A potential methodology for biocontrol of lionfish (*Pterois* spp.)

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Abstract. The control of invasive species require a special approach for their management. Aquaculture production technologies may provide viable alternatives for their control. We propose the production and propagation of YY males as a biocontrol method for use with lionfish. This approach uses reproductive and genetic intervention to produce all-male populations; the males having two Y-chromosomes. Mathematical models developed by other researchers support the possibility of driving fish populations to local extinction through releases of YY males. In this article we provide a simplified description of the methodology that will be involved of producing an all-male population of lionfish with two Y-chromosomes, and discuss major challenges facing implementation of this technology.
Key Words: aquaculture production technology, controlling invasive species, daughterless technology, sex-skewing methods, Trojan Y chromosome, YY males.

The lionfish invasion. Lionfish, *Pterois* spp., are venomous tropical scorpionfish native to the Indian and western Pacific Oceans that are now present in the western Atlantic Ocean (Kimball et al 2004). There are speculations about how lionfish were first introduced into the western Atlantic, however, recent studies point to one most likely vector: aquarium releases in south Florida in the USA in the 1980s (Morris & Whitfield 2009). Since the first reported sightings off the coast of Dania Beach, Florida in 1985, lionfish have expanded their population range increasingly rapid into the Caribbean, the Gulf of Mexico, and the western North Atlantic (Schofield 2009). In the USA, lionfish have been observed as far north as the Rhode Island coast in New York, but have only established as far north as North Carolina, probably due to inability to survive cold temperatures (Kimball et al 2004). This invasive species is capable of persisting in a variety of habitats, including reefs, mangroves, and estuarine areas (Jud et al 2011), and have been found down to 1,000 feet deep. They have not many known predators in the Atlantic Ocean, and they have a high reproductive rate. Additionally, they are generalist consumers who prey on a wide variety of organisms. Studies have shown that lionfish may have negative effects on native fauna through competition for food sources and predation (Lönnstedt & McCormick 2013). They are predators who feed on a variety of prey, including juvenile reef fish, and implicated of reducing populations of reef fish (Green et al 2012; Cerino et al 2013). Herbivorous reef fishes as well as grazing invertebrates are a necessity to maintaining the balance of the coral reef ecosystem, as they reduce the amount of macroalgae on the reef. Any imbalance in herbivory could lead to seaweeds outcompeting corals or interfering with coral recruitment (Albins & Hixon 2013). Additionally, lionfish populate mangrove habitats, which are essential nursery grounds for reef fishes and numerous other species (Barbour et al 2010).

Current management attempts to control the lionfish invasion. Lionfish may have strong negative effects outside their native range; therefore, it is important that they are effectively controlled from their non-native range. Management techniques are limited to the removal of lionfish through recreational fishing, including organized tournaments. There are even organizations which receive donations in order to fund lionfish fishers. Studies suggest that fishing, starvation, a specific disease, or native biotic resistance are the only pathways which can lead towards eventual lionfish eradication (Albins & Hixon 2013). While derby and recreational fishing have slightly reduced local populations of lionfish, the effects of recruitment from areas where there is no fishing have not been evaluated (De Leon et al 2013). Local removal of lionfish populations may slightly alleviate their predatory pressure on local populations of economically and ecologically important species. However, more analysis is needed to address the impacts of lionfish reproduction, recruitment, and immigration (Frazer et al 2012).

An alternative management technique – skewing sex ratios using YY males. A novel management practice is essential for ecosystem-wide eradication of lionfish. We propose the production and propagation of YY males as a biocontrol method for use with lionfish. This approach uses reproductive and genetic intervention to produce males with two Y- chromosomes. Fish scientists and aquaculturalists historically produced transgender fish of specific gender to increase their production (Yamamoto 1967; Purdom 1993; Mair et al 1997; Liu et al 2013). The production and propagation of YY males is more ethically acceptable since it does not produce transgenic individuals. Transgenic individuals result when genes, the hereditary units of living things, are inserted into an organism from another species to give the species improved or new traits. Mathematical models support the effectiveness and theoretical possibility of driving fish populations to local extinction through releases of YY males (Gutierrez & Teem 2006).

Understanding the details of the YY male biocontrol technique. Most vertebrate species examined, including fishes, have an XY sex-determination system. Their sex is determined by a pair of chromosomes, in which case the presence of a Y chromosome will result in a genetic male. However, although their internal genetic sex is fixed, in many fishes their phenotypic (i.e., expressed) sex characteristics are said to be 'plastic or malleable'. In other words, an individual fish may be male genetically but functionally be a female. Fishes can undergo natural sex changes influenced by their own hormones, hormone levels in their food and environment, or other outside stimuli like temperature. With the right stimulus, therefore, fishes with typical male XY chromosomes can develop into fully functional females. This results in a different phenotype (physical traits) than genotype (genetic blueprint). As such, researchers can induce a desired mating event through the right combination of breeding and rearing techniques (Figure 1).

For example, under natural circumstances crosses between females with genotype XX and males with genotype XY will produce progeny that typically consist of 1/2 females and 1/2 males. However, in the laboratory taking advantage of the 'plasticity' of fish for sex expression, males with two Y chromosomes can be produced. This is easily accomplished by feeding young fish, before they sexually differentiate, with a potent feminization hormone like estrogen or derivatives. The adult, feminized offspring, can be crossed with wild type males with resulting progeny being 25% females (XX), 50% males (XY) and 25% males having a YY genotype (Figure 1). Mating with a male having a YY genotype will result in all-male offspring (i.e., XX + YY = XY + XY). The analogous Y chromosome is also referred to as a "trojan" sex chromosome. In aquaculture practice males with the YY chromosomal complement are commonly referred to as 'YY males' or 'Supermales'. These YY males can still breed with normal females, however, they will be daughterless – only spawn male offspring – because they will always pass on a Y chromosome (Figure 1). The YY males can also be feminized with estrogens and produce YY brood stocks for further use. These YY females can also be released into the wild and possibly produce 1/2 YY males that can also assist in skewing the sex ratio.

How YY males can control lionfish populations. The use of YY males as a biocontrol is designed to affect a target species by changing the sex ratio of males to females in the wild. To do this, YY males are released into the wild population. Theoretically, they will breed with wild females and produce only male offspring, resulting in a sex ratio skewed towards males (Figure 1). Initially, the introduction of YY males will not noticeably affect the lionfish population. However, after several generations and introductions of YY males, the population should diminish and the native ecosystem can begin to recover. This biocontrol technique is a long-term endeavor as it will take many generations to see the effects on the sex ratio; although the time required is unknown. Also, it is important to consider the need for continual release of YY males. The YY genotype will not persist in the wild population without continuous release of YY males, so its effects on the sex ratio will begin to reverse if releases cease.

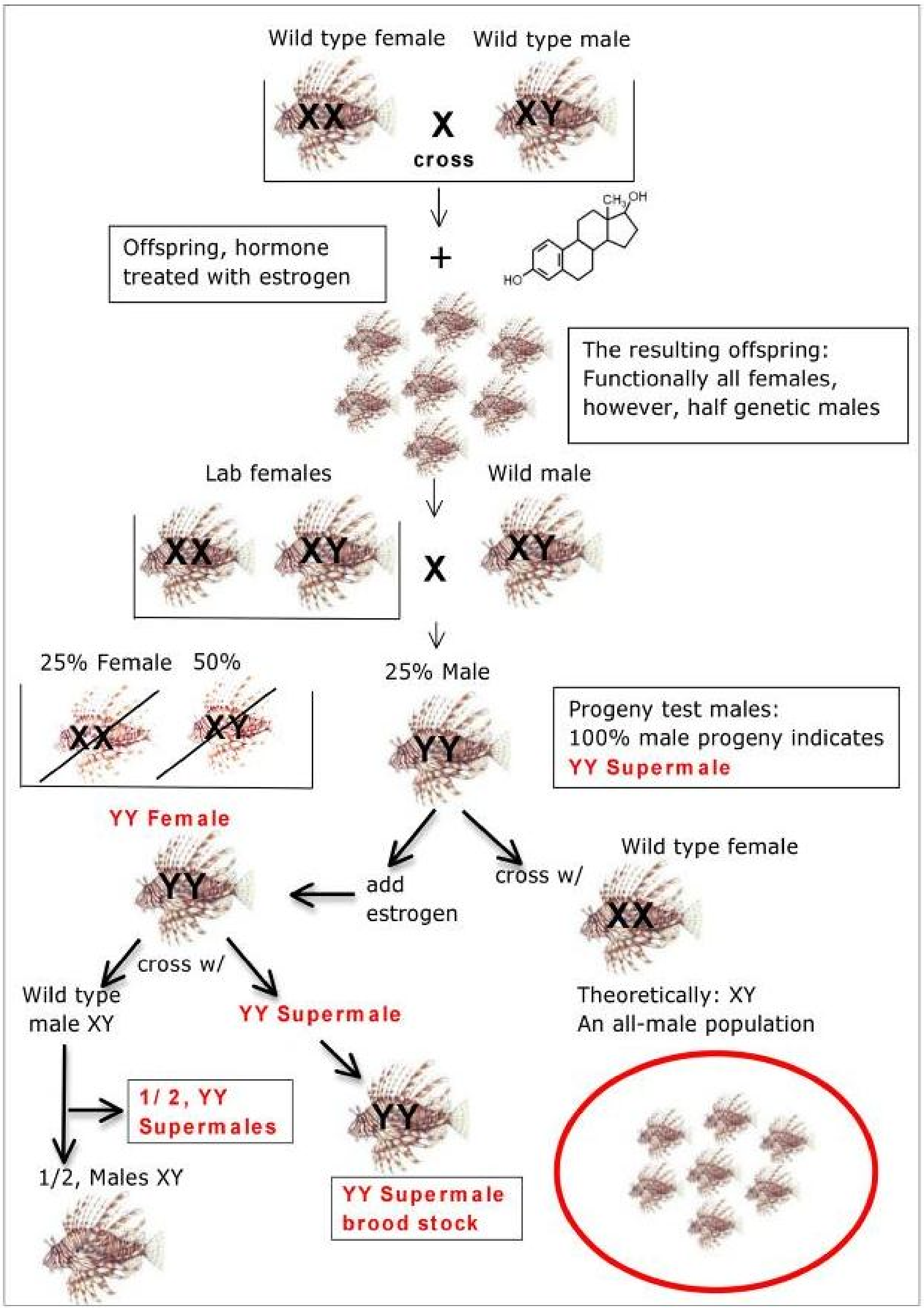

Figure 1. Theoretical mating scheme of lionfish having XY sex-determination system and the outcome on the production of YY males.

Challenges and outlook. The main purpose of this document is to bring to light and provide readers with an introductory synopsis and explanation of the technique of producing YY male fish that can be applied for control of invasive lionfish. The production of YY males has been given little attention in the environmental realm but has great potential to help control invasive species. However, like with any novel technique or tool, one starts with a basic premise some readers will investigate further, and others will not.

Further, when factual evidence shows there is further need for exploration, or no turn-key technique is yet available, these constraints produce a wide range of viewpoints, so consensus among scientists and regulators in the foreseeable future is unlikely. In part, uncertainties open up questions of fundamental and practical relevance to interested stakeholders and assessing them involves extrapolating from the available evidence and drawing conclusions that are inherently subjective.

We recognize that validation of a technique is required before acceptance into practice. To this end we would encourage further studies, in particular those that are of immediate concern and have practical value. These important challenges are beyond the charge of this document and require separate in-depth treatment elsewhere. Below is a summary of these important studies to be conducted:

- lionfish are popular fish in the aquarium and those who have tried to spawn them have been unsuccessful. The production of YY males will require full control of their life cycle, especially artificial breeding and raising of the larvae. Ironically, restrictions have been recently imposed in the USA for those interested in breeding lionfish. Recent progress of reproductive and larval physiology of fish and persistent efforts to develop larval rearing techniques in other marine species will greatly facilitate the artificial induction of spawning and development of rearing techniques of larvae in lionfish;
- the theory behind production of YY males is based on an XY sex-determination system wherein the sperm determines the sex. The XY sex determination system is found in humans, most mammals, some insects and plants, and in most of the fishes for which it has been determined. In lionfish the sex determination pathway is unknown. If lionfish had a ZW system of sex determination, where the egg determines the sex, the idea will be to produce all-female populations instead of males, skewing the population towards females, achieving population control.

The production of YY males on a large scale is first viewed as a complex and unattainable process. However, the production of YY males has begun to be implemented for the commercial production of all-male tilapia fish populations; a necessity since female tilapias mature quickly and stunt. Tilapia fish have become one of the top-ten fish consumed in the USA and the world, therefore requiring cost effective means of mass production of millions of young-of-the year fish. Ironically as well, the sex determination system in tilapia has not been well studied, and in those that have been studied is believed to have a ZW system of sex determination. Nevertheless, large-scale commercial production of tilapia YY males has been achieved by the proposed method for the production of YY male lionfish described in this document. The same approach for the eradication of invasive Asian carps (e.g., *Hypophthalmichthys nobilis*, *H. molitrix*, *Ctenopharyngodon idella*) using the YY male technology was suggested by Teem & Gutierrez (2010). Most recently, the technique has also been proposed and implemented for eradication of brook trout (*Salvelinus fontinalis*) populations where they are not desired (Schill et al 2016).

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