Evaluation of the use of Sterile and YY Male Release Programs for managing North American catfish (*Ameiurus nebulosus*) in Lakes Rotoiti and Rotorua

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Final Report

Executive Summary

- This report details the results of an investigation commissioned by NIWA on behalf of the Bay of Plenty Regional Council on the logistical feasibility of using either a sterile male or YY male release program to eradicate North American brown bullhead catfish in Lakes Rotoiti and Rotorua

- The investigation consisted of two phases: scenario testing and review of the relevant technical literature

- Scenario testing was done using a customized version of a generic fish population/genetics model (Bax and Thresher 2009). The model was customized for catfish based on published information in New Zealand, on information from studies in the catfish's native range where New Zealand data were not available, and on population sizes and structure provided by NIWA.

- In all scenarios tested, the sterile male release program outperformed release of YY males at the same stocking rates. Production of large numbers of YY males is also problematical.

- Only Lake Rotoiti was modelled, as population data for Lake Rotorua were not provided. Two estimates of adult population size were tested: 37,000 and 93,000. Assuming the population is at carrying capacity, eradication of catfish from Lake Rotoiti is modeled to take about 60 years at an annual stocking rate of 7,500 juveniles for the population of 37,000 adults, and 20,000 juveniles for 93,000 adults. Substantial suppression takes about 20 years.

- The modelling makes three key assumptions: that the catfish population is stable and at carrying capacity, that the rate of juvenile survival is very low, and that, consequently, sterile or YY fish released as large juveniles have a much higher rate of survival than the wild fish. Violation of these assumptions drastically alters the outcomes of the modelling, requiring much higher stocking rates to effect eradication.

- In the base model (assumptions listed above), annual removal of adult catfish of 30% of the population or more coupled with a sterile male release program significantly reduces the time to eradication. Selective removal (in which sterile or YY males are returned to the population, but wild fish removed) further improves the control program.

- There is an extensive literature on culturing North American catfish. The latter is based on the genus *Ictalurus*, which is closely related to *Ameiurus*, but much of the information should be directly applicable. Procedures of producing sterile males in catfish are well established and logistically straight-forward.

- Sufficient fingerlings of a large enough size for an effective stocking regime could be produced annually from as few as four females at the low population estimate and thirteen at the higher value, at relatively low effort spanning a four-six month period.

- Cost of production is difficult to determine in the absence of specific information on New Zealand hatchery facilities and availability. However, overseas data suggests fingerlings of a size suitable for stocking at the required low mortality rates can be produced at marginal (operating) costs of less than NZ$0.25 @, when mass reared.

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Background

Historically, invasive fish can be eradicated if they detected soon after establishment and if the invaded area and targeted population are small. At small scales, invasive fish can be controlled by use of biocides, physical removal, barriers and environmental modification, such as blocking access to spawning grounds. This window of opportunity, unfortunately, has probably passed for catfish (*Ameiurus nebulosus*) in Lakes Rotoiti and Rotorua. For the now established populations, there are three broad classes of possible control mechanisms: classical biological control, a sterile male release program, and genetic technology.

<u>Classical biological control</u>, involving the release of an exotic predator, parasite, or pathogen to control an alien species, has not been widely used against invasive fish, mainly because of difficulties in finding suitable, species-specific agents. Peacock bass (*Cichla ocellaris*), a generalist predator, have been successfully introduced in Florida (USA) to control other invasive cichlid populations, but have also impacted native fish species; a similar effort to use peacock bass to control stunted cichlids in Kenya was apparently less successful. 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. More recently, Australian scientists are vigorously pursuing the option of using Koi Herpes Virus (otherwise known as CyHV-3) as another option to control carp. The virus appears to affect only common carp (and its ornamental variant – koi), but release of the virus remains controversial because of concerns about how effective it will be, the effects of dead carp on water quality, and the possibility that the virus will mutate and attack native species (Kopf et al., 2019). To date and unfortunately, the only known catfish-specific disease (channel catfish herpes disease [Ictalurid herpesvirus 1]) does not affect *A. nebulosus* (Plumb 1989).

<u>A sterile male release program</u> is modelled after the widespread use of the technology to control insect pests. Released males are reproductively active, and hence mate with wildtype females, but none of the offspring survive. As a result, the pest population declines, more or less in proportion to the number of sterile males released. In the

case of insects, historically males were typically sterilized by gamma irradiation, which, unfortunately, also reduces their competiveness. As a result, successful sterile male release programs typically had to release vast numbers of sterile males, to the point where they outnumber normal, wildtype males by 100s to 1 (Dyke, et al., 2005). More recently, a suite of recombinant genetic techniques has been developed that cause male insect sterility without substantially affecting the male's ability to compete for matings (Harris, et al., 2012). Under such conditions, logistically more realistic stocking rates are possible (typically around 10 sterile males for every normal wildtype male in the targeted population). In either case, considerable effort also has to be expended to release only males. While both the radiation and recombinant methods also sterilise females, releasing females into the targeted population dilutes the impact of the released males and makes control of the pest population more difficult.

At this stage, only one attempt at release of sterilized males for purposes of pest control has been carried out in fish. Chemical sterilants have been used to laboriously produce sterile male sea lampreys for release into the St. Marys River (North American Great Lakes). Analysis of spawning success in the system following several years of releases indicated successful suppression of local ammocoete production (Bergstedt et al. 2003), but the effect of the releases on adult lamprey populations was unclear (Bravener and Twohey 2016).

Genetic technology provides another suite of potential control options. As early as the 1960s, entomologists observed that a mutant Y chromosome had apparently driven some insect populations to extinction, and suggested that genetics could be used to manage insect populations. 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 modelled a suite of options for pest control, highlighting their potential strengths and weaknesses from a theoretical perspective, while the technical feasibility of at least three recombinant methods (repressible male sterility, virally or parasitically vectored immuno-contraception, and female-biased sex ratio distortion) have been tested in the laboratory. Field trials have recently been undertaken in several countries to test the impacts of sterile recombinant males on insect pest populations.

Genetic options can be broadly divided into two categories: recombinant genetics (= genetic/genome engineering or genetic modification) and chromosome-manipulation. Despite recent developments in recombinant approaches to managing pest species (e.g., mosquitoes - Harris et al. 2012, Lacroix et al. 2012) and a successful trial of a sex-ratio-distorting, inherited construct in fish (Thresher et al., 2014a), the use of such technology remains problematical. Although "synthetic biology" has been hailed as a possible transformative technology for conservation, the field remains nascent, there have been no field trials to date, and possible applications remain contentious. In the absence of a well-established social license and international regulatory frameworks for such technology (Sustainability Council of New Zealand 2018), proposals for its use against catfish in New Zealand would likely be very controversial.

In contrast, chromosome manipulation in fishes is a well-established technology, inherently species-specific (aside from hybridization among closely related species, which would not be the case in New Zealand), and in the form of triploid fish, widely used non-contentiously globally for recreational fisheries and in aquaculture (Thresher et al. 2014b). Chromosome manipulation takes two forms: triploidy and Trojan Y.

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). For most fish species, the manipulations themselves are simple and inexpensive, involving either physical treatment (thermal or hydrostatic pressure) or the application of chemicals (e.g., cytochalasin B) and have advanced to the point of commercial application in many fish species. Because triploidy does not involve manipulations of individual chromosomes or genes, triploids are not widely considered by the public or regulators to be "genetically modified". Triploids are sterile because their odd number of chromosome sets results in dysfunctional development of fertilized eggs. This dysfunction does not prevent gonad development, but germ cells produced in the gonads either fail to develop or they develop into cells with abnormal chromosome numbers, 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 sperm, but their offspring die shortly after fertilization. Triploid females, however, have much smaller ovaries than diploid females, throughout life retain the characteristics of juvenile fish and rarely, if ever, spawn.

Trojan Y is based on the release of phenotypically female fish that have two Y chromosomes. The combination male-biases offspring sex ratios, all of which are either XY or YYs. Given an aggressive stocking regime, this bias could result in extinction of the pest population (Guitierrez and Teem 2006). A first step in producing YY females is generating YY "supermales", through selective breeding and the use of hormones to sex-reverse juveniles. These YYs are dosed with oestrogens or cold shocked as fry to convert them, theoretically, into functional Trojan Y females. In practice, even in the species for which it works best, the system can be inefficient. In Nile Tilapia, both hormones and irradiation result in only about a third of the progeny developing as YY females; the rest are intersex or supermales (Karayucel et al. 2003). YY females have also been produced in brook char (*Salvelinus fontinalis*) for a control program in Idaho (USA) (Kennedy et al. 2017), but few details are available regarding survival rates or fertility. Overall, the viability and fertility of YY females in fishes is highly variable and often poor (Thresher et al. 2014b). An alternative, though less efficient control program can be based on the YY supermales themselves, the offspring of which are all XY males. Supermales often grow larger than XY males, are more aggressive and can outcompete normal males for breedings (Hamilton et al. 1969; Kirankumar and Pandian 2003), but otherwise reproduce like normal males.

Evaluating the options for managing catfish in Lake Rotoiti

Selected options

As a result of discussions with NIWA, two options were selected for consideration: a sterile male release program because it already has social licence and could be quick and cheap to get off the ground, and release of YY "supermales". As the latter is a variant on extant chromosomal manipulations in fish, it is also likely to be socially acceptable, and could be based on existing studies of chromosomal manipulations in catfish (discussed below). Evaluating the two approaches proceeded in two steps: (1) modelling the possible efficacy and logistical constraints on their deployment in Lake Rotoiti (there was insufficient data to model the Lake Rotorua catfish population), and (2) a review of the available technology to produce sterile and YY males in catfish.

Scenario testing

To do a first pass evaluation of whether the release of sterile or YY males at logistically feasible rates could significantly impact the invasive catfish population, the population was simulated using a customized variant of a deterministic, generic fish population dynamics/genetics model described by Bax and Thresher (2009). In brief, the age-structured model simulates births, mortality, sex ratios and chromosome frequencies in a freely interbreeding population. Numbers of individuals in each age class are determined using discrete-time population equations that incorporate parameters for births at the start of each time interval (in this case, years) and instantaneous (= annual) rates of natural mortality. Harvest/fishing mortality rates can also be incorporated into the model structure. The number of new individuals born each year is determined by a Ricker stock-recruitment relationship. Stocking is assumed to be continued annually until there is a less than 1% chance of a surviving female in the targeted feral population; beyond this, the stocking alone sustains a pest population, which is deemed undesirable.

Population parameters for NZ catfish are based primarily on Patchell (1977). To simulate population dynamics in Lake Rotoiti, the model was parameterized as follows:

- An stable estimated population size of 186,000 fish, of which two ratios of juveniles to adults were applied – 80% and 50%, resulting in target adult populations of 37,000 adults and 93,000 adults, respectively. These values were provided by C. Baker (5 Feb. 2020).
- a 50:50 female-to-male sex ratio,
- 50% sexual maturity at an age of 2 years for both sexes and 100% sexual maturity at 3 years,
- a maximum age (99% mortality) of 8 years,
- an instantaneous (annualized) mortality rate (m) of 4.9 for wildtype juveniles (= 0.6% annual survival) and 0.65 (52% annual survival) for all adults, based on Sinnott and Ringler (1986).
- 90% survival (m = 0.1) of stocked juvenile males

The effects of varying the first and the last two assumptions, in particular, are discussed below. The model further assumes these parameters do not change as population size varies, and no density-independent environmental effects on recruitment, mortality rate or carrying capacity. The last set of assumptions simplifies the model outputs without significantly changing the outcomes. The Ricker stock-recruitment relationship was assumed to reflect a carrying capacity (zero population growth) when the population exceeded roughly 50% of its maximum. The model was run on yearly time steps for a total of 100 years.

Results are shown in Figure 1. In all cases, a sterile male release program is more effective than a YY male release program at the same stocking rates. At the lower value for the adult population, eradication within 100 years requires annual stocking of about 7,000 fry, the time to eradication roughly halving if stocking rates are doubled. The figure increases to 18,000 fry per year at the higher estimate of adult catfish in the target population. The basic population trajectory consists of a relatively steep decline followed by a long tail, prior to termination of stocking. The steep decline reflects the depressing effects of a stocking program on population

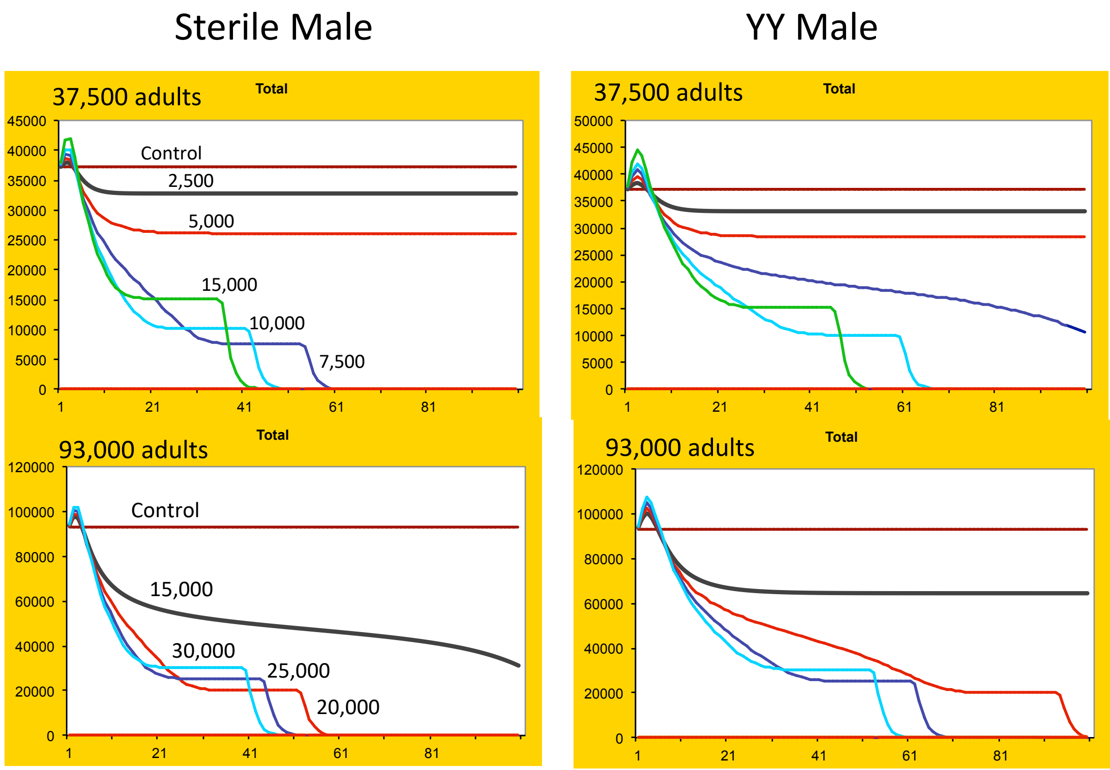


Figure 1. Population trajectories for low and high estimates of catfish abundance at different annual stocking rates and 90% post-stocking survival, for the sterile male and YY male options

fecundity, whereas the long tail reflects the extra effort needed to drive the number of females down to less than a 1% probability of a single female remaining. This endpoint is chosen as conservative.

The Key Assumptions

The model makes three key assumptions.

Assumption 1. The catfish population is stable and at or near carrying capacity. Recent information (C. Baker, 30 March 2020) indicates the population is recently invasive, with recruitment likely to be density-independent (that is, recruitment for an individual female is not being depressed as a result of, for example, competition for food by a population that has reached its growth limits). The model can be re-run using this assumption, but a possibly simpler approach is to estimate the rate at which the invasive population increases each year and then calculate the number of juveniles needed to be stocked to stop that increase. For example, if the population doubles each year, then all things being equal stopping that increase requires half of the breeding male population to be sterile. This figure can be calculated, as an example, as follows:

- Adult population 37,000, 50% male = 18,500 males
- 50% reduction requires 18,500 sterile males to match wildtype males
- Assumed age-specific mortality rates as above (0.6% annual survival as juveniles, 52% annual survival as adults)
- For simplicity, assume maturity occurs at age 3, continues for 5 years (until age 8) and that all mature age classes contribute to the recruitment in proportion to their numbers in the adult male population. Under those conditions, 18,500 sterile males in the breeding population requires annual recruitment at age 3 of 17,626 males.
- At 90% survival of stocked males during the juvenile stage, the required stocking rate required each year to insert 17,626 sterile 3 year-old males into the population is 10,308.

The analysis above is simplified by assuming the population size is static over the original stocking period, but illustrates the approach. The population is depressed proportionately by any stocking rate higher than 10,308. In practice, the rate at which the catfish populations in the lakes are increasing could be difficult to determine and, if rapid, could make this approach logistically impractical, but it could be a useful piece of information upon which the Council could base decisions on management options. <u>The rate at which the population is increasing or decreasing is a critical piece of information, and determines the nature of the stock-recruitment relationship used in scenario testing.</u>

Assumption 2. The survival rate of stocked juveniles is 90%, as compared to 0.6% for wild fry and juveniles. There is no specific size/age at which catfish males can be stocked into the lake. The decision depends on the cost of rearing the fry/juveniles and their subsequent impact on the targeted population. In principle, stocking small fry into the lake is not likely to be cost-effective. If the males are stocked as newly hatched fry and hence subject to the same mortality schedule as wild catfish fry, eradication within a century and at an assumed population size of 37,500 adults requires stocking 50 million fry each year. In practice, hatchery managers always rear fish to a larger size, exactly in order to avoid the extremely high mortality rates suffered by smaller fish. The literature suggests that in its native range, catfish longer than 10 cm are subject to very low mortality rates. Anecdotal information on-line suggests this size in *A. nebulosus* can be reached in as little as 4 months post-hatching (https://www.monsterfishkeepers.com/forums/threads/bullhead-growth-rate.21946/) although growth in field conditions can be slower (e.g., Raney and Webster 1940, Patchell 1977). The model assumes this equates to a 90% survival rate, which is likely to be conservative. Increasing the survival rate further, to 99% for example, has relatively little effect on modelled scenarios.

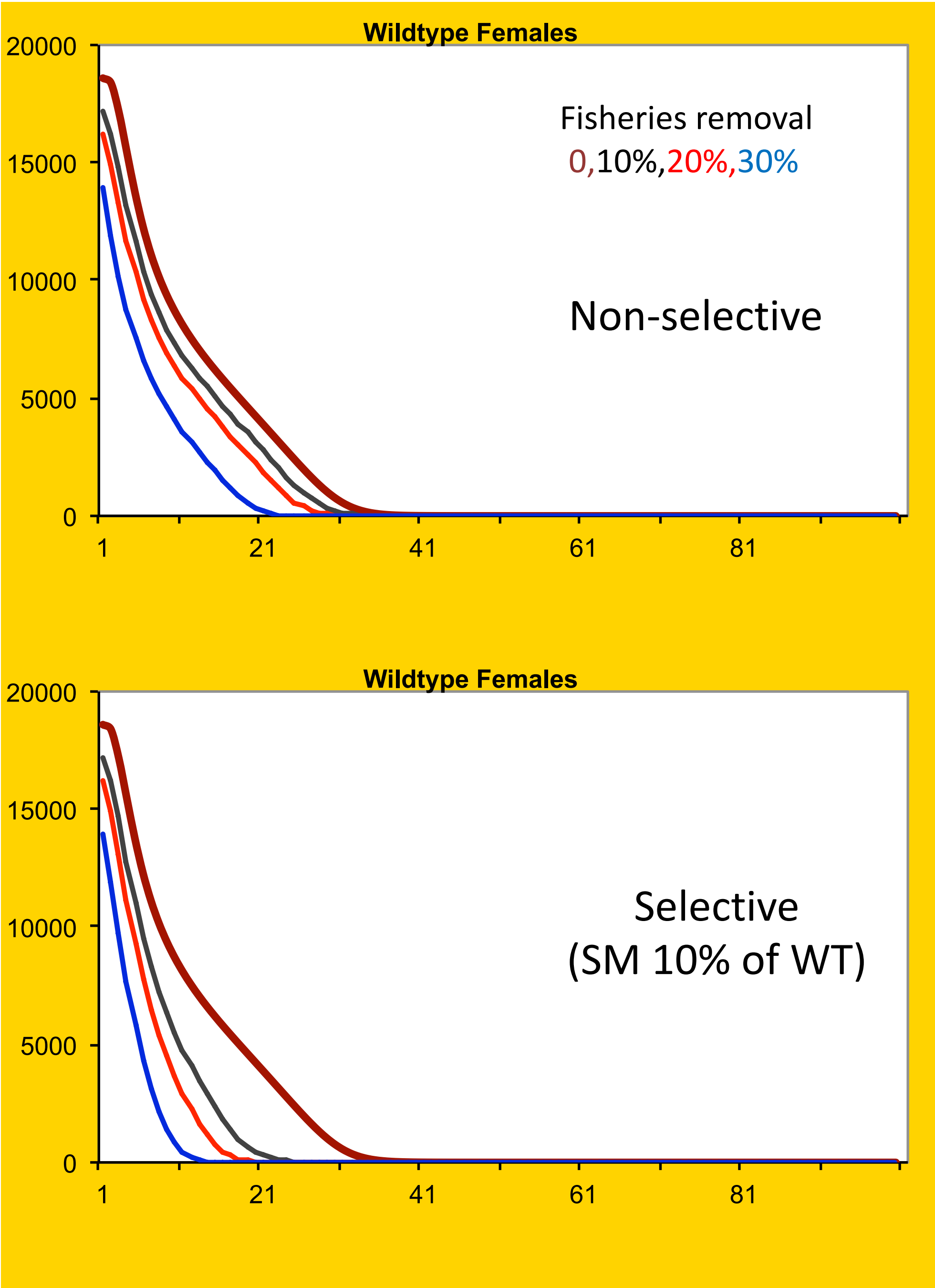
Assumption 3. Post-hatch survival of wild catfish fry and juveniles is very low. The model assumes 99.4% of the wild fry and juveniles die each year. This figure is based on sparse information on age-specific mortality of *A. nebulosus* in its native range (Sinnott and Ringler 1986) and is almost certainly optimistic. In its native range, small catfish are subject to predation by a wide range of piscivores that have co-evolved with the species. Even so, predation rates appear to decline steeply once the juveniles reach a size of 10 cm, by which size the sharp dorsal and pelvic spines of the species that make it unpalatable have hardened up. In New Zealand, very few piscivores attack small catfish. Patchell (1977) reported that in the Waikato River, only large longfin eels (*Anguilla dieffenbachi*) were known to consume large numbers of juvenile catfish, while also noting that the species was at that stage, and presumably since then still is, rare in the system. Nothing else appeared to pose a significant predatory threat to the juveniles. Longfin eels are present in Lake Rotoiti (C. Baker, pers. comm., 30 March 2020), but given the overall decline in the species abundance in New Zealand (Parliamentary Commissioner for the Environment 2013) and their very low densities in the lake (C. Baker, pers. comm.), predation on catfish by piscivores in the lake is likely to be low. Such a scenario could account for the apparent rapid increase in catfish in the lake. If so, it has a large impact on the number of stocked sterile males required to effect population control. The structure of the model is such that the lower the rate of wildtype fry and juvenile survival, the larger the impact of stocking large juveniles and consequently the lower the stocking regime required to affect control. If wild catfish fry and juveniles survive at a rate equal to that of the stocked males (90% per year), then eradication within a century using either the sterile male or YY male options requires sustained stocking of around 5 million males every year.

Integrated management of catfish in Lake Rotoiti

Whether the catfish population in the lake is increasing or stable, reducing the growth or size of the targeted populations appears to be a high priority for any integrated management plan. Reducing spawning success by release of sterile males in the catfish could be effectively combined with a program reducing adult numbers (by, for example, netting or ghost trapping) in order to minimize rates of population growth and drive the population towards eradication. The smaller the target population, the greater the impact of a quantum of released sterile males on population fecundity.

The effect of continued netting of catfish in the lake can be assessed using the models. Only the sterile male approach was run, as under all scenarios it outperforms the YY male release option. The effect of different adult removal rates on a sterile male release program (7,500 stocked male juveniles per year, at an assumed 90% survival and 0.6% survival of wild juveniles) is shown in Figure 2. Two options were modelled: non-selective removals (sterile male and wild type adults removed in proportion to their abundance in the population) and selective removals (removals target wildtype fish with sterile males released; for the model, 90% of caught sterile males are assumed to be released). Under the non-selective option, removing 30% of the catfish population every year reduces by about a third the time required to achieve effective eradication (less than 0.01 female remaining). If mostly wildtype fish are removed, the impact is greater as the selectively increases the proportion of sterile individuals in the remaining male population; under that scenario, a 30% removal rate reduces the time to effective eradication to just over 10 years. Selective removal requires a means of identifying stocked males. Possibilities include pit tagging (e.g., Bodine and Fleming 2014) or, perhaps more simply, clipping the adipose fin, along the lines used in many programs to mark stocked trout (see numerous on-line sources). Equivalently, a removal program that targeted juvenile small catfish, if possible, would accomplish both selective removal (all small juveniles would be wildtype fish) and depress population fecundity, further speeding up an eradication program. Numerous trap designs exist that target small fish, including bullhead catfish, but their impacts on native species would need to be determined before widespread application in the lake.

Figure 2. Effect of removing adults on the number of remaining females for a population of 37,500 adults when combined with a sterile male release program of 7500 males/yr at 90% survival



Summary of Scenario Testing

The modelling suggests several points. First, both a sterile male release program and a program based on YY males can, in theory at least, eradicate catfish from the invaded lakes. Second, in all cases, at the same stocking rates the sterile male release approach is more efficient than one based on YY males. Third, the time to eradication, not surprisingly, depends on the rate at which carriers are stocked into the lake; in almost all cases, however, eradication takes more than a decade and at what might be realistic stocking rates, more often 30-60 years. Significant suppression of the pest populations, however, can occur in two decades. Fourth, a critical parameter is the rate of juvenile mortality; the higher this mortality rate, the greater the gain in efficiency from stocking larger sterile or YY male juveniles.

"Essentially, all models are wrong, but some are useful"
George E.P. Box

Like all such models, those above involve a set of simplifying assumptions and best guesses in the absence of detailed biological information about catfish in Lakes Rotorua and Rotoiti. Their usefulness, in a George Box sense, lies in two things. First, they broadly outine the utility of possible control options prior to, and may help inform, decisions about investment in either of the options. Second, they help identify aspects of the biology of the target species that are most in need of firming up. If desired, a future study could undertake a sensitivity analysis of the assumptions and guesses, to determine which uncertainties have the largest effect on the outcomes and hence which areas would best be focal in order to improve the performance and "real world" applicability of the model. At a first glance, however, there appear to be three priorities that could help inform decision-making by managers: refining estimates and determining the rate of change of population sizes for the catfish, determining the rates and sources of age-specific catfish mortality in the lakes, and developing options for selective removal of wild type individuals.

Technical feasibility

Sterile male and chromosomal approaches are mature technologies, well studied in fishes because of the desirability of mono-sex populations in the aquaculture industry. Single sex populations preclude the possibility of in-pond breeding, explosive population growth and "stunting" by cultured fish. Males are usually preferred because they do not "waste" production by investing heavily in large gonads and eggs. In this regard, NZ initiatives to use either sterile or YY males as pest control options benefit greatly from well-established aquaculture industries for North American catfish. *A. nebulosus* itself is not cultured, but there is a huge literature and industry based on the culture of its close relatives in the genus *Ictalurus*. The methods described below, in that regard, are more or less routine for most finfish studied.

Sterile males

The conventional method to produce sterile males in fish is triploidy, followed by hormonal treatment. The former usually involves "shocking" early stage, developing eggs with either temperatures (usually high temperatures) or hydrostatic pressure, which forces retention of the second polar body following fertilization, resulting in 3N individuals. Hormonal treatment causes all genotypes to develop into functional males (or females, depending on the hormones used). For a useful review of the methodology, see Dunham (2011). In *Ictalurus* catfish, high frequencies of triploids can be produced using cold shocks (4ºC. for 60 minutes starting 5 minutes after fertilization) (Wolters et al. 1981), heat shocks (40-43ºC for 1-3 minutes starting 80-90 minutes after fertilization) (Bidwell et al. 1985), or pressure shocks (8000 psi, for 3 minutes starting 5 minutes after fertilization) (Goudie et al. 1995). The last is now routinely used in catfish aquaculture (R. Dunham, pers. comm.). Masculinization of the triploids can be done by feeding the fry trenbolone acetate for 60 days immediately following yolk sac resorbtion, at doses of 50-150 mg/kg of dry food (Galvez et al., 1995).

An alternative approach to producing sterile triploids involves crossing a tetraploid adult with a diploid one. All progeny are triploid, without the need for the procedures outlined above. The viability and fertility of tetraploids in fishes varies widely. The production of tetraploid catfish fry was reported by Goudie et al. (1995), with the reported intent to determine if they could be used as brood stock. Results of the efforts were never published, however, and subsequent studies on tetraploids as brood stock consistently refer to success in only a few taxa (notably salmonids), suggesting that the approach was not successful.

Recent studies suggest another approach for producing sterile male fish that may be less time consuming and applicable to catfish. In early fish embryogenesis, two types of cell lineages develop. The large majority of cells develop into somatic cells, which essentially constitute the animal's body; a very small number of cells, however, develop into germ line cells, which proliferate in the gonads (which themselves are formed by somatic cells) to produce sperm and ova in males and females, respectively. Genetic developmental studies show that disrupting the development of the primordial germ line cells results in, first, gonads that are otherwise normal, but lack sperm or ova, and second, that all such fish are seemingly normal males, albeit sterile (Zhou et al. 2018). Disruption of the germ line cells is accomplished by micro-injecting an antisense oligonucleide (a morpholino) into the developing egg. This is a standard, non-transgenic technique for shutting down the effects (knocking-down) specific genetic sequences, in this case targeting a gene critical for migration of the primordial germ line cells. The technique used in the experimental study is labour-intensive, and designed only to test the functionality of the knock-down. However, the method lends itself to mass application by means of mass transfection methods such as electroporation, in which thousands of embryos can be modified rapidly and inexpensively. In that regard, Wong and Zohar (2015) show that using the germ line knock-down approach, sterile male zebrafish can be mass produced by immersing the newly fertilized eggs in a water bath containing the morpholino mixed with a "molecular transporter" compound ("Vivo"). The targeted gene sequence and the methods used are widespread in fish and fish genetic studies, respectively, and are likely to be applicable with some modification to catfish. If so, it could constitute a very simple and cost effective way to generate large numbers of sterile male fry to on-grow in hatcheries prior to field release.

YY Males

YY supermales have been produced in a variety of fish species by using steroids or cold shock to produce XY females and then crossing them with normal XY males: the resulting progeny are 25% XX females, 50% XY males, and 25% YY males (e.g., Scott et al., 1989). Crossing YY males with hormonally induced XY females produces broods that are 50% YY males and 50% XY males. YY adults can also be produced by androgenesis, in which irradiation is used to destroy chromosomes in eggs and then the eggs are heat shocked following fertilization to cause duplication of the single viable chromosome present in the egg. The resulting offspring are YY and XX, the phenotypic sex of which can be manipulated using hormonal treatments (typically methyltestosterone to produce males, oestrogens [e.g., diethylstilbestrol] to produce females) or temperature shocks.

In *Ictalurus* catfish, hormonal treatments have been used to produce XY females (Goudie et al. 1983), and back-crossed with normal males to produce YY males (Dunham 2011). The latter were fertile and when mated with normal females produced all male (XY) progeny. YY males have also been produced in *Ictalurus* using pressure-induced androgenesis, but the offspring sex ratios for most individuals, when crossed with normal females, were less than 100%, suggesting an effect of the treatment on the sex-determination system (Goudie et al. 1995).

<u>Efficient use of YY males to manage catfish in NZ requires that all stocked individuals be YY supermales. The current state of the technology does not easily allow this option.</u> The only method guaranteed to produce all YY male progeny is crossing YY males with YY females. Thus far, fertile "normal" YY females have only been produced in two species, Nile Tilapia (Karayucel et al. 2003) and brook trout (Kennedy et al. 2017), despite work on a number of fish species. In both cases, the families involved have relatively labile sexual development, which possibly accounts for the success in producing YY females. YY female *Ictalurus* catfish have been produced, but are severely reproductively compromised (Dunham 2011). Consequently, although YY males have been produced in catfish, the methods used do not lend themselves to mass production. Progeny survival following androgenesis is usually very low (ca. 1%) (Dunham 2011), such that even if supermales could be

used as sperm donors (for which there are no reports in the literature), the low survival rates limit their use for mass producing stockable all-male fingerlings. For the alternative approach, crossing a YY male with a hormonally-induced XY female produces only 50% YY males. Use of androgenesis to mass produce male progeny, following hormonal treatment, produces 50% YY males and 50% XX males. For either approach, release of only YY males requires the male progeny of the crosses to be screened for genotype prior to release, such that XY and XX males, respectively, can be excluded from the stocking program. There are no reports of obvious external differences between the male genotypes in catfish and thus far no described karyotypic, DNA or protein differences that might be used in a rapid screening process. Without a process to cost effectively remove XY males, at best the number of stocked individuals would need to be doubled in order to meet target stocking rates of YY males. More fundamentally, stocking XY or XX males along with YY males dilutes the impact of the supermales on population sex ratios, and effectively renders the approach non-viable.

Husbandry

An extensive literature and experience base exists for culturing North American catfishes. The latter is based on *Ictalurus*, but many of the methods developed would be applicable to *A. nebulosus*. Methods for mass producing catfish are described by Tucker and Hargreaves (2004) and Engle and Stone (2014). The model suggests effective control could be achieved by stocking about 7,000 male fingerlings per year at the low estimate of population size, if they had a post-stocking survival rate of 90% and wild type survival is only 0.6%, and 18,000 at the higher estimate. The size of the stocked fingerlings required to achieve that survival rate needs to be determined empirically for the Lake Rotoiti population, but is likely to be on the order of 10 cm. The scale of the effort required to sustain this stocking rate can be estimated from available literature sources as follows:

- Fecundity of a large female catfish 2,500 – 6,000 eggs (Patchell 1977), assuming only a single group of ova are matured at time of stripping; for simplicity, assume 4,000 eggs

- 66% immediate survival following pressure shock to induce triploidy (Goudie et al. 1995)

- 50% mortality during the 4-6 month hatchery period prior to release

The last is guesswork at this stage, but probably reasonable. The parameters indicate roughly 7,500 fingerlings of a size suitable for stocking could be produced from the eggs obtained from four females. Progeny of thirteen females would be required for the higher population estimate. The effort involved would likely be comprised of less than 1-2 week's effort in stripping brood stock and applying heat shocks to eggs to induce triploidy, a subsequent month-long period feeding post-yolk sac larvae live food before weaning them on to hormone-treated dry food, a several month, relatively low intensity effort to feed and maintain the developing fingerlings, and then a final, relatively short effort to tag fish to be stocked, if desired.

Cost of production of 12 cm catfish fingerlings, excluding infrastructure cost and the cost of producing fry, has been reported at approximately US$ 0.04 (Ligeon et al. 2004) and US$ 0.08 (Engle and Stone 2014) each when mass produced (i.e., tens of thousands of fingerlings). How these figures would translate into production in NZ is uncertain, based on variables such as cost of access to hatcheries and marginal labour costs of producing a relatively small number of fingerlings.

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