Fisheries

VOL 33 NO 3
MARCH 2008

AFS

Fisheries News
Legislative Update
Journal Highlights
Calendar
Job Center

American Fisheries Society • www.fisheries.org

Autocidal Technology for the Control of Invasive Fish

Can Soft Water Limit Bighead Carp and Silver Carp (*Hypophthalmichthys* spp.) Invasions?

FEATURE:
INTRODUCED FISH

Ronald E. Thresher
Thresher is a senior research scientist at the Invasive Animal Cooperative Research Centre and the Commonwealth Scientific and Industrial Research Organisation Marine and Atmospheric Research, Hobart, Tasmania, Australia. He can be reached at Ron.Thresher@csiro.au.

Autocidal Technology for the Control of Invasive Fish

ABSTRACT: Recombinant genetic technology offers considerable potential for the safe, cost-effective control of invasive fish. This article reviews the range of genetic approaches suggested for controlling invasive species, and considers their practicality, likely efficacy, and the risks they entail. Implementation of any genetic method to control invasive fish will be heavily influenced by an as yet unknown level of public acceptability and a regulatory and policy framework for managing environmental applications of genetic technology that is confused at almost all jurisdictional levels.

Tecnologías de Control Biológico de Especies de Peces Invasoras

RESUMEN: La tecnología de recombinación genética tiene un potencial considerable para el control seguro y económicamente efectivo de las especies invasoras de peces. En este artículo se revisan los diferentes enfoques genéticos que se sugieren para el control de especies invasoras, su practicidad, eficacia y los riesgos que éstos suponen. La implementación de cualquier método genético para controlar especies invasoras estará fuertemente influenciada por un nivel aún desconocido de aceptación pública y por un marco político y de regulación de aplicaciones de tecnologías genéticas en problemas ambientales, que se confunde o malinterpreta en casi todos los niveles de jurisdicción.

JASON JENKINS, UNIVERSITY OF MISSOURI

Invasive plants and animals are one of the greatest threats to species and community conservation worldwide (www.cbd.int/programmes/cross-cutting/alien/). Virtually every terrestrial and aquatic environment suffers the impacts of invasive species. Fish are a large part of the problem. Of the "100 worst invasive alien species" recently compiled by the World Conservation Union (IUCN), 9 are fish (Lowe et al. 2001), without even including the sea lamprey (*Petromyzon marinus*) or recent concerns about snakeheads (*Channa striata*). Over 200 fish species have established non-native populations around the world (Lever 2002), while Fuller et al. (1999) report 536 fish species as "non-indigenous" in the United States alone (a category that includes both species exotic to the United States and native species that have been introduced or have spread outside of their original ranges). When these species cause problems, there is a need for cost effective and reliable options for managing their abundance.

Currently, these options are extremely limited. At small scales, invasive fish can be controlled by use of biocides, physical removal, barriers, and environmental modification, e.g., lowering water levels to disrupt spawning (reviewed by Meronek et al. 1996; Rayner and Creese 2006). Pheromones as spawning disrupters or species-specific attractants or repellents have also attracted interest, but so far have only been used in test situations (Sorenson and Stacey 2004; Wagner et al. 2006). For widely dispersed species, the only practical option is biological control (Thresher and Kuris 2004). Classical biological control, involving the release of an exotic predator, parasite, or pathogen to control an alien species, has not been widely used against fish, mainly because of difficulties in finding suitable agents. One of the few successful applications has been the release of peacock bass (*Cichla ocellaris*) in Florida to control other introduced cichlids and create a sport fishery (myfwc.com/fishing/offices/boca.html); a similar effort to use it to control stunted cichlids in Kenya was apparently less successful (Lever 2002). Spring Viraemia was considered for the control of carp in Australia, but rejected on the basis of uncertain efficacy and species-specificity (Crane and Eaton 1997). Australian scientists are currently looking into Koi Herpes Virus as another option.

Another form of biological control, a sterile male release program, has been implemented against sea lampreys in the St. Marys River (Bergstedt et al. 2003), but it is not clear that the effort has significantly reduced adult lamprey populations. For the vast majority of invasive fish, even those causing major ecological or economic damage, there is still no cost-effective or practical means of control at anything other than local scales.

Recombinant genetics could change this. Advocates suggest that genetic manipulation offers the potential for cost-effective, safe, and complete eradication of invasive species. If this potential can be realised, it changes fundamentally the operational and policy contexts in which pest management operates. The potential of genetics was first noted in the 1960s by entomologists, who suggested that using genetics to manipulate sex ratios could be a powerful means of controlling mosquitoes (Hamilton 1967). The idea was based on the observation that naturally occurring genes that favor production of one sex over the other (meiotic drive) had apparently caused several insect populations to go extinct. The idea languished, however, in the absence of a practical way to genetically manipulate sex ratios. We are now close to having that capability. The last decade has seen immense development in genetic technology, resulting in, among other things, a renewed interest in the possibility of controlling invasive species using genetic techniques. In this article, I review the options that have been proposed, outline some of the risks involved, and comment on the issues of public acceptability and the policy implications of genetic approaches. The goals of the article are to critically synthesize this rapidly developing field in the specific context of controlling invasive fish, to summarize recent grey literature not widely available that is relevant to the application, and to stimulate discussion about the desirability of the technology and the policy frameworks that will be needed to manage it.

GENETIC OPTIONS FOR CONTROLLING INVASIVE SPECIES

Three approaches to control invasive species using recombinant genetics have been investigated: genetically engineered viruses that, when incorporated into a bait, act as a species-specific toxin or sterilizer; engineered viral diseases; and "autocidal" genes. Viruses as toxins will not be considered further here, as its dynamics and efficacy largely parallel those of a conventional baiting program (see Torres et al. 2001). Genetically engineered diseases have been examined principally in the context of controlling mammals. "Immunocontraception" is based on modifying an otherwise low impact vector (usually a virus) so that it expresses a gene critical for its host's reproduction. The host's immune system raises antibodies against the artificially induced proteins, which non-discriminately also attack the host's own proteins, causing sterility. The approach has been extensively investigated for rabbits, mice, foxes (Hardy et al. 2006), and cane toads (Robinson 2006) in Australia and brushtail possums in New Zealand (Cowan 1996), and has been suggested for use against carp (Hinds and Pech 1997). Despite promising results in the laboratory, a decade long program in Australia to develop immuno-contraception against introduced mice has recently been terminated, in part because of perceived difficulties in obtaining public approval to release a genetically modified virus and in part because of problems finding a suitable virus. The approach is very likely to run into similar problems if applied to fish. Focus group studies indicate that the use of genetically engineered viruses to control invasive species would face considerable public resistance (Thresher and Kuris 2004).

"Autocidal" refers to the concept of modifying a species genome such tht its impacts or abundance are controlled (Gould and Schliekelman 2004). Five autocidal approaches have thus far been suggested in the literature as having the potential to control invasive species (Table 1). All appear to be genetically feasible. Projects to develop or trial three on fishes are currently in progress: "daughterless" and female-specific sterility are being investigated at the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO; Thresher et al. 2007), and the population effects of a pleiotropic "Trojan" gene are being studied at the University of Minnesota (A. Kapuscinski, pers. comm.). A pleiotropic gene is one that has more than one effect. In the case of a Trojan gene, the effects are to raise the reproductive attractiveness of the carrier while simultaneously lowering the viability of its offspring (Muir and Howard 1999). A sex-specific lethal gene as a potential control agent also has recently been demonstrated in insects (Thomas et al. 2000). Autocidal techniques are of interest in part because they offer possibilities for control where none now

Table 1. Autocidal approaches suggested in the literature as possible options for controlling invasive pests.

Approach	Description	References
Sex or stage-specific lethality/sterility	Construct induces death of offspring at specified stage, or kills or sterilises offspring of one sex in which case the gene is transmitted through the other sex.	Thomas et al. 2000
Gender distortion ("daughterless" or "sonless")	Construct causes offspring to develop as specified sex irrespective of sexual genotype.	Hamilton 1967, Thresher et al. 2007
Inducible mortality	Construct causes death when externally triggered by, e.g., extreme environmental variability or artificial trigger; construct maintained in population by further stocking	Grewe 1997, Schliekelman and Gould 2002
Pleiotropy "Trojan gene"	Construct pleiotropically has positive effect on one or more fitness components, and negative effects on others, e.g., increases mating advantage while decreasing viability of genetically modified offspring	Muir and Howard 1999
Selfish genes	Operational construct (e.g., one that causes gender distortion or sex-specific lethality) is packaged into a genetic element that has a high probability of reproducing itself within a genome, increasing both its spread and that of the construct	Burt 2003, Burt and Trivers 2006

exist (e.g., most established invasive species), but also because they have several intrinsic advantages over conventional biological control. Theoretically at least,

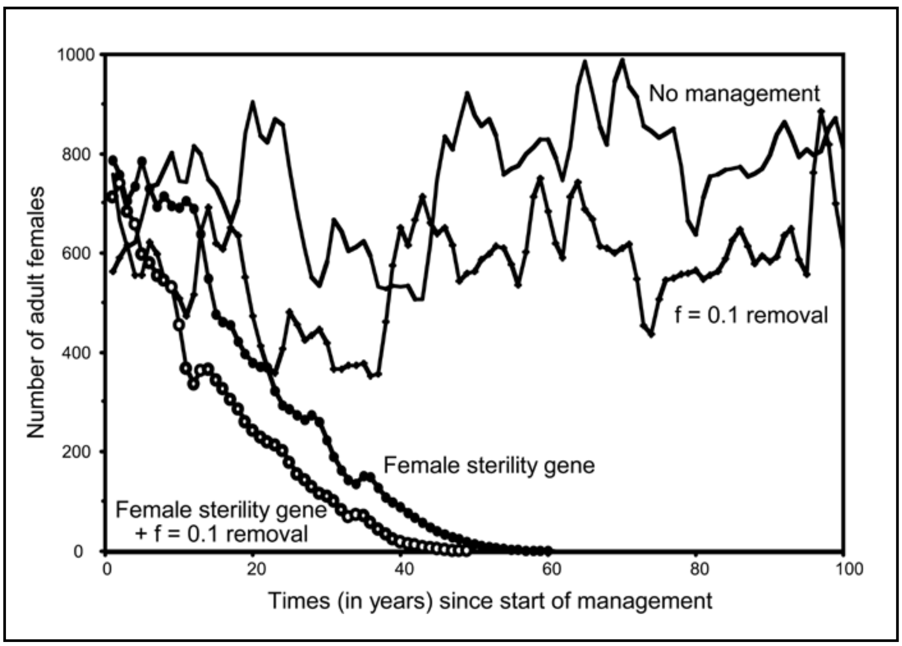
1. The genes can be constructed to be species-specific;
2. They can target particular life-history stages or only one sex, so as to maximise efficacy or minimise damage to non-target species;
3. Their effects can potentially be reversed if something goes wrong; and
4. Some approaches lend themselves to relatively quick and inexpensive modification to target other species, while retaining species-specificity for each.

The last contrasts with the need to find a new control agent for each species targeted by conventional biological control, and spreads the benefits of the high-cost genetic program required to develop an autocidal approach. Modelling studies also show that autocidal pest control programs can be very effective (Gould and Schliekelman 2004; Bax and Thresher in press; Figure 1).

Modelling also indicates three other features of autocidal control programs. First, they are inherently slow acting. To be effective, the gene construct has to spread through the target population. The rate at which it spreads depends on the genetic approach used, stocking rates, generation time, fitness effects, and population structure (Bax and Thresher in press). Even under optimal conditions, effective population control (e.g., populations reduced to $<1\%$ of virgin biomass) typically requires more than 10 generations, and can take much longer. This slow impact has both positive and negative implications. On the plus side, the impacted ecosystem has time to adjust to the absence of the invasive species; there are no mass mortalities and consequent environmental (e.g., water quality) or health problems; and if a problem develops, there is adequate time to launch counter-measures, such as a second gene construct that effectively shuts off the first. On the minus side, the public (and funding agencies) may not be impressed with such a slow response to the problem; for most techniques, gene carriers need to be stocked at high levels for a long time; and most approaches essentially require the stocked carriers to have multiple copies of the gene construct for control to occur in any reasonable time frame. Minimum stocking rates equivalent to 3–5% of annual mean natural recruitment are indicated by most models, sustained for at least 5 generations. Although routine production of carriers may not be a problem given current hatchery capabilities, achieving a stocking rate equivalent to 5% of natural recruitment is not a trivial task for any highly successful invasive species. Stocking strategies that take advantage of metapopulation dynamics could substantially reduce the magnitude of the task, however. Stocking, for example, could be concentrated on a single breeding population, for example, pushing it rapidly towards fixation of the construct, and then allowing emigrants from that population to seed adjacent areas at no additional expense. The need for sustained stocking does have a plus side; it makes it very unlikely that the accidental release of a few carriers would significantly affect a species in its native range.

With regard to copy number, the more independently inherited copies of the construct in the stocked carriers, the faster the gene spreads in the target population and the less stocking effort is required. Constraints on copy number have not been studied in fish, but in some plants at least, genetic mechanisms silence introduced constructs when numerous copies are present (Schubert et al. 2004). If this is also true in fish, then constraints on copy number could severely limit the efficacy of genetic options. Two ways to avoid this problem have been suggested. One is to incorporate the population-controlling construct into a "selfish" gene element. A selfish gene is one that duplicates itself within the genome and so spreads as if present at very high copy numbers (Burt 2003). The approach has never been tried, but is a theoretical possibility. Another option would be to use simultaneously several different constructs that bring about the same functional outcome, e.g., male sterility.

Figure 1. A modelling comparison of methods for reducing invasive fish populations. Population dynamics are based on common carp (*Cyprinus carpio*). Physical removal targets all mature adults and occurs annually at a mortality rate of f = 0.1. The genetic manipulation assumes a copy number of 8, annual stocking equivalent to 5% of annual mean natural recruitment, and no adverse effects of the construct on individual fitness. The modelled carp population has a mean generation time of 4 years, a natural mortality (m) of 0.33, a maximum carp age of 20 years, and moderate levels of density dependence (Ricker parameter = 0.75) and environmental variability. Model details are given in Bax and Thresher (in press).



JASON JENKINS, UNIVERSITY OF MISSOURI

RISK AND PUBLIC ACCEPTABILITY

From its onset, the Australian "daughterless carp" project emphasized public awareness and consultation. This included extensive use of the media, frequent public presentations, and consultative workshops (see Lapidge 2003), a formal communications plan, and a professional survey to assess community concerns about the project. In general, public response to the project has been broadly positive. The community's attitudes were assessed in detail by the survey, which used telephone, mail, and focus group methods to query the public on perceived benefits and risks/costs of the daughterless carp project, and whether the research was worthwhile (Fisher and Crib 2005). On a scale of 1–10 (10 = best or highest), respondents rated the overall benefits of the research at 8.3. The highest perceived benefits of the project were environmental (recovery of native species, bank vegetation, and water quality), but respondents also rated highly the humane aspects of the control method, its safety to humans and other species (discussed below), and economic benefits from improved aquatic environments. Concerns about the research rated an average of 5.9, with the highest concern being the risk that the genetic construct could jump to other species, followed by a related concern that the technology, once fully developed, could be mis-used against humans. In the end, 65% of respondents strongly supported research on the development of a daughterless genetic method to control carp in Australia. However, this was coupled with an even stronger feeling (average rating 8.8) that the community be regularly consulted on work like this. Other key messages were that there must be a fully transparent, public discussion and approval process before this technology is released, and that criticism from those who oppose any genetic modification technology should be expected and even welcomed, as helping identify areas of possible public concern.

The survey confirmed conclusions from an earlier focus group study (Thresher and Kuris 2004) that genetic techniques that modified only the invasive species would be publicly acceptable. More broadly, both studies indicate two elements strongly influence the degree of public acceptability—the extent or risk of damage to other species and the environment and the extent to which the program could be stopped and even reversed if an unforeseen problem arises.

For recombinant approaches, the factors that determine the level of risk to other than the target species still need to be rigorously examined. However, one key factor is likely to be whether or not the method used is species-specific, which in turn depends on the risk that the construct will be passed between species and then whether it will function in its new cellular environment. The usual means by which a gene spreads is from parent to offspring ("vertical transfer"), so that in part the likelihood of inter-specific transfer is constrained by the usual barriers to hybridisation, and hence depends on whether or not the target species co-occurs with closely related species. However, genes can also be spread by "horizontal transfer," i.e., between individuals by, for example, a virus. The extent to which horizontal transfer occurs is debated by geneticists and probably varies widely amongst taxa, as well as depending on the size and nature of the gene itself (Burt and Trivers 2006). The subject is not well studied in fish, though horizontal transfer of selfish gene elements has been suggested to have occurred in the group (Leaver 2001; Pocwierz-Kotus et al. 2007).

The likelihood that a gene will function when transferred to another species depends on the genetic similarities (broadly equated with relatedness) of the source and recipient species and the gene involved. In general, at the level of translation (making proteins), genes often function effectively in diverse recipient species and hence show little or no species-specificity, e.g., a construct synthesized from ocean pout, salmon, and carp genes increases growth hormone production and growth rates in tilapia (Caelers et al. 2005). At the other extreme, at the level of transcription, genes that repress the expression of other genes can require an exact match between the repressor and target gene to work. As genetic sequences of even closely related species often differ by 10% or more, repressors can in theory be designed to function in only a single species. Hence, from a specificity perspective, the safest application of a recombinant approach would use a repressor against a species at a site where it had no close relatives (e.g., common carp in Australia) and, broadly speaking, the riskiest would be a construct that codes directly for a product, such as a growth hormone, against a species in an area full of its close relatives (e.g., a sunfish outside its native range in North America). Genetically modified viruses or selfish gene elements, which have many virus-like features, could be riskier still, due to the apparently higher likelihood of horizontal gene transfer.

The second determinant of perceived risk—the extent to which a program could be stopped if something goes wrong—appears to differ intrinsically between autocidal approaches. At one extreme, some techniques require sustained stocking of carriers before a critical point of no return is reached. For such techniques, stopping the stocking will not eliminate the introduced gene, but does allow the wild-type gene to reassert itself and the targeted population essentially reverts to normal. At the other extreme, it has been suggested that the release of even a single male carrying a Trojan gene dooms an affected population to extinction (Muir and Howard 1999). Whether or not this is true in practice is still being studied. In theory, the effects of a genetic construct could also be reversed by releasing a second construct that shuts down the first and restores the population. Such an approach has never been tried, but the slow action of an autocidal control program makes it conceptually feasible. Susceptibility to a "negating" construct could be an important, and potentially mandatory design feature activated if, for example, a gene construct jumped to a native species. It is worth noting that the option to stop autocidal technology could make it inherently safer than classical biological control, in that a released predator, pathogen, or parasite is very often an irreversible addition to the biota that can cause problems in its own right.

Ultimately, the extent to which the public would accept any of these approaches is likely to depend on how effective and how risky they are. Efficacy we can begin to estimate from modelling studies, but there are still too many unknowns to quantify risk. For these reasons, a staged, adaptive management approach to developing the technology is appropriate, working up from small-scale experimental trials in bio-secure facilities (e.g., large closed-system aquaria), to more realistic trials in isolated and secured pond habitats, before finally risking wider scale releases. Such a staged approach allows predictions about efficacy and risk to non-target species to be tested at each level, models to be refined to improve predictability and increase reality at local and larger scales, and the public to be informed and its approval sought before moving to the next, larger scale of investigation.

POLICY FRAMEWORKS FOR GENETICALLY-BASED PEST CONTROL

The use of genetically modified (GM) fish for environmental remediation is outside the scope of many of the policy and legislative frameworks established to deal with GM organisms. In part, this is because most regulations to date have been developed and applied principally towards plants, given initial and current concerns about contamination of commercial crops with GM strains. A second reason is that where animals have been addressed, the underlying assumption is that they would be used principally as food or sources of biological products (e.g., serums or vaccines). Hence, the emphasis of regulation has primarily been human health considerations and containment of farmed animals. The deliberate release of a genetically modified fish with the express intent of maximizing its spread through an ecosystem is not likely to be what the framers had in mind when policy and legislation were drafted.

The policy and legislative frameworks that might apply to release of a recombinant fish for purposes of pest control in North America, in general, and the United States in particular, have been examined in detail by Anne Kapuscinski and her colleagues, and are summarized in Kapuscinski and Patronski (2005). In brief, it is unclear what regulations would apply at the national level, or even what the appropriate regulating agency would be. The U.S. Food and Drug Administration (FDA) has dealt with GM fish intended for human consumption, but lacks expertise to assess environmental issues. The agency recently declined to be involved in regulation of "Glo-fish," a zebrafish (*Danio rerio*) strain genetically modified to express a fluorescent jellyfish pigment, on the basis that it would not be consumed by humans and hence fell outside FDA's purview. Whether this applies to recombinant fish released to control invasives is vague: small species, such as unwanted populations of *Gambusia*, could impact human consumption only as prey for larger sports fish, whereas human health issues could be of direct concern for taxa like the carps, which are consumed. As noted by Kapuscinski and Patronski (2005), the FDA's explicit exclusion of public input into its environmental assessments, due to commercial considerations, is also directly at odds with the National Environmental Policy Act (NEPA), which requires both an impact assessment and public review in seeking to make enlightened decisions. It also conflicts with the public's demand for transparent decision making about releasing a recombinant species, noted above. Ultimately, however, both FDA and NEPA regulatory frameworks are engaged only if the recombinant species is an inter-state issue or explicitly involves elements of the federal government, such as funding, lands, or permits. In principle, a private individual could release a GM fish into a 10-acre pond with no mandated federal oversight at all.

Regulation at the international, national, state, and local levels varies widely among jurisdictions. Internationally, elements of the Convention on Biological Diversity, and specifically, the Cartagena Protocol on Biosafety, provide mandatory regulations on the international movement of GM organisms, and require risk management and environmental assessments of releases that might span national boundaries, mandatory international consultation in such cases, and legal redress. Other trans-national frameworks that could come into play, though they are not specific to the issue and in many cases, would cope poorly with it, include those intended to regulate trade in food products and seeds, minimize impacts of ballast water, protect endangered species, and even regulate bio-warfare. At the state level, several states have introduced legislation to manage GM organisms (e.g., California, Michigan, Minnesota), but others have not. The regulations that are in place are broadly similar, but would benefit from harmonisation in order to deal with control efforts that would inevitably, if not by design, span state boundaries. The number of jurisdictions that would be impinged by the release of daughterless Asian carp into the Mississippi Basin is staggering. Legal impediments to the release of the fish could also be staggering.

As the release of such fish constitutes a form of biological control, regulatory frameworks based on those in place for biological control may be appropriate, suitably modified to encompass expert input on recombinant genetics. Unfortunately, regulatory frameworks for biological control in the United States also appear to be confusing and inconsistently applied (Strong and Pemberton 2000).

NEXT STEPS

The science of controlling invasive pest fish by recombinant genetics is developing slowly, in part due to funding constraints and in part due to relatively long generation times in fish. At this stage, theory is more advanced than the empirical science, but in turn, models are still poorly parameterised due to the plethora of assumptions that need to be incorporated into them. Similarly, we can begin to outline an appropriate risk assessment framework, but are constrained by large uncertainties about even the nature of the genetic approaches to be used.

Investment in the science is a prerequisite if the field is to progress. However, two other areas are equally in need of development: public consultation and policy development. With regard to the former, discussions with North American environmental managers indicate that many feel that the public will not accept recombinant approaches. This opinion appears to derive from a perceived widespread public opposition to GM foods. However, the basis for this perception is ambiguous. Surveys suggest the public is relatively sophisticated in terms of what it will and will not accept with regard to modern biotechnology (Gaskell et al. 1999). The U.S. public, for example, broadly supports biotechnology for GM medicines, while opposing its use in xenotransplantation. The failed realization of the dire predictions some groups made about the consequences of GM foods may also have reduced negative reactions by the public towards genetic technologies. Given this, a formal public consultation process would allow public input into potentially contentious (but also potentially not) investment decisions in recombinant R&D for high profile invasives like Asian carp, and into the management of the technology.

The second issue is the need to remove ambiguities in the policy and legislative framework(s) that would regulate environmental applications of recombinant technology. The central issue is not the promulgation or advocacy of regulatory frameworks that might facilitate the recombinant approaches. Rather, current uncertainties could obstruct development and application of the technology inadvertently, while still not being sufficient to prevent its un-wise use. A detailed review and evaluation of relevant North American legislation that could pertain to genetic approaches to controlling invasives would appear to be a sensible step towards effective management and regulation of the technology.

CSIRO has just built a first generation daughterless carp construct. It is ready to be tested, and could be in field trial in as little as five years. In Australia, GM technology is managed by a national Office of the Gene Technology Regulator (OGTR), based on national legislation that is mirrored at the state level. The prospect of releasing a daughterless carp is very different from the crop and medically oriented issues OGTR has principally dealt with to date, but our discussions with the agency indicate that a relatively clear path to assessment, consultation, and approval (or rejection) is in place. If autocidal technology is ever to be used against invasive species in North America, the development of a similarly informed and transparent decision-making process to regulate and manage it needs to start. My discussions with U.S. federal authorities strongly suggest that modifying the national regulatory framework for GM products to accommodate genetic control of invasive species will be politically difficult. As such, it is not likely to happen without the active engagement of fisheries and wildlife managers. The American Fisheries Society and allied organizations, such as the Association of Fish and Wildlife Agencies, can play an important role in getting this process started at national and local levels, in informing the fisheries community and public of key issues and developments, and in providing expert advice at all levels. ♋

ACKNOWLEDGEMENTS

This article is based on a pair of presentations at the 2006 meeting of the Association of Fisheries and Wildlife Agencies. I thank the Mississippi Interstate Cooperative Resource Association and particularly I. J. Rivers and L. Riley for the invitation and support to attend this meeting. The concepts presented have developed during extensive discussions with numerous colleagues, including but not limited to N. Bax, P. Grewe, C. Hardy, K. Hayes, L. Hinds, A. Kapuscinski, J. Patil, and S. Whyard. All are gratefully acknowledged. N. Bax, P. Grewe, K. Hayes, M. Jones, J. Patil, L. Riley, two anonymous referees, and an AFS editor provided valuable input to the manuscript. This work has been supported by the CSIRO, the Murray-Darling Basin Commission, and Australasian Invasive Species CRC.

REFERENCES

Bax, N. J., and **R. E. Thresher.** In press. The effects of ecological, behavioural and genetic factors on the use of recombinant techniques to control invasive pests. Ecological Applications.

Bergstedt, R. A., R. B. McDonald, M. B. Twohey, K. M. Mullet, R. J. Young, and **J. W. Heinrich.** 2003. Reduction in sea lamprey hatching success due to release of sterilized males. Journal of Great Lakes Research 29 (Suppl. 1):435-444.

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

Burt, A., and **R. Trivers.** 2006. Genes in conflict. Harvard University, Cambridge, Massachusetts.

Caelers, A., N. Maclean, G. Hwang, E. Eppler, and **M. Reinecke.** 2005. Expression of endogenous and exogenous growth hormone (GH) messenger (m)RNA in a GH-transgenic tilapia (*Oreochromis niloticus*). Transgenic Research 14:95-104.

Cowan, P. E. 1996. Possum biocontrol: prospects for fertility regulation. Reproductive Fertility and Development 8:655-660.

Crane, M. S., and **B. T. Eaton.** 1997. Spring viraemia of carp virus (*Rhabdovirus carpio*): a biological control agent. Pages 87-107 *in* J. Roberts and R. Tilzey, eds. Controlling carp: exploring the options for Australia. Murray-Darling Basin Comm., Canberra, Australia.

Fisher, N., and **J. Cribb.** 2005. Monitoring community attitudes to using gene technology methods (daughterless carp) for managing common carp. Report prepared for the Cooperative Research Centre for Pest Animal Control, Valuemetrics Australia. Canberra, Australia.

Fuller, P. L., L. G. Nico and **J. D. Williams.** 1999. Non-indigenous fishes introduced into inland waters of the United States. American Fisheries Society Special Publication 27, Bethesda, Maryland.

Gaskell, G., M. W. Bauer, J. Durrant, and **N. C. Allum.** 1999. Worlds apart? The reception of genetically modified foods in Europe and the U.S. Science 285:384-387.

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

Grewe, P. 1997. Potential of molecular approaches for the environmentally benign management of carp. Pages 119-127 *in* J. Roberts and R. Tilzey, eds. Controlling carp: exploring the options for Australia. Murray-Darling Basin Comm., Canberra, Australia.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477-488.

Hardy, C. M., L. A. Hinds, P. J. Kerr, M. L. Lloyd, A. J. Redwood, G. R. Sgelam, and **T. Strive.** 2006. Biological control of vertebrate pests using virally vectored immunocontraception. Journal of Reproductive Biology 71:102-111.

Hinds, L. A., and **R. P. Pech.** 1997. Immuno-contraceptive control for carp. Pages 108-118 *in* J. Roberts and R. Tilzey, eds. Controlling carp: exploring the options for Australia. Murray-Darling Basin Comm., Canberra, Australia.

Kapuscinski, A. R, and **T. J. Patronski.** 2005. Genetic methods for biological control of non-native fish in the Gila River system. Contract report to the U.S. Fish and Wildlife Service. University of Minnesota, Inst. Social, Economic and Ecological Sustainability, St. Paul, Minnesota. Minnesota Sea Grant Publication F20.

Lapidge, K. L. (editor). 2003. Proceedings of the National Carp Control Workshop, March 2003, Canberra. Cooperative Research Centre for Pest Animal Control, Canberra, Australia.

Leaver, M. J. 2001. A family of Tc1-like transposons from the genomes of fishes and frogs: evidence for horizontal transmission. Gene 271:203-214.

Lever, C. 2002. Naturalized fishes of the world. Academic Press, New York.

Lowe, S., M. Browne, S. Boudjelas, and **M. DePoorter.** 2001. 100 of the world's worst invasive alien species, a selection from the Global Invasive Species Database. IUCN-ISSG, Aukland, New Zealand.

Meronek, T. G., P. M. Bouchard, E. R. Buckner, R. M. Burri, K. K. Demmerly, D. C. Hayleli, R. A. Klumb, S. H. Schmidt, and **D. W. Coble.** 1996. A review of fish control projects. North American Journal of Fisheries Management 16:63-74.

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 U.S. National Academy of Science 96:13853-13856.

_____. 2002. Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. Transgenic Research 11:101-114.

Pocwierz-Kotus, A., A. Burzynski, and **R. Wenne.** 2007. Family of Tc-1-like elements from fish genomes and horizontal transfer. Gene 390:243-251.

Rayner, T. S., and R. G. Creese. 2006. A review of rotenone use for the control of non-indigenous fish in Australian fresh waters, and an attempted eradication of the noxious fish, *Phalloceros caudimaculatus*. New Zealand Journal of Marine and Freshwater Research 40:477-486.

Robinson, T., N. Siddon, S. Tarmo, D. Halliday, T. Shanmuganathan and **D. Venables.** 2006. CSIRO biocontrol project: concept and progress. Pages 86-88 *in* K. L. Mollay and W. R. Henderson, eds. Science of cane toad invasion and control. Invasive Animals Cooperative Research Centre, Canberra, Australia.

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

Schubert, D., B. Lechtenberg, A. Forsbach, M. Gils, S. Bahadur, and **R. Schmidt.** 2004. Silencing in *Arabidopsis* T-DNA transformants: the predominant role of a gene-specific RNA sensing mechanism versus position effects. Plant Cell 16:2561-2572.

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

Strong, D. R., and **R. W. Pemberton**. 2000. Biological control of invading species—risk and reform. Science 288:1969-1970.

Thomas, D. D., C. A. Donnelly, R. J. Wood, and **L. S. Alphey.** 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287:2474-2476.

Thresher, R. E., and **A. Kuris.** 2004. Options for managing invasive marine species. Biological Invasions 6:295-300.

Thresher, R. E., P. Grewe, J. Patil, and **L. Hinds.** 2007. Genetic control of sex ratio in animal populations. Australian Patent 2001291520, Commonwealth Scientific and Industrial Research Organisation. Hobart, Tasmania, Australia.

Torres, J. M., C. Sanchez, M. A. Ramierez, M. Morales, J. Barcena, J. Ferrer, E. Espuna, A. Pages-Mante and **J. M. Sanchez-Vizcaino.** 2001. First field trial of a transmissible recominbinant vaccine against myxomatosis and rabbit hemorrhagic disease. Vaccine 19: 4536-4543.

Wagner, C. M., M. L. Jones, M. B. Twohey, and **P. W. Sorenson.** 2006. A field test verifies that pheromones can be useful for sea lamprey (*Petromyzon marinus*) control in the Great Lakes. Canadian Journal of Fisheries and Aquatic Science 63:475-479.