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Sterile 'Judas' carp—Surgical sterilisation does not impair growth, endocrine and behavioural responses of male carp

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ABSTRACT

Use of 'Judas' fish to betray the locations of conspecifics is a powerful tool in management of invasive pest fish but poses a risk of contributing to recruitment. Our aim therefore was to generate surgically sterilised male common carp (*Cyprinus carpio*) and test whether they readily assimilate into wild populations, retain sexual behaviour and successfully betray the locations of feral carp. Male common carp were surgically sterilised ($n = 44$) adopting a two-point nip technique, using either a haemoclip, suture or electro cautery to tie each of the testicular ducts about 2.5 cm cranial to urogenital sinus—retaining all of the glandular testis tissue. Observed survival (95%) and success (>70%) rates were relatively high. Plasma steroids (11-keto testosterone and 17β-estradiol) were quantified by immunoassay. A subset of sterile and control male fish ($n = 7$ each) were implanted with radio-transmitters and released into Lake Sorell (50 km^2) and their ability to betray the location of feral carp was assessed by radio tracking and targeted fishing. There was a statistically significant difference in 11-keto testosterone and 17β-estradiol levels over time ($P < 0.05$), but not between the sterile and control groups within each sampling time ($P > 0.05$), implying that surgery did not compromise the animals physiologically. The sterile Judas fish integrated well into the population—behaving similarly to control Judas males and assisted in the capture of feral carp. The study marks a significant breakthrough in the management of this pest fish with potential adoption to the management of other pest fish globally.

1. Introduction

The Judas technique is a method that has proven successful in controlling, and in some cases eradicating a range of terrestrial and aquatic invasive species (Taylor and Katahira, 1988; Cruz et al., 2009; Taylor et al., 2012). The method relies on tracking individuals within a population to reveal population level behaviour, such as aggregative behaviour, to facilitate selective removal (Taylor and Katahira, 1988). Particularly, radio-implanted 'Judas fish' have been successfully employed to manage (e.g. Baijer et al., 2011) and eradicate (Taylor et al., 2012 and Diggle et al., 2012) carp. The technique is known to directly increase the effectiveness of fishing by signalling when and where an aggregation is occurring and in predicting the broad scale seasonal and inter-annual habitat preferences that can be exploited for capture and reproductive sabotage (Taylor et al., 2012). Whilst use of Judas fish of either sex is feasible, a preferential use of male Judas has proven effective for the common carp on count of reducing spawning risk and more ready betrayal of feral fish locations (Diggle et al., 2012). For instance male Judas fish contributed to about 63% of the total fish caught in Lake Crescent, Tasmania, from the time of the technique's introduction in 1997 to eventual eradication of carp in 2008 (Diggle et al., 2012). Given the efficiency of these Judas males, it is deemed impossible to achieve control let alone eradication in their absence (Diggle et al., 2012). Whilst the current practice of deploying mature Judas males is a powerful tool, it has inherent risk of contributing to recruitment resulting in disastrous environmental and economic consequences. For example, they (fertile Judas males) were attributed to all recruitment of carp in Lake Sorell, Tasmania in 2009, setting-back the eradication program by an estimated 7 years (Diggle et al., 2012). Largely driven by this compelling need to prevent repeat and inadvertent contributions of Judas fish to recruitment we asked the question if surgically sterilised male Judas fish could be generated? If so would they assimilate into the populations seamlessly and reliably betray the locations of the feral animals?

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Although surgical gonadectomy or castration has been carried out in many species of fish (e.g. Brown and Richards, 1979; Schulz et al., 2012; Baker et al., 2013) most of these are limited to short term physiological and behavioural observations with little emphasis on long term survival and their use in fisheries management. The survival and sterilisation success rates reported thus far also appear very low with even complete gonadectomy resulting in regeneration and discharge of gametes (Underwood et al., 1986). Barring the early attempts, the field has remained relatively fallow perhaps owing to limited early success or lack of feasible application. However, a recent study on koi carp has shown that use of analgesics can alleviate surgery related pain (Baker et al., 2013). But little else is known about the long-term consequences of surgical removal of gonads on the physiology, fitness and behaviour of the operated animals.

In contrast, it is well known that gonads produce steroid hormones in response to a plethora of environmental and social cues and stimulate changes in cellular metabolism, resulting in a variety of effects on important life history traits. Particularly, gonadal steroid such as testosterone, 11-keto testosterone (11-KT) and 17-β-estradiol (E2) have critical consequences not only for reproduction, but their trade-offs with immune function and growth exert concomitant effects on other life-history traits such as survival and the organism's overall fitness (e.g. Grossman, 1985; Mills et al., 2010). Conceivably, surgical removal of gonads would significantly compromise the physiology, behaviour and fitness of the target animals, compromising the utility of such sterilised Judas fish in managing pest fish such as the carp in Australia.

In Australia, the introduction and spread of non-native fish has been identified as a major factor in the classification of threatened status for 42% of native fish species (Lintermans, 2004). The introduction of the common carp (*Cyprinus carpio*) is of particular concern because it has become the most abundant freshwater fish species in the country (Koehn, 2004). Worldwide, freshwater systems are recognised as being particularly vulnerable to the impacts of biological invasions (Cohen and Carlton, 1998; Sala et al., 2000). Therefore eradication is recognised as a key option for mitigating the effects of invasive species and facilitating ecosystem restoration (Genovesi, 2005; Lorvelec and Pascal, 2005; Convention on Biological Diversity 2007). In this context developing targeted and efficient eradication programs centered on selective removal methods such as the 'Judas fish' with a low impact on native species, are essential.

On the basis of previous observations including anatomy of the fish gonads and the critical role they play in overall fitness of the animals we hypothesised that (1) removing a small tubular portion of the testis would effectively block sperm discharge with minimal compromise to the physiology and behaviour of the fish and (2) securing the severed wounds would prevent their regeneration and re-establishment of the tracts, thus providing a reliable and perpetual sterility.

Here we demonstrate for the first time that it is not only feasible to surgically and reliably sterilise carp, but that the techniques had no measurable impact on the growth, physiology and behaviour of the animals. Further we demonstrate that these sterile individuals when deployed as Judas fish assisted in betraying the locations of the conspecifics and their subsequent removal in a Tasmanian lake.

2. Methods

The aim of the project was to determine the feasibility of surgical sterilisation of carp with a view to deploy sterile Judas-fish for assisting capture and eradication of feral carp from Lake Sorell, Tasmania. An ability of the technique to block the expression of milt/sperm was used as a key indicator of success. Sperm quality, gonad morphology, and circulating levels of two steroid hormones—(E2) and 11-KT—were monitored to detect subtle effects. Growth indices were used as surrogates to monitor potential side or ill effects of the treatments. The movement, distribution and assimilation of the sterile individuals were evaluated as surrogates for behavioural responses using radio-telemetry and ARcGIS techniques. All experimental procedures were approved by an Animal Ethics Committee of the Department of Primary Industries, Parks, Water and Environment, Government of Tasmania, Australia.

2.1. Experimental fish and their maintenance

The surgical sterilization experiments were carried out on male common carp (*C. carpio*). The animals (0.5–0.9 kg and 260–375 mm) were captured at Lake Sorell and individually tagged with 2 floy (T-bar) tags—one on each side of the fish—for subsequent identification. A PIT tag was also inserted under anaesthesia (0.3 mg/l AQUI-S®) into the dorsal musculature to further assist individual identification. The fish were then transported to a secure holding facility and held in a 26,000 L capacity circular tank at ambient photoperiod and temperature adopting standard protocols. The water quality, including temperature (2–15.3 °C), dissolved oxygen, pH (6.6–7.2), total ammonia (0–0.4 mg/L), and nitrates (0.00–0.05 mg/L) was monitored regularly. The experimental fish were fed daily with 6 mm-artificial salmon pellets (*Skretting-RK 3111-7*). Prior to experimentation, fish were acclimated for 3 weeks to recover from netting and transport stress. A subset of the males ($n = 16$) was randomly allocated to serve as controls.

2.2. Sexing fish

Because the population of carp in Lake Sorell consisted of a single year-class (2009–10) just coming into puberty, it was difficult to sex fish based on external morphology—attempt to sex based on secondary sexual characters i.e. the shape of vent, tubercles and gamete discharge were unreliable. Therefore sexing was carried out using ultrasound using a portable device (Esaote, MYLabFive) fitted with an 8 MHz linear transducer.

2.3. Surgical sterilisation

A set of photographs showing select steps in the surgical procedure is presented in Fig. 1. Holding fish in a cradle suspended in the anesthetic bath was found to be most efficient and convenient. Predominantly sutures ($n = 17$) or clips ($n = 24$) were used to tie the severed ends of the sperm ducts (tubal ligation). Cautery was attempted in only two individuals using electro cautery surgical unit (CGS Electro 70, 1.9 MHz) as per the manufacturer's instructions; in one individual both the testicular lobes were secured using electro-cautery and in the other, one lobe was electro-cauterised and the other lobe clipped.

The basic surgical operations were carried out following standard protocols that have been previously established for radio-transmitter implantation of carp (Macdonald and Wisniewski, 2003). Prior to surgery fish were starved for a day and all surgical operations were carried out in a make shift outdoor-operation setup, under anesthesia (0.3 mg/l AQUI-S®). Following deep anesthesia, the fish were held upside down in a cradle with gills submerged in water with just the belly exposed above the water level. A ventral midline surgery approach was adopted, wherein scales were first removed and local anesthesia (0.5% bupivacaine) was administered via a hypodermic injection. Then an incision of about 3 cm (about 1–2 cm cranial to the anal pore) along the ventral midline was made and the body cavity held open with the help of retractors. The surgery visualisation was assisted by a head