, and T. Dilworth. 1997. The occurrence and spawning of cultured Atlantic salmon (*Salmo salar*) in a Canadian river. International Council for the Exploration of the Seas Journal of Marine Science 54:1064–1073.

Cook, J. T., M. A. McNiven, G. F. Richardson, and A. M. Sutterlin. 2000. Growth rate, body composition, and food digestibility/conversion of growth-enhanced Atlantic salmon (*Salmo salar*). Aquaculture 188:15–32.

Dangel, J. R., P. T. Macey, and F. C .Withler. 1973. An annotated bibliography of interspecific hybridization of fishes of the subfamily Salmoninae. U.S. Department of Commerce, National Oceanic and Atmospheric Administration (NOAA) Technical Memorandum WNMFSFC-1, U.S.

Devlin, R. H., C. A. Biagi, T. Y. Yesaki, D. E. Smailus, and J. C. Byatt. 2001. A growth-hormone transgene boosts the size of wild but not domesticated trout. Nature 409:781–782.

Devlin, R. H., J. I. Johnsson, D. E. Smailus, C. A. Biagi, E. Jonsson, and B. T. Bjornsson. 1999. Increased ability to compete for food by growth hormone-transgenic coho salmon *Oncorhynchus kisutch* (Walbaum). Aquaculture Research 30:479–482.

Devlin, R. H., T. Y. Yesaki, C. A. Biagi, E. M. Donaldson, P. Swanson, and W. K. Chan. 1994. Extraordinary salmon growth. Nature 371:209–210.

Devlin, R. H., T. Y. Yesaki, E. Donaldson, S. Du, and C. Hew. 1995. a. Production of germline transgenic pacific salmonids with dramatically increased growth-performance. Canadian Journal of Fisheries and Aquatic Science 52:1376–1384.

Devlin, R. H., T. Y. Yesaki, E. M. Donaldson, and C. L. Hew. 1995. b. Transmission and phenotypic effects of an antifreeze/GH gene construct in coho salmon (*Oncorhynchus kisutch*). Aquaculture 137:161–169.

Droge, M., A. Puhler, and W. Selbitschka. 1998. Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. Journal of Biotechnology 64:75–90.

Dunham, R. A. 1994. Predator avoidance, spawning, and foraging ability of transgenic catfish. In The 1994 Risk Assessment Research Symposium. Available online at http://www.nbiap.vt.edu/brarg/brasym94/dunham.htm .

Dunham, R. A. 1996. Results of early pond-based studies of risk assessment regarding aquatic GMOs. In The 126th Annual Meeting of the American Fisheries Society, Dearborn, MI, August 26–29, 1996. Abstract #381.

Dunhan, R. A., G. W. Warr, A. Nichols, P. L. Duncan, B. Argue, D. Middleton, and H. Kucuktas. 2002. Enhanced bacterial disease resistance of transgenic channel catfish Ictalurus punctatus possessing cecropin genes. Marine Biotechnology 4:338–344.

Farrell, A. P., W. Bennett, and R. H. Devlin. 1997. Growth-enhanced transgenic salmon can be inferior swimmers. Canadian Journal of Zoology 75:335–337.

Fiske, P., and R. A. Lund. 1999. Escapees of reared salmon in coastal and riverine fisheries in the period 1989–1998. NINA Offdragsmelding 603:1–23 (in Norwegian with an English abstract).

Fletcher, G. L., A. Alderson, E. A. Chin-Dixon, M. A. Shears, S. V. Goddard, and C. L. Hew. 2000. Transgenic fish for sustainable aquaculture. Pp. 193–201 in Sustainable Aquaculture, N. Svennevig, editor; , H. Reinertsen, editor; , and M. New, editor. . Rotterdam, Netherlands: AA. Bolkema.

Fletcher, G. L., P. L. Davies, and C. L. Hew. 1992. Genetic engineering of freeze-resistant Atlantic salmon. Pp. 190–208 in Transgenic fish, C. L Hew, editor; and G. L. Fletcher, editor. , eds. River Edge, NJ: World Scientific Publishing.

Gerardi, M. H., and J. K. Grimm. 1979. The History, Biology, Damage, and Control of the Gypsy Moth, *Porthetria dispar*. Cranberry, NJ: Associated University Press.

Golovan S. P., R. G. Meidinger, A. Ajakaiye, M. Cottrill, M. Z. Wiederkehr, D. J. Barney, C. Plante, P. W. Pollard, M. Z. Fan, M. A. Hayes, J. Laursen, J. P. Hjorth, R. R. Hacker, J. P. Phillips, and C. W. Forsberg. 2001. a. Pigs expressing salivary phytase produce low-phosphorus manure. Nature Biotechnology 19:741–745.

Golovan S. P., M. A. Hayes, J. P. Phillips, and C. W. Forsberg. 2001. b. Transgenic mice expressing bacterial phytase as a model for phosphorus pollution control. Nature Biotechnology 19:429–433.

Groot C., and L. Margolis. 1991. Pacific salmon life histories. Vancouver: University of British Columbia Press.

Hallerman, E. M., and A. R. Kapuscinski. 1992. a. Ecological and regulatory uncertainties associated with transgenic fish. Pp. 209–228 in Transgenic Fish, C. L. Hew, editor; and G. L. Fletcher, editor. , eds. Singapore: World Science Publishing Company.

Hallerman, E. M., and A. R. Kapuscinski. 1992. b. Ecological implications of using transgenic fishes in aquaculture. International Council for the Exploration of the Seas Marine Science Symposium 194:56–66.

Hallerman, E. M., and A. R. Kapuscinski. 1993. Potential impacts of transgenic and genetically manipulated fish on wild populations: Addressing the uncertainties through field testing. Pp. 93–112 in Genetic Conservation of Salmonid Fishes, J. G. Cloud, editor; and G. H. Thorgaard, editor. , eds. New York: Plenum Press.

Hammer, R. E., R. L. Brinster, and R. D. Palmiter. 1985. Use of gene transfer to increase animal growth. Cold Spring Harbor Symposia on Quantitative Biology 50:379–387.

Handler, A. M. 2001. A current perspective on insect gene transformation. Insect Biochemistry and Molecular Biology 31(2):111–128.

Hansen, L. P., T. Håstein, G. Nævdal, R. L. Saunders, and J. E. Thorpe. 1991. Interactions between cultured and wild Atlantic salmon. Aquaculture 98(1/3):1–324.

Harvey, A. J., G. Speksnijder, L. R. Baugh, J. A. Morris, and R. Ivarie. 2002. Expresion of exogenous protein in the egg white of transgenic chickens. Nature Biotechnology 19:396–399.

Hedrick, P. W. 2000. Genetics of Populations, 2nd edition. Sudbury, MA: Jones & Bartlett.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Science 48:945–957.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Berreli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. Proceedings of the National Academy of Sciences of the United States of America 98:9157–9160.

Hone, J. 2002. Feral pigs in Namadgi National Park, Australia: Dynamics, impacts and management. Biological Conservation 105:231–242.

Houdebine, L. M. 2000. Transgenic animal bioreactors. Transgenic Research 9:305–320.

Howard, R. D., R. S. Martens, S. A. Innes, J. M. Drnevich, and J. Hale. 1998. Mate choice and mate competition influence male body size in Japanese medaka. Animal Behaviour 55:1151–1163.

Hoy, M. A. 1992. a. Commentary: Biological control of arthropods: Genetic engineering and environmental risks. Biological Control 2:166–170.

Hoy, M. A. 1992. b. Criteria for release of genetically improved phytoseiids: An examination of the risks associated with release of biological control agents. Experimental and Applied Acarology 14:393–416.

Hoy, M. A. 1995. Impact of risk analyses on pest management programs employing transgenic arthropods. Parasitology Today 11:229–232.

Hoy, M. A. 1997. Laboratory containment of transgenic arthropods. American Entomology 43:206–256.

Hoy, M. A. 2000. Transgenic arthropods for pest management programs risk and realties. Experimental and Applied Acarology 24:463–495.

Ito, J., A. Ghosh, L. A. Moreira, E. A. Wimmer, and M. Jacobs-Lorena. 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. Nature 417:452–455.

Jarvi, T. 1990. The effects of male dominance, secondary sexual characteristics and female mate choice on the mating success of male Atlantic salmon *Salmo salar* . Ethology 84:123–132.

Jeffries, C. 1974. Quantitative stability and digraphics in model ecosystems. Ecology 55:1414–1419.

Jia, X., A. Patrzykat, R. H. Devlin, P. A. Ackerman, G. K. Iwama, and R. E. Hancock. 2000. Antimicrobial peptides protect coho salmon from *Vibrio anguillarum* infections. Applied and Environmental Microbiology 66:1928–1932.

Johnsson, J. I., E. Jönsson, E. Petersson, T. Jarvi, and B. T. Björnsson. 2000. Fitness-related effects of growth investment in brown trout under field and hatchery conditions. Journal of Fish Biology 57:326.

Johnsson, J. I., E. Petersson, E. Jonsson, and B. T. Bjornsson. 1999. Growth hormone-induced effects on mortality, energy status, and growth: A field study on brown trout (*Salmo trutta*). Functional Ecology 13:514–522.

Jones, J. W. 1959. The Salmon. London: Collins.

Kaplan, D. L. 2002. Spiderless spider webs. Nature Biotechnology 20:239–240.

Kapuscinski, A. R. 2002. Controversies in designing useful ecological assessments of genetically engineered organisms. Pp. 385–415 in Genetically Engineered Organisms: Assessing Environmental and Human Health Effects, D. Letourneau, editor; and B. Burrows, editor. , eds. Boca Raton, FL: CRC Press.

Kapuscinski, A. R., and E. M. Hallerman. 1990. Transgenic fish and public policy: Anticipating environmental impacts of transgenic fish. Fisheries 15:2–11.

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

Kapuscinski, A. R., T. Nega, and E. M. Hallerman. 1999. Adaptive biosafety assessment and management regimes for aquatic genetically modified organisms in the environment. Pp. 225–251 in Towards Policies for Conservation and Sustainable Use of Aquatic Genetic Resources, Conference Proceedings 59, R. S. V. Pullin, editor; , D. M. Bartley, editor; , and J. Kooiman, editor. , eds. Makati City, Philippines: ICLARM (International Center for Living Aquatic Resources Management).

Kempthorne O., and E. Pollak. 1970. Concepts of fitness in Mendelian populations. Genetics 64:125–145.

Kidwell, M. G. 1993. Lateral transfer in natural populations of eukaryotes. Annual Review of Genetics 27:235–056.

King, C. M., J. G. Innes, M. Flux, and M. O. Kimberley 1996. Population biology of small mammals in Pureora Forest Park: The feral house mouse (Mus musculus). New Zealand Journal Of Ecology 20:253–269.

Krebs, C. J., A. J. Kenney, and G. R. Singleton 1995. Movements of feral house mice in agricultural landscapes. Australian Journal of Zoology 43:293–302.

Lai, L., D. Kolber-Simonds, K. W. Park, H. T. Cheong, J. L. Greenstein, G. S. Im, M. Samuel, A. Bonk, A. Rieke, B. N. Day, C. N. Murphy, D. B Carter, R. J. Hawley, and R. S. Prather. 2002. Production of α-1, 3-galactosyltransferase-knockout inbred miniature swine by nuclear transfer cloning. Science 295(5557):1089–1092.

Lande, R. 1983. The response to selection on major and minor mutations affecting a metrical trait. Heredity 50:47–65.

Lynch M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associates.

Lynch, M., and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. Conservation Genetics 2:363–378.

Muir, W. M., and R. D. Howard. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis. Proceedings of the National Academy of Sciences of the United States of America 24:13853–13856.

Muir, W. M., and R. D. Howard. 2001. Fitness components and ecological risk of transgenic release: A model using Japanese medaka (*Oryzias latipes*). American Naturalist 158:1–16.

Muir, W. M., and R. D. Howard. 2002. a. Methods to assess ecological risks of transgenic fish releases. Pp. 355–383 in Genetically Engineered Organisms: Assessing Environmental and Human Health Effects, D.K. Letourneau, editor; and B. E. Burrows, editor. , eds. Boca Raton, FL: CRC Press.

Muir, W. M., and R. D. Howard. 2002. b. Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. Transgenic Research 11(2):101–114.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. Washington, DC: National Academy Press.

NRC (National Research Council). 1996. Understanding Risk: Informing Decisions in a Democratic Society. Washington, DC: National Academy Press.

Paine, R. T. 1966. Food web complexity and species diversity. The American Naturalist 100:65–75.

Parks, K. R., E. J. Eisen, and J. D. Murray. 2000. a. Correlated responses to selection for large body size in oMt1a-oGH mice: Growth, feed efficiency, and body composition. Journal of Animal Breeding Genetics 117:385–405.

Parks, K. R., E. J. Eisen, I. J. Parker, L. G. Hester, and J. D. Murray. 2000. b. Correlated responses to selection for large body size in oMt1a-oGH transgenic mice: Reproductive traits. Genetical Research (Cambridge) 75:199–208.

Pimm, S. L. 1984. The complexity and stability of ecosystems. Nature 307:321–326.

Pursel, V. G., C. E. Rexroad, J. Bolt, K. F. Miller, R. J. Wall, R. E. Hammer, K. A. Pinkert, R. D. Palmiter, and R. L. Brinster. 1987. Progress on gene transfer in farm animals. Veterinary Immunology and Immunopathology 17:303–312.

Rahman, M. A., and N. Maclean. 1999. Growth performance of transgenic tilapia containing an exogenous piscine growth hormone gene. Aquaculture 173:333–346.

Regal, P. J. 1986. Models of genetically engineered organisms and their ecological impact. Pp. 111–129 in Ecology of Biological Invasions of North America and Hawaii, H. A. Mooney, editor; and J. A. Drake, editor. , eds. New York: Spring-Verlag.

Ricklefs, R. E. 1990. Ecology, Third Edition. New York: W. H. Freeman.

Sandler, L., and E. Novitski. 1957. Meiotic drive as an evolutionary force. American Naturalist 91:105.

Sarmasik A., G. Warr, and T. T. Chen. 2002. Production of transgenic medaka with increased resistance to bacterial pathogens. Marine Biotechnology 4(3):310–322.

Schroder, S. L. 1982. The Role of Sexual Selection in Determining Overall Mating Patterns and Mate Choice in Chum Salmon. Ph.D. dissertation. Seattle: University of Washington.

Schwartz, F. J. 1972. World literature to fish hybrids with an analysis by family, species and hybrid: Supplement 1. National Oceanic and Atmospheric Administration Technical Report NMFS SSRF-750. Washington, DC: National Marine Fisheries Service.

Scientists' Working Group on Biosafety. 1998. Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms. Part One: Introductory Text and Flowcharts. Part Two: Flowcharts and Worksheets. Edmonds Institute; 245. Available online at www.edmonds-institute.org/manual.html .

Siewerdt, F., E. J. Eisen, and J. D. Murray. 2000. a. Correlated changes in fertility and fitness traits in lines of oMt1a-oGH transgenic mice selected for increased 8-week body weight. Journal of Animal Breeding and Genetics 117:83–95.

Siewerdt, F., E. J. Eisen, J. D. Murray, and I. J. Parker. 2000. b. Response to 13 generations of selection for increased 8-week body weight in lines of mice carrying a sheep growth hormone-based transgene. Journal of Animal Science 78:832–845.

Spielman, A. 1994. Why entomological anti-malarial research should not focus on transgenic mosquitos. Parasitology Today 10:374.

Spielman, A., J. C. Beier, and A. E. Kiszewski. 2002. Ecological and community considerations in engineering arthropods to suppress vector-borne disease. Pp. 315– 329 in Genetically Engineered Organisms: Assessing Environmental and Human Health Effects, D. K. Letourneau, editor; and B. E. Burrows, editor. , eds. Boca Raton, FL: CRC Press.

Stevens, E. D., and A. Sutterlin. 1999. Gill morphology in growth hormone transgenic salmon. Environmental Biology of Fishes 54:415–411.

Tearle, R. G., M. J. Tange, Z. L. Zannettino, M. Katerelos, T. A. Shinkel, B. J. W. Van Denderen, A. J. Lonie, I. Lyons, M. B. Nottle, T. Cox, C. Becker, A. M. Peura, P. L. Wigley, R. J. Crawford, A. J. Robins, M. J. Pearse, and A. J. F. d'Apice. 1996. The α-1,3-Galactosyltransferase knockout mouse: Implications for xenotransplantation. Transplantation 61:13–19.

Tiedje, J. M., R. K. Colwell, Y. L. Grossman, R. E. Hodson, R. E. Lenski, R. N. Mack, and P. J. Regal. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70:298–315.

USDA. 1995. Performance standards for safely conducting research with genetically modified fish and shellfish. In Agricultural Biotechnology Research Advisory Committee (ABRAC), working group on Aquatic Biotechnology and Environmental Safety, United States Department of Agriculture. Document No. 95–01. Washington, DC: U.S. Department of Agriculture.

Utter, F. 2002. Genetic impacts of fish introductions. In Population Genetics: Principles and Practices for Fisheries Scientists. American Fisheries Society. Available online at http://www.fisheries.org/publications//epubs/genetics/chapter15.pdf .

Vtorov, I. P. 1993. Feral pig removal: Effects on soil microarthropods in a Hawaiian rain forest. Journal of Wildlife Management 57:875–880.

Wright, G., A. Carver, D. Cottom, D. Reeves, A. Scott, P. Simons, I. Wilmut, I. Garner, and A. Colman. 1991. High level expression of active human alpha-1-antitrypsin in the milk of transgenic sheep. Biotechnology 9:830–834.

Youngson, A. F., J. H. Webb, J. C. MacLean, and B. M. Whyte. 1997. Frequency of occurrence of reared Atlantic salmon in Scottish salmon fisheries. International Council for the Exploration of the Seas Journal of Marine Science 54:1216–1220.

Zaneveld, L. J. D., D. P. Waller, R. A. Anderson, C. Chany, W. F. Rencher, K. Feathergill, X. H. Diao, G. F. Doncel, B. Herold, and M. Cooper. 2002. Efficacy and safety of a new vaginal contraceptive antimicrobial formulation containing high molecular weight poly (sodium 4-styrenesulfonate). Biology of Reproduction 66:886–894.

§This article was reproduced, with permission, from a Workshop Summary of the Institute of Medicine and published by NCBI Bookshelf of the National Library of Medicine, National Institutes of Health, USA.