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Genetic control of invasive fish: technological options and its role in integrated pest management

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Abstract Genetic options for the control of invasive fishes were recently reviewed and synthesized at a 2010 international symposium, held in Minneapolis/St. Paul, MN, USA. The only option currently available “off-the-shelf” is triploidy, which can be used to produce sterile males for a release program analogous to those widely and successfully used for biological control of insect pests. However, the Trojan Y and several recombinant options that heritably distort pest population sex ratios are technologically feasible, are at or are close to proof-of-concept stage and are potentially much more effective than sterile male release programs. All genetic options at this stage require prolonged stocking programs to be effective, though gene drive systems are a potential for recombinant approaches. They are also likely to differ in their current degree of social acceptability, with chromosomal approaches (triploidy and Trojan Y) likely to be the most readily acceptable to the public and least likely to require changes in legislative

or policy settings to be implemented. Modelling also suggests that the efficacy of any of these genetic techniques is enhanced by, and in turn non-additively enhance, conventional methods of pest fish control.

Keywords Biological control · Daughterless · Genetic control · Insect · Recombinant · Triploidy · Trojan Y

Introduction

Invasive species are changing the world's natural environment and our use of it at an unprecedented rate (Vitousek et al. 1996; Mack et al. 2000). As many as half a million species have been introduced to new geographical regions as a result of human activities, with an estimated annual cost globally to agriculture alone of \$248 billion (Pimentel et al. 2001, 2005). Fish

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are a large part of this problem. Nine of the “100 Worst Invasive Alien Species” are fish (Lowe et al. 2001), impacts of the sea lamprey (*Petromyzon marinus*) are still being managed at a significant annual cost to Canada and the United States, and snakeheads (*Channa striata*) have established themselves in both the Potomac and Mississippi drainages, with potentially severe ecosystem consequences. More broadly, over 200 fish species have established non-native populations around the world (Lever 2002).

Invasive pests can be eradicated if detected soon after establishment or if the invaded area is small and the targeted population closed (Dahlsten and Garcia 1989; Bax et al. 2002; Culver and Kuris 2000; Veitch and Clout 2002). At small scales, invasive fish can be controlled by use of biocides, physical removal, barriers and environmental modification (reviewed by Meronek et al. 1996; Rayner and Creese 2006). For well established and widely distributed pests, however, the only realistic options to date have been augmentative and classical biological control (Van Driesche and Bellows 1996) and sterile male release programs (Krafsur 1998; Benedict and Robinson 2003) (see Box 1).

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. Peacock bass (*Cichla ocellaris*) have been successfully introduced in Florida to control other invasive cichlid populations (myfwc.com/fishing/offices/boca.html) and augmenting local predator populations has been proposed to control common carp (*Cyprinus carpio*) in midwestern USA lakes (P. Sorensen unpublished data). A virus, Spring Viraemia, was considered for the control of common carp in Australia, but rejected on the basis of uncertain efficacy and low species-specificity (Crane and Eaton 1997). Australian scientists are currently conducting specificity trials of Koi Herpes Virus as another option. With regard to other approaches, we are aware of only one attempt at release of sterilized male fish for purposes of pest control—sea lampreys in the St. Marys River (Bergstedt et al. 2003)—but the effect of this effort on adult lamprey populations is still unclear.

Genetic technology provides another suite of potential control options for invasive fish. As early as the 1960s, entomologists observed that meiotic

drive acting through a mutant Y chromosome had apparently driven some insect populations to extinction, and suggested that genetics could be used to manage insect populations (Hamilton 1967). Recent advances in recombinant genetics, as well as the lack of other options to effectively control established pest populations, have led to renewed interest in this idea. Several studies have modeled a suite of options for pest control, highlighting their potential strengths and weaknesses from a theoretical perspective (e.g., Krafsur 1998; Schliekelman and Gould 2000a, b; Gould and Schliekelman 2004; Schliekelman et al. 2005; Gutierrez and Teem 2006; Phuc et al. 2007; Bax and Thresher 2009) and the technical feasibility of at least three recombinant methods (repressible male sterility, virally or parasitically vectored immune-contraception, and female-biased sex ratio distortion) are being tested in the laboratory (Thomas et al. 2000; Hinds et al. 2002; Horn and Wimmer 2003; Thresher et al. 2005; Gong et al. 2005; Phuc et al. 2007) (Box 1). Field trials have recently been undertaken in several countries to test the impacts of sterile recombinant females on insect pest populations (Thomas et al. 2000; Enserink 2010) (see Box 1), as well as the use of sterile triploid salmonids to reduce the possible genetic impacts of non-native species or strains on wild stocks (Warrillow et al. 1997; Cotter et al. 2000; Kozfkay et al. 2006; Wagner et al. 2006; High and Meyer 2009).

Whether any of these methods are effective in the “real world” remains unknown, either as a sole control method or as part of an integrated pest management (IPM) approach (Bax and Thresher 2009). To further the development of the field, genetic options for controlling pest fish were recently examined by a broad suite of international stakeholders, including geneticists, conservationists and management agencies, at an International Symposium on Genetic Biocontrol of Invasive Fish (Minneapolis/St. Paul, MN, USA, 21–24 June 2010). This paper summarizes and synthesizes the outcomes of those discussions. Specifically, it reviews the current state of technical development of the genetic options, and assesses their potential use in an IPM context. Companion papers in this volume address the development of an appropriate risk analysis framework and the public and political acceptability of the technology (Dana et al. 2013; Hayes et al. 2013; Sharpe in press).

Box 1 Genetic Control of Insect Pests

Use of the sterile insect technique (SIT) has a long history starting with the eradication of the screwworm fly from the Southern USA and later from Mexico and Central America (Knippling 1960; Krafssur 1998). Application of SIT to other Diptera (flies) as well as to Lepidoptera (moths) and Coleoptera (beetles) has resulted in a number of other important successes (Klassen and Curtis 2005), but there have also been notable failures (Curtis 1985; Gould and Schliekelman 2004). A major driver of research on SIT has been the UN International Atomic Energy Agency (IAEA) which has focused on tephritid fly pests of fruits and vegetables as well as on disease vectors such as mosquitoes and tsetse flies (Dyke et al. 2005). The tephritid programs, including the internationally backed Medfly eradication program have yielded major benefits to agriculture (Enkrlin 2005). The tsetse fly has been eradicated from Zanzibar, but progress in other parts of Africa has been slow (Feldman et al. 2005). A number of attempts have been undertaken to eradicate the mosquito, *Aedes aegypti*, which transmits dengue fever, but none have succeeded (Asman et al. 1981)

More complex, classical genetic approaches have been attempted with hopeful results but no major successes (Curtis 1985). One early attempt that used hybridization of two tsetse fly species to create a system with under dominance was successful on a small scale but was never used on a larger landscape (Vanderplank 1947; Klassen and Curtis 2005). A number of projects involved creation of mutagen-induced translocations. These also resulted in under dominance properties, and in some cases where the translocation involved the sex-determining chromosome, they caused selective death of females (Foster and Whitten 1974). The major stumbling block in these projects was the low fitness of translocation homozygotes

After a period of diminished research on SIT, the advent of new transgenesis methods in *Drosophila* led to rekindled interest in genetic manipulation of insect pests (Gould and Schliekelman 2004). Early work focused on mosquitoes that transmit dengue and malaria. The *Drosophila P*-element transformation systems did not work in other insects, but molecular geneticists discovered a number of transposable elements with broader taxonomic range (Handler 2002). One early idea was to use transposable elements to spread either anti-pathogen genes (to disable the dengue virus and malaria plasmodium) or genes that would put a genetic load on the mosquito population. Unfortunately, none of the transposons discovered to date have high enough rates of non-local transposition to be useful in this context

More recently, the work in this area has split into two approaches, one that simply uses transformation to create conditional lethality in females or in both sexes, and a second, more sophisticated approach, the goal of which is to create synthetic selfish genetic elements that could drive desirable genes into pest populations. The first approach has moved ahead more rapidly (Heinrich and Scott 2000; Thomas et al. 2000) and there are now a number of strains of mosquitoes and tephritid fruit flies with conditional lethality genes affecting either only females or both males and females (Schetelig et al. 2009; Fu et al. 2010). For *A. aegypti*, the most advanced transgenic strain is one that causes females to be flightless (i.e. death inducing in the wild) when reared as larvae on a diet without tetracycline (Fu et al. 2010). This mosquito strain was tested in large enclosures to see if it could cause eradication of a wild type strain of the mosquito. The experiments demonstrated eradication in about 8–13 generations (de Valdez et al. 2011). A somewhat less complex strain in which males and females both die when tetracycline is not in the diet (i.e. under field conditions) was recently tested in a small field release on the Grand Cayman Islands (Enserink 2010). The results have recently published and demonstrate at least partial success in decreasing the density of the wild population (Harris et al. 2012). More releases in a number of countries are planned. A number of recent advances have been made in engineering tephritid strains with either conditional female lethality or lethality in both sexes (Gong et al. 2005; Schetelig et al. 2009; Schetelig and Handler 2012). These strains have yet to be tested for efficacy in large enclosures or in the field

The more sophisticated gene drive systems are, as expected, not as far along in development as the simpler systems, although some significant breakthroughs have been made. In 2007, Bruce Hay and colleagues succeeded in transforming a *Drosophila* strain with a synthetic *Medea* element (Chen et al. 2007). Population cage experiments with the resultant fly lines demonstrated that the *Medea* element could increase in frequency on its own. The experiment started with the *Medea* allele at a frequency of approximately 0.25 and ended with fixation. Attempts to build a similar *Medea* gene drive system in disease-vectoring mosquitoes have met many challenges, in part because the genomic tools for the mosquitoes are much more primitive than for *Drosophila*

In 2003, Austin Burt proposed using another class selfish genetic element for gene drive. This class is called the homing endonucleases. They function by turning insects that are hemizygous for the element into homozygotes during meiosis (Burt 2003). Although naturally occurring homing endonucleases had never been found in insects or other complex diploid species, Burt and his colleagues recently succeeded in engineering a synthetic homing endonuclease into the malaria-vectoring mosquito, *Anopheles gambiae* (Windbichler et al. 2011). As with the work on *Medea*, the research team was able to demonstrate that the homing endonuclease could increase in frequency in a lab population even though it conferred no fitness benefit to the individuals that carried it

The experimental gene drive systems described above are based on novel transgenic constructs. In contrast, a major project funded by the Gates Foundation has used the transfer of a strain of *Wolbachia* bacteria into the mosquito vector of dengue, *Aedes aegypti*, to suppress that insect's ability to transmit the dengue virus (Walker et al. 2011). This *Wolbachia* strain's combined virus suppression and selfish gene drive properties enabled its establishment in two small Australian towns following its intentional release (Hoffmann et al. 2011). These recent breakthroughs have resulted in some enthusiasm for future projects that will broaden the use of engineering techniques to invasive insect taxa that harm biodiversity and to other insects that impact human health or nutrition (Gould 2008)

Genetic pest control technologies

Self-propagating approaches

Self-propagating options use a genetically modified agent (usually a virus) to spread a recombinant lethal or sterilizing construct. The agent in the most developed option (immuno-contraception) expresses a protein critical for its host's reproduction. The host's immune defence system reacts to the virus by raising antibodies against the artificially-induced proteins, which indiscriminately attack both introduced and the host's own proteins, thereby causing sterility. Immuno-contraception has been suggested for use against carp (Hinds and Pech 1997), but is primarily being developed to control invasive mammals (Cowan 1996; Hardy et al. 2006). Predictive models suggest that it could be an extremely effective method of pest control, but stakeholder analysis suggests that self-disseminating, genetically modified viruses are unlikely to be acceptable to the public, at least in Australia (Thresher and Kuris 2004). In part for that reason, a long-term program to develop immuno-contraception against introduced mice in Australia was terminated, despite successful laboratory trials. Scientists in New Zealand, however, are still developing the technology for use against introduced brushtail possums, using nematodes to spread a sterilising gene construct (Cowan 1996).

Chromosomal approaches

Triploidy

Triploids are animals in which the normal diploid chromosome set is artificially augmented with a third unpaired chromosome set. Triploidy induction can be "direct" (by the manipulation of meiosis) or "indirect" (by the manipulation of mitosis to produce tetraploids and then crossing tetraploids of one sex with diploids of the other to yield triploid offspring). These manipulations involve the retention of entire unpaired chromosome sets that would normally be separated between polar bodies and daughter cells. The manipulations themselves are simple and inexpensive, involving either physical treatment (thermal or hydrostatic pressure) or the application of chemicals (e.g., cytochalasin B). The technology for the production of triploids has

advanced to the point of commercial application in fishes and bivalves (Benfey 2009; Guo et al. 2009; Piferrer et al. 2009; Fraser et al. 2012), and substantial progress has also been made on the production of triploid crustaceans (Sellars et al. 2010). Because triploidy does not involve manipulations of individual chromosomes or genes, triploids are not widely considered to be "genetically modified". They are widely used in recreational fisheries and aquaculture, suggesting there is likely to be little or no public concern about their possible use in a pest control context.

Triploids are sterile because their odd number of chromosome sets results in meiotic dysfunction during germ cell development, when homologous chromosomes would normally recombine and segregate, as this process can only work when homologous chromosomes can be arranged in pairs (including duplicate pairs in the case of tetraploids). This dysfunction does not prevent gonadal development, but germ cells either fail to develop or they develop into cells with abnormal chromosome numbers (aneuploidy), which produce offspring that fail to develop to maturity. In fishes, triploid males are generally morphologically indistinguishable from diploid males at maturity and produce functional spermatozoa, but their offspring die shortly after fertilization (Piferrer et al. 2009; Benfey 2011). Triploid females, however, have much smaller ovaries than diploid females and throughout life retain the characteristics of juvenile fish (Piferrer et al. 2009; Benfey 2011).

To date, triploidy has primarily been used to maximise production in recreational fisheries and aquaculture by minimizing the investment of energy into gonadal growth (Benfey 2009), but it has also been suggested as a means of minimizing, although not eliminating the risk of feral populations establishing from escaped aquaculture stock (National Research Council of the National Academies 2004). Triploid animals are not always 100 % sterile (e.g., Normand et al. 2008). In cases where undesirable species have already established, triploid males could be released to mate with wild females, reducing population fecundity. Such a sterile-male approach has not yet been tried in fish and based on insect parallels (Krafsur 1998) is likely to require that large numbers of triploids be released in order to swamp the male breeding component of the established wild populations. A consequence of this is that the high pest

densities during the lifetimes of the stocked fish could have substantial, possibly undesirable ecological effects. If applied when pest populations are small and just establishing, however, the numbers of males required could be tractable and temporary ecological impacts preferable to the permanent damage caused by a widely distributed pest.

Trojan Y chromosome

The Trojan Y Chromosome (TYC) approach uses female fish with two Y chromosomes (YY) to shift the sex-ratio of a target population towards males. Carried to its limit, this could result in extinction of the pest population (Gutierrez and Teem 2006). In fish with an XY sex-determination system, the presence of a Y chromosome normally results in a male phenotype. For some species, such as Nile tilapia (*Oreochromis niloticus*), YY “super males” can be generated by a combination of selective breeding techniques and the use of hormones to sex-reverse juveniles. These super males nominally have the same mating characteristics as an XY male, but produce only male progeny. In the TYC strategy, YY fish are induced, via hormone treatment in the laboratory, to develop into phenotypic females, which are then introduced into the pest population. These females cause increased production of males in two ways. First, all of their progeny are males: XY males and YY males. Second, these YY progeny in turn generate only male offspring: XY males if mating occurs with a normal XX female, but YY males if mating occurs with the stocked Trojan YY female. This latter is a feedback loop that increases even further the number of YY males in the system. Modelling studies suggest that a continued addition of YY females to a target population skews population sex ratios to the point of extinction of XX females. At this point, discontinuing the addition of Trojan YY females causes the population go to extinct (Gutierrez and Teem 2006).

The TYC strategy remains untested at this time, and is in need of further research. A key unknown is whether complications might arise as a result of altered mating behaviour of the YY fish. Aside from *O. niloticus*, only a few other fish species have been manipulated to produce YY variants (e.g., Bongers et al. 1999) and detailed studies on their fitness in a competitive environment have not been carried out. It is also not yet clear whether all species can tolerate a

YY genotype. YY female channel catfish (*Ictalurus punctatus*), for example, “do not reproduce or have severe reproductive problems” (Dunham 2011). There are also potential complications in some species due to sterility of offspring of YY males (e.g., Bongers et al. 1999) and the effects of autosomal sex modifiers (Kallman 1984; Nanda et al. 2003) and environmental temperatures (Mylonas et al. 2005; Rougeot et al. 2007) on offspring sex ratios. Finally, the Trojan Y approach requires genetic sex determination be either XY or, possibly, ZW. The extent to which this is the case in fish species is not yet clear (e.g. Cnanni et al. 2008), particularly given common environmentally mediated sex determination in some groups (Devlin and Nagahama 2002).

Recombinant approaches

“Sterile feral” technology

Sterile feral technology is the recombinant analogue of triploidy. As currently configured in fish, sterile feral constructs consist of a stage-specific promoter, a blocker for a critical developmental gene and a repressible element. The inheritable gene construct renders both males and females sterile (unless repressed for hatchery breeding purposes) by triggering the lethal blocker in their eggs or fry, including those produced by any wild type fish with which a homozygous carrier breeds. A number of different combinations of promoters, target genes and repressible elements are currently being trialled (Su et al. in preparation). The only successful system reported thus far consists of an early embryonic promoter (*SMAD5*), the repressible element Tet-off, and a sequence that either over-expresses a dorsalisating gene (*BMP2*) or expresses an RNA interference sequence that nominally inhibits *BMP2* transcription (Thresher et al. 2009). Both over- and under-expression of *BMP2* during embryogenesis causes lethal disruption of embryonic dorsal–ventral patterning. Tet-off (“tetracycline-controlled transcriptional activation”) is a commercially available construct based on bacterial and viral elements that stops gene expression (in this case, the lethal element) in the presence of dietary or water-borne tetracycline or doxycycline (Gossen and Bujard 1992). The prototype sterile feral technology has been tested successfully in transient assays in zebrafish (*Danio rerio*) and channel catfish (Thresher et al. 2009) and in integrated channel catfish lines (Chaimongkol 2008).

In the latter, 95.6 % of embryos in catfish lines that had integrated the construct up-regulating *BMP2* died in the absence of doxycycline. In comparison, mortality rates of siblings dosed with 100 ppm of doxycycline in their rearing water were less than 50 % and did not differ significantly from those of wild type fry (Chaimongkol 2008).

Sterile feral technology is being developed for the aquaculture industry, as a means of preventing the establishment of feral populations should exotic or genetically modified fish escape containment. However, as the sterility is similar to that produced by triploidy, it can potentially be used for many of the same applications and can also be used in a preventative mode—an area that is about to be invaded can be stocked with a small number of sterile feral males and females, as to soak up the reproductive inoculum of invading individuals and thereby prevent establishment. The use of the sterile feral technology for long-term pest control can potentially be enhanced by delaying expression of the construct and allowing it to spread (via inheritance) throughout a population prior to expression. Technical options for delaying across generations the onset of gene expression, however, are currently theoretical.

Autocidal technology

Autocidal (“self killing”) refers to modifying a species genome such that as the modification spreads through the population the species’ impacts or abundance are reduced (Gould and Schliekelman 2004). To date, eight autocidal approaches have been identified as having the potential to control invasive species (Table 1). All appear to be genetically feasible. The furthest developed at this stage is a construct that biases offspring sex ratios towards, and is inherited through, males. Severe skewing of population sex ratios, in theory at least, can result in population collapse and even pest extinction. Several variants of a female-specific lethal gene have been demonstrated as a potential control agent for insects (Thomas et al. 2000; Fu et al. 2007) and one has recently been successfully tested on caged populations of fruitflies (Ant et al. 2012). In fish, prototype sex-ratio distorting constructs (“daughterless”) that bias sexual differentiation towards the male phenotype or cause female-specific sterility or lethality have been demonstrated in laboratory populations of medaka (*Oryzias latipes*)

and zebrafish (Thresher et al. unpublished data) and are currently being tested in common carp. Sex ratios in an integrated line of zebrafish carrying a female lethal construct are strongly male-biased through at least four generations, with little evident effect on the fitness of male carriers.

Several modelling studies have examined the potential strengths and weaknesses of autocidal approaches (e.g., Gould and Schliekelman 2004; Bax and Thresher 2009; Thresher et al. 2013). Chief among the weaknesses are the need to stock large numbers of carriers (see below) and the desirability of high numbers of independently segregating copies in each carrier, to maximise the rate of spread of the genes. 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 the case in fish, it could significantly reduce the efficacy of the approach. Two ways to avoid this problem, both theoretical, have been suggested: incorporate the population-controlling construct into a “driven” genetic system (Burt 2003; Chen et al. 2007) and stock fish that carry several different constructs that bring about the same functional outcome, e.g., male sterility.

Comparison of approaches

Genetic options are attractive because they offer possibilities for control where none currently exist (i.e. most established invasive species), and because in theory they have several intrinsic advantages over conventional biological control: (1) they can be very effective; (2) they are (chromosomally) or potentially are (recombinantly) species-specific; (3) recombinant options can target one sex or a particular life-history stage in order to maximise efficacy or minimise damage to non-target species; (4) their effects are potentially reversible if something goes wrong; and (5) some recombinant approaches lend themselves to relatively quick and inexpensive modification to target different species, while retaining species-specificity for each (Gould and Schliekelman 2004; Bax and Thresher 2009; Teem et al. 2013). The last characteristic helps to spread the high-cost of developing autocidal technology across many species, in stark contrast to conventional biological control programs that must target each new species individually.

Table 1 Recombinant methods considered to date

Method	Description	Reference(s)
Lethal construct	Construct induces embryonic death of offspring. When homozygous results in sterility and is equivalent to a sterile male/female release	Thomas et al. (2000), Horn and Wimmer (2003), Phuc et al. (2007), Thresher et al. (2009), Harris et al. (2012)
Sex-specific lethality	As above, but male or female-specific; transmitted through male or female line	Heinrich and Scott (2000), Schliekelman and Gould (2000a), Fu et al. (2007, 2010), Ant et al. (2012)
Sex-specific sterility	Construct causes offspring of one sex to be sterile; transmitted through male or female line	Schliekelman et al. (2005), Thresher (2008)
Gender distortion (“daughterless” or “sonless”)	Construct causes offspring to develop as specified sex irrespective of sexual genotype	Hamilton (1967), Schliekelman et al. (2005), Thresher et al. (2005)
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 (2000b)
“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 (2004)
Mutual incompatibility	Construct is lethal when present in 2 or more copies (unless genes are identical)	
Engineered under-dominance	Construct is lethal when only 1 copy present (or more than one copy but genes are identical)	Davis et al. (2001), Magori and Gould (2006)

Modelling also indicates two other features of genetic pest control programs. First, Trojan Y and autocidal approaches are slow acting. The spread of the control modification is inherently a function of generation time, though it also depends on genetic approach used, stocking rates, fitness effects, and population structure (Bax and Thresher 2009). Even under optimal conditions, effective population control 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 and if a problem develops, there is adequate time to launch counter-measures, such as stopping the stocking program or releasing a second gene construct that effectively shuts off the first. On the minus side, the public and funding agencies may not support such long-term approaches, particularly if the final outcome is uncertain. The long timeframe may also result in natural selection against

the biocontrol agent if the genetic manipulations negatively impact fitness. Fitness effects could be direct (i.e., on sperm mobility), indirect (i.e., on growth rates) or mediated by selection for individuals that avoid matings with the biocontrol fish. Options involving sterile male or female releases (triploidy and sterile feral technology) and self-propagating agents are potentially much quicker, but the former at least require very high stocking rates. In that regard, most techniques require gene carriers to be stocked at high levels for a long time. Experience with insects (see Box 1) indicates that success with a sterile-release program requires that released sterile males outnumber fertile males in the target population by at least a ratio of 10 to 1. If the number of invasive fish in the target population is at the carrying capacity, sterile fish must be added in numbers that exceed the carrying capacity by tenfold to reach the desired ratio. For other approaches, minimum stocking rates equivalent to 3–5 % of annual mean natural recruitment are

indicated by most models, sustained for at least 5 generations. These rates could be substantially reduced, however, by combining genetic approaches with complementary pest management activities (see below). However, the need for sustained stocking also makes it very unlikely that the accidental release of a few carriers would significantly affect a species in its native range.

The strengths and weaknesses of different genetic options are summarised in Table 2. Of the options we consider, self-propagating recombinant agents (e.g., virally vectored immuno-contraception) are likely to be by far the most effective when released into a pest population, but, we suggest, are unlikely to be used because of the very high cost of their development and likely public opposition. Chromosomal approaches (triploidy and Trojan Y) have substantial advantages over recombinant techniques in that they are relatively inexpensive to develop, are based on existing and proven technology (though the fitness of YY carriers needs to be demonstrated, and is a critical unknown in the Trojan Y option), are likely to be publicly acceptable, and in most jurisdictions would probably not require legislative or policy changes specific to the technology. The Trojan Y approach could potentially be particularly effective, as relative stocking rates increase as stocked female carriers progressively compete over time with increasingly fewer wild type females (Teem et al. 2013; Thresher et al. 2013). However, chromosomal approaches overall are likely to be technically limited, among vertebrates, to fish and amphibians. They also allow only a limited suite of intervention strategies (essentially only female sterility and gender distortion), and have limited scope for enhancement. Recombinant approaches are potentially applicable to a wide variety of vertebrate pests, are likely to be generalisable among pests while retaining species-specificity, can be tuned to target particular physiological or life history vulnerabilities in the target species, and have the potential to have their efficacy enhanced through use of ancillary genetic approaches, such as incorporation into selfish gene elements or combination with growth enhancing constructs (increasing carrier male reproductive success relative to smaller wild-type males). However, at least in the early stages of the technology, recombinant autocidal techniques face uncertain public acceptability, still require considerable technical development for vertebrates, and in some jurisdictions may require

changes in legislation that has been designed and adopted specifically to limit, rather than facilitate, the spread of recombinant animals.

Adaptive management and the role of genetic options in IPM

IPM seeks to control invasive species by targeting weaknesses in their life histories using combinations of mechanical, chemical and/or biological controls guided by statistical modelling (Box 2). Because invasiveness often appears to be determined by specific combinations of local factors (e.g., low susceptibility of invaders to predation, disease or competition in the invaded habitats) that can vary with environmental conditions and pest population density (Li and Moyle 1981; Mooney and Drake 1986), an optimal IPM framework should be adaptive, along the lines described by Holling (1978). Ideally, an IPM program starts with well-defined management objectives and detailed knowledge of the pest's life history and role in the impacted ecosystem, then develops management strategies and sustainable actions, and finally, before these actions start, establishes a program to monitor performance indicators. Monitoring is critical in this context, as once intervention is underway, results of the monitoring program are used to evaluate the effectiveness of, and adjust if necessary, management strategies and actions.

IPM has been successfully employed to control numerous insect pests. Most programs to control pest fish focus on the use of a single technique, e.g., locally applied biocides, though complementary methods and continuous performance monitoring characterise management of the sea lamprey in the North American Great Lakes (Bergstedt et al. 2003) and common carp in lakes in Tasmania (Australia) (Inland Fisheries Service 2009) and have been proposed for management of carp in the American midwest (Weber et al. 2011; Sorensen and Bajer 2011). To assess the potential role of genetic options in such efforts, below, we approximate, model and then manipulate a scenario similar to that described in the American midwest to determine synergies between conventional and genetic options for reducing the impacts of common carp. Specifically, we combine the release of males carrying a female-lethal construct—a form of sex-ratio distortion—with physical removal of carp,

Table 2 Strengths and weaknesses of different genetic options for the control of invasive pest fish

	Chromosomal	Recombinant
Self-propagating	NA	<p>+</p> <p>Potentially very high efficiency</p> <p>Potentially applicable to a wide range of vertebrates</p> <p>Low maintenance costs after release</p> <p>–</p> <p>High developmental costs</p> <p>Species-specificity uncertain (particularly for viral agents)</p> <p>Public acceptability is likely to be low</p> <p>Legislative/policy impediments in some jurisdictions</p> <p>Introduction to native range may result in unintended extinction</p>
Sterile male/ female release	<p>+</p> <p>Technology available (triploidy) for fish and amphibians; applicability to other vertebrate taxa is unlikely</p> <p>Species-specific outside of naturally occurring hybridization cases (e.g., carp-goldfish)</p> <p>Public acceptability high</p> <p>Few legislative/policy impediments</p> <p>Low cost</p> <p>–</p> <p>Only males participate in matings</p> <p>High stocking rates required</p> <p>Reduced growth rate in some taxa, potentially resulting in low competitiveness and/or survival</p>	<p>+</p> <p>Prototype available for fish</p> <p>Potentially applicable to wide range of vertebrates</p> <p>Both sexes fertile and sterile</p> <p>Species-specific</p> <p>Potential recombinant options for increasing efficacy</p> <p>–</p> <p>Developmental work still required</p> <p>Moderately high cost to produce and maintain brood lines</p> <p>High stocking rates required</p> <p>Public acceptability uncertain</p> <p>Legislative/policy impediments in some jurisdictions</p>
Autocidal	<p>+</p> <p>Technology (Trojan Y) available in part (YY males); viability of YY females uncertain and likely to differ between species</p> <p>Applicable to fish and amphibians; applicability to other vertebrates unknown</p> <p>Applicable to live-bearing fish</p> <p>Species-specific outside of naturally occurring hybridization cases (e.g., carp-goldfish)</p> <p>Publicly acceptable</p> <p>Few legislative/policy impediments</p> <p>Low cost</p> <p>Efficacy is unknown, but potentially moderate</p> <p>–</p> <p>Sex ratio manipulation only</p> <p>Carrier fitness and competitiveness unknown</p> <p>Effects of autosomal sex modifying genes</p> <p>Effects of environmental temperature on sex ratios unknown</p> <p>Developmental work required, although basic technology for chromosome and gender manipulations available</p>	<p>+</p> <p>Efficacy?, but potentially moderate to high, depending on copy number</p> <p>Potential recombinant options for increasing efficacy</p> <p>Potentially applicable to wide range of vertebrates</p> <p>Alternative and complementary genetic strategies can be targeted at vulnerable life history stages/traits</p> <p>Alternative and complementary genetic strategies can be applied sequentially or simultaneously to increase efficacy</p> <p>–</p> <p>High developmental costs</p> <p>Developmental work still required</p> <p>Carrier fitness and competitiveness unknown and likely to vary depending on construct</p> <p>Species-specificity is likely to vary depending on construct</p> <p>Public acceptability is uncertain</p> <p>Legislative/policy impediments in some jurisdictions</p>

See also Dana et al. (2013), Hayes et al. (2013) and Sharpe (in press)

Box 2 Integrated pest management

Integrated pest management (IPM) was developed by Californian entomologists in the 1950s and 1960s in response to increasing concern about the overuse of synthetic pesticides and subsequent insect resistance (Perkins 1989). It sought to identify the best mix of chemical and biological controls based on an ecological understanding of the pest species in its local environment (often a particular field) and accepted that the goal was control and not total eradication. The approach was extended to include additional controls. It became part of US national policy in February 1972 with President Nixon directing agencies to establish IPM in all relevant sectors. In 1997 Perry Adkisson and Ray Smith received the 1997 World Food Prize for their leadership in developing IPM

There are three main components of IPM—prevention, monitoring and intervention—and (at least) three underlying beliefs. First, the emphasis is on control, not eradication. Second, understanding the ecology of the target species is important to success. Third, interventions causing minimum environmental harm (at low cost) should be considered first. Under this hierarchy, simple mechanical controls would be the first to consider, followed by biological controls and lastly synthetic pesticides. Where would genetic control fit in this hierarchy of desirable controls? Many scientists would place it third—after biological control due to its (to date) longer development time and greater cost; many (but not all) public interest groups might place it last due to a perceived risk of negative consequences due to a genetic modification. Focus group analyses to date are broadly supportive of genetic approaches to control invasive pests, but are also ambiguous due to considerable unknowns about the technology. Support is also likely to vary widely across jurisdictions due to attitudes towards genetic modification in general (Thresher and Kuris 2004; Fisher and Cribb 2005)

In the context of the genetic control of invasive pest fish, IPM as originally conceived has three key lessons: (1) Goals and objectives need to be clearly articulated. Is eradication or control the goal, and if the latter, what constitutes adequate control? Conversely, what is the acceptable risk to achieve this goal? (2) An ecological approach will be more successful than a “one-size-fits-all” approach. Understanding the ecology and reproductive biology/behaviour of the target species is essential to determine the mix, timing and application of management techniques. This may be especially relevant in patchy distributions spread over a variety of environmental conditions and population densities; (3) Monitoring is critical to the success of IPM. Monitoring the progress of an intervention will provide information that can be used to adapt further control efforts (or indeed cease particular efforts that are found to be ineffective or antagonistic)

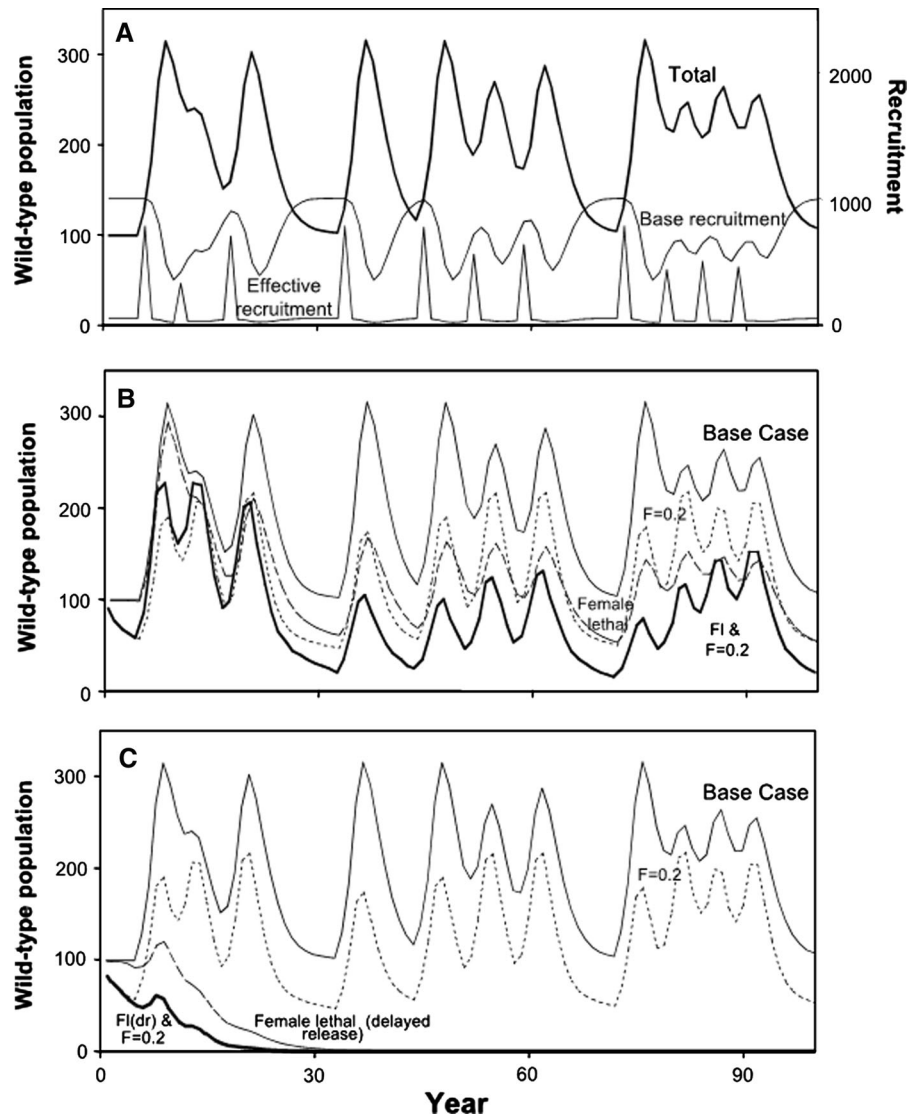
with and without adaptive changes in stocking strategies.

Characteristics of the modelled carp population are based in part on Bajer and Sorensen (2010), Sorensen and Bajer (2011) and Weber et al. (2011) and in part on generic carp characteristics (e.g., Koehn et al. 2000). In brief, the model assumes carp are well established, live to be up to 50 years and are extremely fecund. Recruitment, however, is sporadic, perhaps only occurring once every 5–10 years. This is thought to be a result of irregularly occurring winter-kills of small predatory native fish in the interconnected wetlands and shallow lakes. In years without winter-kills, mortality of carp eggs and fry can approach 100 % (Bajer and Sorensen 2010). In the absence of detailed information, we include in the model as simplifying assumptions that (1) density dependence is “moderate” (recruitment reaches an asymptote as carp population densities increase), (2) environmental (e.g., climate) effects on mortality rates and recruitment are negligible other than as a result of winter kills for the latter, (3) the natural mortality rate for juveniles and adults is constant at 25 % per annum and fish reach sexual maturity by age 5, (4) all adults are subject to harvesting without a size

bias, and (5) the carp population is closed. To simulate the effects of winter kills on recruitment, in most years new recruits (eggs and young-of-the-year) experience 90 % mortality in addition to the natural mortality rate; the combined first year mortality rate in those years is close to 100 %. At irregular 5–10 year intervals, however, this additional mortality is removed, i.e., young-of-the-year are subject only to the natural constant mortality rate. The model structure is deterministic and adapted from Bax and Thresher (2009), which details the model’s conceptual and mathematical underpinnings. This hypothetical simulation starts with a carp population of about 1300 adults and an equal sex ratio.

Basic carp population dynamics as simulated are shown in Fig. 1a. Base recruitment (essentially population fecundity) varies irregularly as a function of total adult population size and the density-dependent stock-recruitment relationship. Effective recruitment, however, is effectively zero most years, due to high levels of predation on eggs and fry, but peaks irregularly at close to population fecundity levels during years with simulated winter kills of small predators. Total adult population size varies slowly over time as a result of the irregular recruitment pulses, constant mortality rates

Fig. 1 Output of modelled population dynamics of common carp in a hypothetical American midwestern lake. **a** Base dynamics as simulated, showing total number of adult carp (*heavy line*), potential recruitment and effective recruitment, the last being recruitment after the effects of early egg and fry mortality. **b** Effect on long-term adult carp numbers (*base case*) of doubling the rate of natural mortality by non-selective fishing ($F = 0.2$) and of annual stocking of carriers of a female lethal construct at 5 % of natural recruitment, alone and (*dark line*) in combination. **c** As above, but with stocking deferred until released carriers are not subject to high levels of early mortality during non-winter kill years



and the long life span of the fish. This long-term trajectory we refer to as the population “base case”.

To assess the possible effects of genetic control on this base population, with and without complementary management actions (e.g., physical removal), we add male recruits carrying a female-lethal construct to the population, at a copy number of 8 independently segregating copies and a per copy additional mortality rate of 1 % per annum, i.e., the construct has a subtle pleiotropic effect on juvenile or adult survival. We test two alternative stocking regimes. In Scenario 1, carriers are stocked each year at 5 % of the initial natural base recruitment, and are subject to the same early mortality rates as the wild fish. In Scenario 2,

carriers are retained in a hatchery until they are large enough to escape the mortality due to the small-gaped native predators, and then stocked at 5 % of initial base recruitment. Scenario 2 was included as an obvious and cost-effective alternative to stocking out eggs and small fry in the face of a known extremely high level of early stage mortality. Impacts of the two scenarios on carp populations are compared with, and added to, those of a non-selective fishing regime with an instantaneous mortality rate of 0.2, i.e., twice the rate of natural mortality. The fishing effort equates to a physical removal rate of 18 % of the adults annually.

Results of the simulations are shown in Fig. 1b, c. By design, parameter values for Scenario 1 were

chosen such that neither the genetic option nor physical removal alone would eradicate the carp within the 100 years of the simulation. Eradication was defined by <1 adult female carp remaining. By the end of the simulation, each management option alone reduces the carp population by about 50 %, reflecting the high fecundity of the fish, its longevity and, in the case of the genetic option, effective stocking of carriers only at 5–10 year intervals. Combining the two management options, however, is more effective than either alone, reducing the carp population in Scenario 1 to less than 20 % of starting biomass by the end of the run (Fig. 1b). In this scenario, carp can be eradicated in less than 50 years by selective fishing that targets only non-carriers or by increasing fishing pressure for carriers and non-carriers alike to about 35 % per annum ($f = 0.4$). In the latter case, stocking carriers reduces the eradication efficiency, as the stocked fish prop up the number of females in the system. Alternative options (selective removal of females only, increased stocking rates to 50 % of natural recruitment, and selection of brood stock to eliminate the per copy mortality increase) each alone substantially depresses the carp population when added to Scenario 1 and reduces it to less than 0.02 % of starting biomass by the end of the simulation, but do not eradicate it.

The enhancing effect of complementary management efforts is similar in Scenario 2 (Fig. 1c), but the efficacy of the genetic option is considerably greater, eradicating carp from the system within 30–35 years when used alone, and in less than 25 years when combined with non-selective carp removal (Fig. 1c). The enhanced efficiency of the genetic option derives from annual stocking of the carriers and their overwhelming dominance of the recruiting populations in the year-classes subject to high rates of juvenile mortality (non-winter kill years). Enhancing the effort further by adding any of the modifications suggested above effectively eradicates carp from the system within 20 years.

Carp behavior, ecology and mechanism of impacts are inevitably more complex than captured in this model, and the impacts of management regimes will vary accordingly. However, the model highlights three main points in the context of an IPM approach to the problem:

1. There is considerable synergism in use of complementary management tools, e.g., genetics and fishing. In both scenarios, the whole effect of the two management actions is greater than the sum of the parts.
2. Detailed knowledge of pest dynamics and vulnerabilities allowed development of management actions that greatly increases the benefits, i.e., stocking carriers only after they had grown through the high vulnerability stage. The modification is simple, relatively inexpensive and easy to implement. Its use, however, is critically dependent on a sufficient understanding of the ecology and population dynamics of carp in the lakes, highlighting the potentially immense value of even a small amount of local knowledge.
3. The eradication effort can be further enhanced by learning and adapting efforts, by, for example, tracking carrier fitness to select out brood lines with high rates of pleiotropic mortality, better targeted and hence more efficient fishing efforts or selective removal of only some adults, e.g., removing wild type fish but leaving carriers in the system to breed.

Conclusions

For the vast majority of invasive fish (and other taxa), even those causing major ecological or economic damage, logistical considerations and costs prevent large-scale control (Whitten and Foster 1975; Simberloff and Stilling 1996; Follett and Duan 1999). Classical biological control and sterile male release programs have significant constraints on their application, including high developmental costs, potential high risks of direct and indirect collateral damage, irreversibility, and often only partial mitigation of pest impacts. In this context, genetic approaches are an attractive alternative because they are potentially generic (allowing developmental costs to be amortised across numerous target species), species-specific, reversible under a range of different scenarios, and potentially efficient to the point of possible pest eradication in invaded habitats. The choice of options depends on the specifics of the targeted pest (e.g., YY females may not be possible in some taxa), costs, the extent and dynamics of the pest population and, critically, public and political acceptability of the proposed management option (Norton 1983, 1988; Sharpe in press).

As shown above, IPM has enormous—perhaps enabling—abilities to facilitate the use of genetic technologies in controlling invasive fish. Nevertheless, several caveats need to be addressed. First, IPM is not ‘one size fits all’; rather, it is locale and species-specific. As such, IPM or any individual control approach may not be practical in all locations, e.g., locations where there is exceptionally high immigration. Conversely, each species and situation will have particular weaknesses or leverage points that can be targeted for more effective control. These leverage points may change as the population is reduced. A successful control program will almost always require a high level of local knowledge and the ability to monitor and adaptively manage the control or eradication. It is difficult to conceive of a situation where supplementing genetic control options with more established physical removal methods would not hasten control to an acceptable level. Each management action operates on a population already rendered vulnerable by the other control techniques. The combination of selective fishing and genetics might be particularly useful when targeting long-lived, r-selected species, such as the Asian carp (*Hypophthalmichthys* spp.), where the absolute reduction in time to eradication could enhance public interest in such a program, as well as adding value to control efforts based on fishing alone. Risk assessment, modelling and adaptive management should be essential components of any scheme that proposes genetic control (see Dana et al. 2013; Hayes et al. 2013); we suggest, in fact, it should be a prerequisite for any control or eradication program. Such programs should also include well designed monitoring protocols that will not only improve the control program, but also add to the accumulated knowledge in this developing area, informing subsequent control programs.

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