

# Development and deployment of sterile 'Judas fish' to assist carp eradication in Lake Sorell, Tasmania - surgical and chemical sterilisation

Jawahar G Patil, John Purser and Andrew Nicholson • August 2014

FRDC Project No 2012/039





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# Foreword

The primary objective of this project was to develop reliable techniques for sterilisation of male carp, so they could be employed as Judas fish to assist in the ongoing carp management program and eradicate carp from Lake Sorell and hence Tasmania.

The invasive European carp *Cyprinus carpio* has been a significant environmental risk to the Tasmanian freshwater ecosystem since their discovery in two relatively large lakes Crescent (> 23 ha) and Sorell (> 50 ha) in 1995. Several ecological and economic concerns and logistical limitations have precluded the use of drastic control approaches such as draining or mass application of chemicals/poisons. Thus an integrated approach centred on physical removal has remained the mainstay of the management approach despite the challenges of physical eradication.

The efforts to date have resulted in eradication of the species from Lake Crescent. Central to this success was the adoption of Judas technique in 1997, where in radio-transmitter implanted adult males routinely betrayed the location of carp aggregation in the lake—facilitating targeted fishing and efficient removal of large number of carp. For example, the technique contributed to about ~ 63 % of the total carp capture in Lake Crescent since the introduction of the technique in 1997 to the eventual eradication of carp in the lake in 2007. The Judas fish became a valuable tool not only to target carp aggregations but also to understand carp behaviour, movement and habitat choice i.e. they enabled delineation of inter-seasonal and inter-annual patterns of carp movement and habitat choice in response to changes in lake water level and water temperature (Taylor et al 2012). The resulting knowledge was effectively utilised for fish removal, identifying life cycle vulnerabilities, recruitment sabotage and resource rationalization. However, very unfortunately these very Judas males contributed to the mass spawning and recruitment in Lake Sorell in 2009, setting the program back several years. This triggered an urgent need to sterilise the Judas males so that they would continue to assist the eradication program, whilst negating the risks of recruitment.

The sterilisation techniques developed in this project and particularly the demonstrations of sustained sterility over long periods of time (over 10 months) are unprecedented. This reliability allowed the uptake/ deployment of these animals as sterile Judas fish in Lake Sorell. The observations to date suggest that the sterile Judas fish behave similar to control Judas males and have already assisted in the capture of wild/feral carp. The use of the already deployed sterile Judas fish and the reserve pool of sterile fish generated in this project will continue to assist in the ongoing carp eradication efforts at Lake Sorell. Based on the experiences of eradicating carp in Lake Crescent, the importance of sterile Judas fish particularly during the final stages of the eradication cannot be emphasised enough.

In summary the achievements of the projects are unprecedented and commendable and will remain integral to the Tasmanian carp eradication program, with likely applications elsewhere.

John Diggle

Director of Inland Fisheries, Tasmania

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The report is dedicated to the memory of late Paul Donkers, who was a devoted worker and a staunch advocate of carp eradication in Tasmania.

# Abbreviations

11-KT	11-keto Testosterone
AMC	Australian Maritime College
ANOVA	Analysis of Variance
B <sub>0</sub>	Maximum Binding
BLK	Blank
BSA	Bovine Serum Albumin
CASA	Computer Assisted Sperm Analysis
Dec	December
E2	17β-Estradiol
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
Feb	February
FRDC	Fisheries Research and Development Corporation

FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
H 11-KT	High 11-keto Testosterone level population
IFS	Inland Fisheries Service
Jan	January
K	Condition Factor
L 11-KT	Low 11-keto Testosterone level population
LH	Luteinizing Hormone
LHS	Left Hand Side
Mar	March
NBF	Neutral Buffered Formalin
NCMCRS	National Centre for Marine Conservation and Resource Sustainability
No.	Number
NSB	Non-Specific Binding
PBS	Phosphate-Buffered Saline
RHS	Right Hand Side
SE	Standard Error
TA	Total Activity
UTAS	University of Tasmania
VCL	Spermatozoa Curvilinear Velocity

# Executive Summary

In a world first, the project successfully developed reliable procedures for surgical sterilization of male carp and demonstrated that these when deployed as sterile ‘Judas’ fish are as competent as male ‘Judas’ carp in betraying the locations of feral populations thus assisting their capture. Significantly, the sterile Judas fish will mitigate future risks associated inadvertent contributions to spawning and recruitment. The sterile Judas carp are now an integral component of the ongoing carp eradication program at Lake Sorell, Tasmania.

Following the discovery of the carp *Cyprinus carpio* in lakes Crescent and Sorell in 1995, the Inland Fisheries Service (IFS) decided to eradicate both the populations. While, the Lake Crescent population has been successfully eradicated, the Lake Sorell population has been lingering, owing to inadvertent spawning and recruitment in 2009 —contributed by Judas carp, which are otherwise central to the eradication strategy at the lakes. Responding to the crisis and urgent need to mitigate future recruitment risks this project explored chemical and surgical sterilization options with a view to replace male ‘Judas’ carp with sterile ‘Judas’ carp.

The primary objective of the project was to develop reliable sterilization technique/s that did not significantly compromise the behavior of the sterilized individuals. Behavioral integrity of the sterilized individuals when fitted with radio-transmitters and deployed as Judas fish (i.e. to assimilate and integrate seamlessly into the wild populations) was/is critical to betraying the locations of aggregations allowing targeted fishing and removal of feral populations.

As only male fish were the target of sterilisation experiments, it was essential to unambiguously and non-invasively sex the fish prior to experimentation. The secondary-sexual characters were not reliable nor were the fish expressing gametes at the time of experimentation. Therefore utility of ultrasound to sex the fish non-invasively was explored and demonstrated to be reliable.

The chemical sterilization approach involved administration of Superlorin®, a commercially available form of deslorelin, a GnRH agonist. A single dose (9.4 mg deslorelin/animal) of Superlorin® administered to feline and canine animals routinely causes temporary sterility up to 12 months or more. However, in carp administering up to three fold higher doses (9.4, 18.8 or 28.2 mg/animal) did not appear to have significant effect — did not completely block sperm expression or impair sex steroid hormone levels) — during the study period. These observations are in stark contrast to those in higher vertebrates, suggesting an as yet unknown mechanism of deslorin action on teleost GnRH and the reproductive pathway. Nonetheless, the results may have been confounded or masked by asynchronous maturity of individual males in the cohort.

Three different surgical sterilisation procedures were attempted—the first employing sutures and the second metal clips to tie the severed ends of sperm ducts and a third Essure techniques originally developed as female contraceptive in humans. Suturing and clipping were used as preferred techniques for surgical sterilization, wherein distal tubular section of each lobe was tied or clipped at two places (2-3 cm apart) and the intervening tissue excised. The approach completely blocked sperm production in about 77% of the individuals operated. The post-operative mortality rate was low (4.5%) and the surgery did not significantly impair the growth and levels of key steroid hormones, indicative of little or no physiological and behavioral impairment. Individuals that were not successfully sterilised (~23%), showed remarkable testicular repair i.e. re-establishing connections of single or both lobes to urogenital sinus and expressing milt. In a single case the testicular lobes had fused with intestinal tract and the individual expressed milt via anal pore. Despite being least invasive the Essure technique was difficult to perform without the aid of sophisticated endoscopic imaging and was discontinued.

Most significantly, a fraction of the sterilised individuals were fitted with radio-transmitters and deployed into Lake Sorell to serve as sterile Judas fish. Field observations to date suggest that the Judas fish have remained sterile, integrated well into the lake populations and have already contributed to capture of feral carp. All indications are that the sterile Judas carp will not only continue to betray the locations of feral

carp but will also assist in advance forecasting of events such as spawning aggregations. The sterile Judas carp are now an integral part of the IFS's carp control programme, whose utility for eradication of carp from Tasmania will only increase as females in the current cohort (2009) begin to mature whilst their population size is decreasing. The sterile Judas carp and the techniques developed, as part of this research project can be useful elsewhere where there is a similar need.

The chemical sterilisation approach needs significant follow up investigation to confirm its feasibility to effectively sterilise carp using a range of high and low doses of Superlorin® than those tested in this study. It is also possible that Superlorin® is not a direct antagonist of GnRH, but acts via separate intermediate molecule that are specific to one or more multiple downstream pathways to GnRH. In this context the carp may provide an excellent teleost model in discovery of as yet unknown pathways/modulators of vertebrates reproduction.

For the purposes of deploying sterile Judas fish for carp control, we recommend the adoption/use of surgical approach as the preferred method of sterilisation. However, we also recommend that the surgical techniques be further refined with a view to increase success rate of sterilisation. Non-surgical approaches such as the Essure may provide more humane options to sterilise carp, but require more detailed review and investigations.

## **Keywords**

**Common carp, *Cyprinus carpio*, steroid hormones, Superlorin, testis, tubectomy, Radio-tracking, Ultra sound.**

# Introduction

Carp is a major feral pest in Australia. Development of control options has consistently been identified as a high research and management priority. When carp were discovered in lakes Crescent (2365 ha) and Sorell (4770 ha) in 1995, the Inland Fisheries Service (IFS) commenced a program to eradicate – both populations. While, the Lake Crescent population has been successfully eradicated, the extant Lake Sorell population continues to threaten the State’s aquatic biodiversity and the premier trout fishery.

The presence of an endemic galaxiid in lakes Crescent and Sorell prevents the use of poisons and draining the lakes is not possible. The IFS is therefore committed to a continued campaign of containment and eradication through fishing. Effective containment was achieved by placing a weir with a series of mesh screens at the outlet of the lakes. Mesh sizes are small enough to prevent eggs and juveniles leaving the lakes. In addition the Lake Sorell is closed to public including anglers to reduce the risks of translocations.

Eradication through fishing in medium to large lakes is not a quick or easy process but has been proven to be successful in Lake Crescent. Over the years the effectiveness of the fishing operations in the two lakes was increased by using a variety of gears - fyke nets, seine nets, gillnets, traps, backpack electrofishing, boat electrofishing and combinations of these. Initially the fishing gear types were used somewhat randomly. Later, the IFS started targeting habitat favoured by carp and adapted fishing techniques based on previous catch rates and experience. In 1997, radio tagged male fish were first used as tracker or “Judas” fish to identify aggregations and to help understand carp habitat preference and behaviour. Detected aggregations were targeted using fishing techniques most applicable to the situation.

The use of radio-tracked fish (Judas males) increased the effectiveness of the fishing by signalling when an aggregation was occurring. This technique of targeted fishing assisted by Judas fish contributed to about 63% of the total fish caught in Lake Crescent, from the time of its introduction in 1997 to eventual eradication of carp in the lake in 2007 (declared in 2009). The movement of radio-tracked fish also suggests that carp in the lakes can adopt resident or mobile behaviours and therefore have varying degrees of vulnerability to capture (Taylor et al 2012). While mobile fish can be caught in any aggregation, resident fish rarely move from their habitat unless an aggregation is developing. Interestingly, there also seems to be a group of fish that are consistently caught at above the expected rate, leading to a larger number of high multiple recaptures than expected. This varying vulnerability could have serious implications for removing the final fish from the lake. Radio tracking resident and mobile fish is necessary to target the remaining population.

In effect the eradication effort has become a race to fish down the population rapidly before they have a chance to spawn and add further juveniles to the lake. Temperatures in the Tasmanian lakes are at the minimum end of those required for spawning by carp, so spawning is typically restricted to shallow margins of the lake in the summer, preferably during periods of stable or rising water levels. Relatively, Lake Crescent had low water levels, and water levels were manipulated to maintain low and falling water levels during the spawning period and thus reducing the risk of spawning events. Water level manipulation is less feasible for Lake Sorell, where input cannot be controlled and where there is a much wider range of marsh habitat available. However this was addressed by fencing off marshes and more recently deploying barrier nets to restrict access to these spawning sites.

Asynchronous maturity of male and female carp—males maturing 2-3 years earlier than females of a cohort in the lakes—has also been repeatedly exploited to assist eradication of carp in the Tasmanian lakes. Typically mature adults become more mobile during spawning season and hence more susceptible to capture particularly by passive gears. This vulnerability has consistently allowed to fish down males of a cohort in advance of females reaching maturity, hence contributing to reproductive

sabotage i.e. reducing the risk of spawning and recruitment.

Restriction of spawning has not been entirely successful. Uncontrolled spawning events took place in Lake Sorell in 1995/96, 1997/98 and 2000/01, 2006/07 and 2009/10. The last (2009) spawning occurred despite successfully fishing down the previous cohorts to very low numbers (estimated < 50). The spawning coincided with unusually wet summer and high lake levels that exposed a much larger spawning habitat. Restriction to spawning access was further confounded by resource and effort fragmentation between the two lakes—Lake Crescent was then not yet confirmed to be free of carp. Notoriously, all spawning contribution came from male Judas carp mating with the remaining females in the lake.

In the absence of Judas technique, it is deemed impossible to achieve control let alone eradication, prompting the need to develop techniques to sterilize male carp with a view to prevent repeat of the inadvertent recruitment events of 2009. Juveniles and the maturing males from most recent (2009) spawning have been fished intensively using a combination of strategies and gears resulting in substantial reduction in population. The sterile techniques and Judas carp developed in this project will allow the continued use of the Judas technique whilst negating the risk of inadvertent spawning.

# Objectives

The objectives of the project were to:

1. Refine the surgical sterilization (cauterization) technique;
2. Trial two additional sterilization approaches—tubal ligation and Essure;
3. Optimise superlorin mediated chemical sterilization;
4. Evaluate the success of the techniques, including regular assessment of blood testosterone levels, spermiation and fertility of the animals;
5. Record gross anatomical and histological changes following surgical and or chemical sterilization of select animals; and
6. Deploy successfully sterilized carp with radio-transmitters in the lake and map their behaviour —ability to integrate into the population and assist in location of aggregations — general and reproductive.



# Methods

The aim of the project was to determine the feasibility of chemical and 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 techniques 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— 7B-Estradiol (E2) and 11-keto Testosterone (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 using radio-telemetry and ARcGIS techniques.

A summary of each of the methods is presented below.

## Experimental fish and their maintenance

Both chemical and surgical sterilization experiments were carried out on male common carp (*Cyprinus carpio*). The animals (0.5–1kg) were captured at Lake Sorell and individually tagged with 2 floy (T-bar) tags—one each on each side of the fish—for subsequent identification (Figure 1). 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 at Salmon Ponds, and held in 26,000 L capacity circular tank at ambient photoperiod and temperature adopting standard protocols. The water quality, including dissolved oxygen, pH, total ammonia, nitrites, and nitrates 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 a minimum period of 3 weeks to recover from netting and transport stress.

## Sexing the fish

Because the population of carp in Lake Sorell consisted of a single cohort (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 attempted using ultrasound using a portable device (Esaote, MYLabFive) fitted with an 8 MHz linear transducer. A pilot experiment to determine the accuracy of the technique was carried out on carcass of 67 freshly caught carp. Each fish was sexed using the ultrasound and the diagnosis verified by dissection.

## Chemical sterilisation

Chemical sterilization was undertaken using a GnRH agonist Suprelorin® 12 implants (Virbac Pty Ltd, Australia) containing 9.4 mg of the GnRH agonist deslorelin (Figure 4) as per the instruction of the supplier. In all 80 maturing male common carp (500-1000 grams) were used for the experiment. Each fish was given a serial number (1–80) of which sixteen fish each were randomly assigned and treated with sham (positive control), 1, 2, or 3 Suprelorin® 12 implants or no implants (negative control). All inserts were in the dorsal musculature and carried out under anaesthesia (0.3 mg/l AQUI-S®). The fish were held in a single tank to avoid tank effects and for security reasons.

## Surgical sterilisation

Three different surgical sterilisation procedures were attempted. The first employed sutures and the second metal clips to tie the severed ends of sperm ducts (tubal ligation) and a third Essure techniques originally developed as female contraceptive in humans was also attempted. In a single individual each electro cauterization and a combination of electro-cauterisation and metal clipping techniques were

used. Tubal ligation with either suturing (n = 17) or clipping (n = 24) was used as the preferred technique with a single individual receiving a combination of sutures and clips.

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). All surgical operations were carried out in a makeshift outdoor operation setup (Figure 1) and 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 2-3 cm cranial to the anal pore) along the ventral midline was made and the body cavity held open with the help of retractors. The microsurgery was assisted by a head mounted and illuminated eye loupe (Vorotex). Microsurgery was initiated by first locating the posterior margin of the gonad, where it begins to constrict into a tube from a more sac like (glandular) anterior portion of the lobe. After locating the posterior tubular region, the tubes were gently detached/teased out from the dorsal peri peritoneum. A suture (Premiline DS19) or a clip (small haemoclip) was passed from underneath and the ends tied or clipped. A four-point surgical sterilization approach was used, wherein each tube (right and the left) was first tied or clipped at two locations about 2-3 cm apart. Then, each tube (tissue) between the clips was cut, leaving both severed ends either tied or clipped. A set of photographs showing select steps in the procedure is presented in figure 2. Following tubal ligation the site of incision (surgery) was sutured (typically 3 or 4 sutures) using PDS II and the wound sealed by applying Adapt stoma powder 7906 (Hollister Australia). The operated fish were also administered antibiotic (0.2 ml/kg fish of Oxytet-200) and allowed to recover in shallow troughs with flow through water supply. On recovery the fish were monitored overnight in the troughs and transferred to a re-circulatory tank (26000 L) for rearing.



Figure 1. Makeshift out-door surgery theatre. In the foreground are anaesthetic setup fitted with aeration (right) and the measuring table (middle). In the rear are the portable ultrasound setup (right) and the surgery table (middle) with plastic bin (labelled carp) fitted with cradle (with black net skirting) to hold fish during surgery.

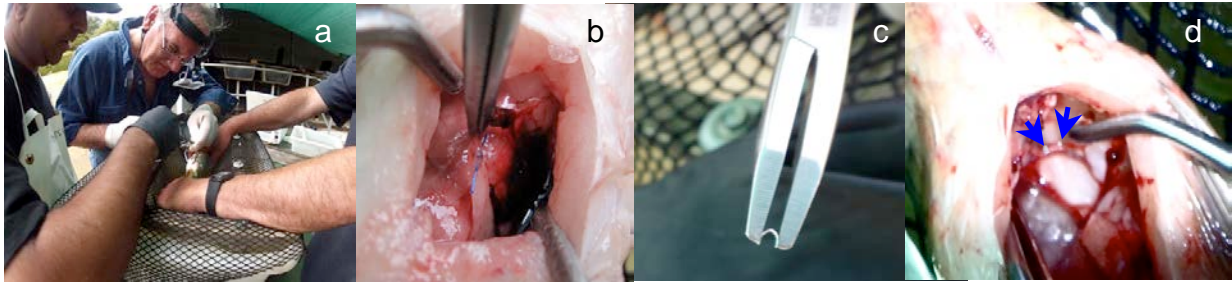


Figure 2. Surgical sterilisation of carp in progress (a), showing severed and sutured distal testicular lobe (b) and metal clips (c) in place (d, blue arrows).

## Fish sampling

Each fish was measured and tested prior to and post chemical and surgical treatments. The post treatment sampling was conducted at monthly intervals; on each occasion length and weight of each fish was measured, tested for sperm expression and, sperm and blood samples collected for sperm quality and hormone assays respectively. Sperm quality assays were limited to those individuals that expressed milt.

## Condition factor

The condition factor was calculated as follows (Ratz and Lloret, 2003):

$$Conditionfactor(K) = 100 \times \frac{Weight(g)}{Lenght(Cm)^3}$$

## Sperm expression and quality measurements

The (non) expression of milt was used as an end point measure of sterilisation success. Where the sperm expression was not completely inhibited, quality analysis of the milt was undertaken to infer subtle effects of the sterilisation procedures.

## Milt sampling

The sperm quality observations and analysis followed those previously described for common carp (Perchec et al., 1995; Linhart et al., 2008; Bastami et al., 2010). Prior to collecting the milt, the area around the urogenital papilla of male common carp was wiped dry with a towel to minimize contamination of milt with urine, faeces, and water. Milt was expressed by gentle pressure on the abdomen and when expressed it was collected using a 1 ml-syringe filled with 0.5 ml Phosphate buffered saline (PBS) solution. The collected milt sample was transferred to a 1.5-ml micro centrifuge tube and a subsample immediately transferred to a slide and examined microscopically for sperm quality including spermatozoa velocity and density. Meanwhile, a 20 µl-capillary tube was used to directly collect the milt of common carp for spermatocrit measurement.

## Sperm preparation and activation for video recording

A 0.1% Bovine Serum Albumin (BSA) solution was used to dilute the collected sperm to prevent adhesion of sperm to the microscope slide. Sperm activation was initiated using hatchery water (filter sterilised, pH 7.01). During spermatozoa activation, 5 µl milt of known dilution was mixed gently with 20 µl of BSA solution in a 1.5 ml micro-centrifuge tube. A volume of 75 µl activation solution was then added and mixed well before transferring to a glass slide for microscopic observations. A timer was used to measure the time between mixing and recording sperm motility microscopically (100X)

for 120 seconds using an eyepiece camera (Dino-Lite Digital Microscope AM7023 Dino-Eye) connected to a laptop. Quicktime 7 Pro software was used to record the sperm motility footage for each milt sample in duplicate.

## **Evaluation of spermatozoa velocity**

To measure sperm velocity, their motility was recorded under a compound microscope within 5 minutes of activation at fixed time points post activation across all samples for 120 seconds. The recorded video files were converted to uncompressed AVI files with Quicktime 7 Pro software and opened in ImageJ with an avi plugin (<http://rsbweb.nih.gov/ij/plugins/avi-reader.html>). The converted B & W binary files were then analysed using freeware ImageJ (<http://rsbweb.nih.gov/ij/download.html>) at every 5 seconds using the Computer Assisted Sperm Analysis (CASA) plugin (Wilson-Leedy and Ingermann 2007) was used to analyse the spermatozoa motility. All quantitative outputs were imported into a Microsoft Excel for comparison.

## **Spermatocrit**

The spermatocrit—percentage volume of packed sperm cells to the total milt volume—was measured as described before (Agarwal and Raghuvanshi 2009). Briefly, micro-haematocrit capillary tubes (20 µl capacity) were filled to approximately 70% of the capillary volume with undiluted common carp milt and sealed with clay sealant at both ends. The capillaries were then centrifuged for 2 minutes, at 10000 g at room temperature in a haematocrit centrifuge (Statspin, Model SS03 S/N 33964, Microhaematocrit Rotor RH12). The packed cell volume was measured using microhaematocrit reader.

## **Sperm density**

A haemocytometer counting chamber was used to determine sperm density. Each milt sample was diluted 200 times using phosphate buffered saline (PBS) and placed in a haematocytometer (depth 0.1 mm) and the sperm allowed to settle for 10 minutes. The spermatozoa were then counted under a compound microscope using a hand-tally counter and sperm density computed as described before (e.g. Alavi et al. 2006).

## **Blood sampling, processing and storage**

The blood from each fish was collected from the caudal vein via caudal puncture using heparinized (90 IU) 3 ml-syringe fitted with 21 gauge needle. About 2 ml blood was collected from each fish at each sampling event and held on ice until processed. Chilled blood samples were centrifuged at 3000 g for 5 minutes at 4 °C to separate the plasma and red blood cells. The plasma from each fish was transferred to two cryovials and frozen in ethanol-liquid nitrogen bath and stored at -80°C.

## **Hormone assays**

The levels of circulating hormones were measured to detect the effects of chemical and surgical treatment on the reproductive physiology of the fish. The hormone assays (11-keto testosterone and 17β-estradiol) were conducted using commercially available enzyme-linked immunosorbent assay (ELISA) kits as per the instruction of the manufacturer (Cayman Chemical Company).

Briefly, the stored blood plasma samples were thawed at room temperature. For 11-keto Testosterone assay the thawed samples were diluted 1:200 with EIA buffer while for 17β-estradiol assays the samples were used undiluted. The assay was carried out in duplicate for each sample in micro-well strips supplied in the kits. Each assay contained a minimum of two blanks (Blk), two non-specific binding wells (NSB), two maximum of binding wells (B<sub>0</sub>), and an eight point standard curve run in

duplicate. All assay reagents were prepared and added to the respective wells as recommended by the supplier and the 96 well-strips sealed with plastic film for incubation. The 17 $\beta$ -estradiol EIA kits were incubated one hour at room temperature, whereas the 11-keto Testosterone EIA kits were incubated 18 hours at 4°C on an orbital shaker. After incubation wells were rinsed five times with wash buffer and 200  $\mu$ l of Ellman's reagent added into each well, except the total activity (TA) wells, which received 5  $\mu$ l of the respective (11-KT or E2) tracer. The strips were again sealed with plastic film and incubated for 45 to 90 minutes on the orbital shaker for the reaction to develop. The intensity of the colorimetric reaction was read using a plate reader at a wavelength 412 nm. The assay results were then calculated with the assistance of a data analysis spread sheet ([www.caymanchem.com/analysis/eia](http://www.caymanchem.com/analysis/eia))—computed using a four-parameter logistic curve-fitting algorithm in Microsoft Excel.

## **Histological processing of testis**

Testis from all non-spermiating males from the chemically sterilised group and select spermiating individuals were subject to histological analysis. Histology was also carried out on excised tubular testicular tissue from those individuals subject to surgical sterilisation. Testis tissue processing and histological examinations followed standard protocols (e.g. Ismail et al. 2011; Hassanin et al. 2002). Briefly, gonad sub samples (approximately 1 cm<sup>3</sup>) of the testis were fixed in 10% neutral buffered formalin (NBF) for 20-24 hours and then transferred to 70% ethyl alcohol. The fixed tissues were then dehydrated with different strengths of ethanol (80%, 95% and 100%), embedded in xylene and paraffin. The processed samples were subjected to histological sectioning (1-2  $\mu$ m) and, stained (haematoxylin and eosin) adopting standard techniques. The stained sections were examined under a compound microscope.

## **Field study area**

Lakes Sorell is a large, shallow, highly turbid lake interconnected to Lake Crescent and is situated on the south-east corner of the Tasmanian Central Plateau at approximately 800m AHD (Figure. 3). Construction of fine mesh screens in 1996 effectively renders the system 'closed' to carp movement between the lakes and catchment downstream. The lake covers 50 km<sup>2</sup> with an average depth of 2.5m at full supply level (Hardie 2003). The habitat of the lakes is dominated by barren areas of sediment, rocky shores, reefs and shallow shoals with areas of aquatic vegetation largely restricted to wetlands associated with the lake—7.8 % surface area of Lake Sorell (Heffer 2003).

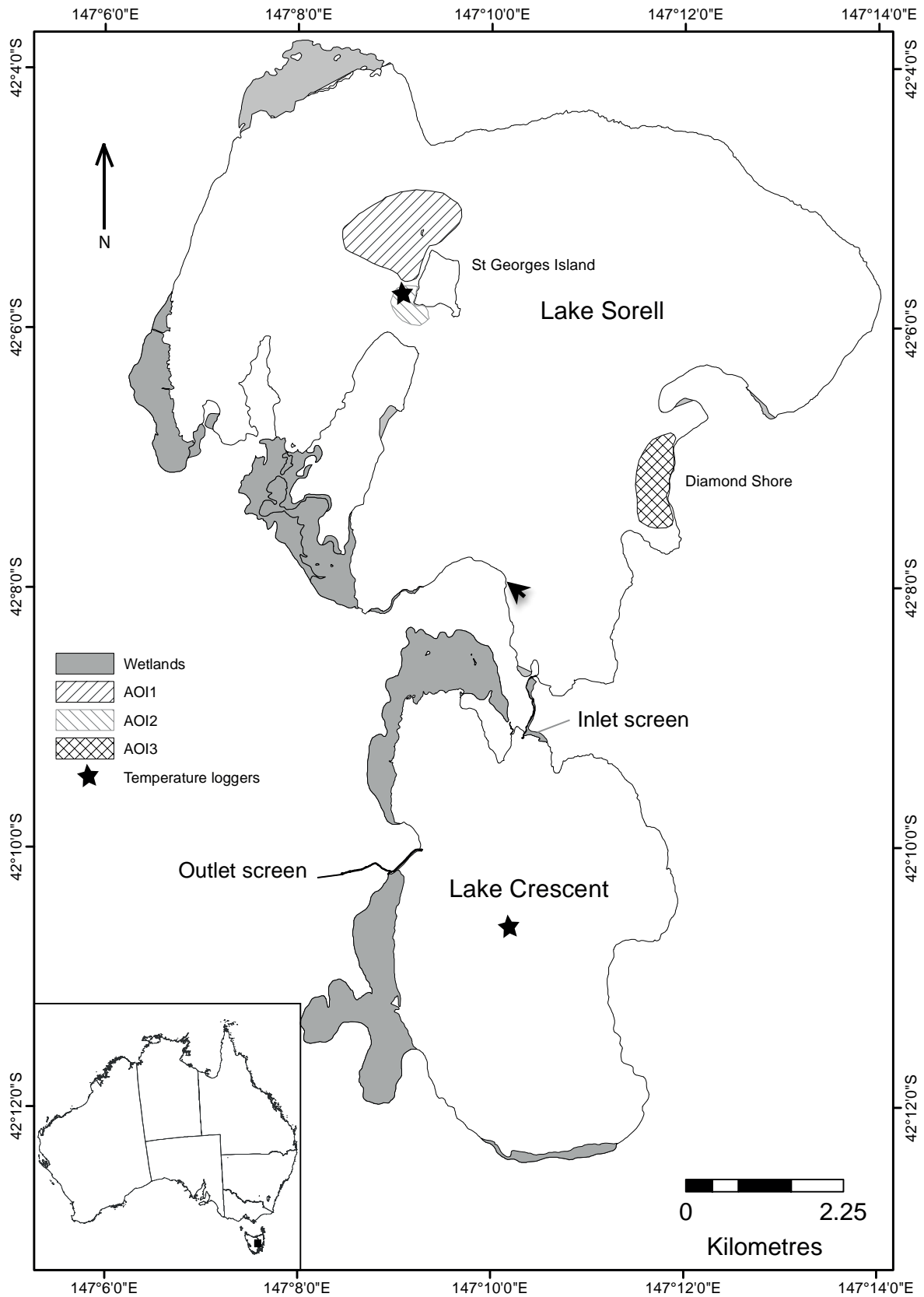


Figure 3: Map of the interconnected lakes Sorell (above) and Crescent (below), showing wetlands and key areas of interest (AOIs 1–3) as defined by habitat preference of adult carp (Taylor et al 2012). Arrow indicates the site (Dago Point) of radio-tagged carp release.

## Radio telemetry

Radio telemetry followed established protocols (Taylor et al 2012). Briefly, radio transmitters (ATS transmitter F1800, Advanced Telemetry Systems, Isanti, Minnesota) were surgically implanted into the body cavity of seven each of chemically and surgically sterilised (not expressing milt) and 7 control male (expressing milt) carp. Prior to surgery, fish were anaesthetised by immersion in 0.3mg/l of AquiS solution (AQUI-S New Zealand Ltd). Once anaesthetised, fish were placed upside down in a cradle with their gills immersed in water. Several scales were then removed slightly along the ventral mid-line, posterior to the pelvic fin and anterior to the anus to prepare the incision site. A small incision was made along the centre of the prepared area long enough to insert the transmitter. A small hole was also punctured through the body wall just posterior to the incision providing an outlet for the external antenna. Following implantation, incision was sutured and fish were allowed to recover before release. Transmitters were set to emit pulses (55 pulses per minute) for all day (24 hours) and the fish tracked using three element yagi antennas connected to a Lotek Suretrack STR1000 receiver. Hand held antennas were used to identify more precise locations when fish were found in shallow wetland habitat and to determine the proximity of transmitter fish relative to each other. Custom-built tracking vessels with an antenna attached to a mast centrepiece were used to track each lake. The locations of each transmitter fish were recorded on a field sheet comprising of a grid reference map and transferred into ArcGIS 9.3.1 software for analysis

## Analysis

The relationships between spermatocrit and spermatozoa density,  $17\beta$ -Estradiol and 11-keto Testosterone were determined using linear regression. A repeated measure Analysis of Variance (ANOVA) was used to analyze the data including the condition indices and hormone assay. The post hoc bonferroni multiple comparison tests were used to compare differences between treatments. A two way ANOVA was used to analyse the data of spermatozoa velocity. Tukey's test was also used as an 'a posteriori' test for comparison amongst averages. A significant level of  $P \leq 0.05$  was used for all analyses. All statistical tests were carried out using SPSS statistical package v. 19 (IBM).

The kernel density distribution plots were constructed, representing the experimental period (October 2013 to February 2014) using a kernel density population analysis option within the Spatial Analyst extension in ArcGIS 9.3.1. 'Areas of interest' (AOI) were defined in Lake Sorell as described before (Taylor et al 2012). These areas were spatially characterised and defined as: 1) 'wetlands', defined by the presence of macro-fauna described by habitat mapping and lake inundation levels. 2) 'AOI1', an area defined bathymetrically as a shallow shoal to the northwest of St Georges Island. 3) 'AOI2', an area defined bathymetrically as a deeper region southwest of St Georges Island. 4) 'AOI3', an area defined bathymetrically as a deeper region on the eastern shore of the lake (see Figure 3).

# Results

## Sexing of carp

As only males were targeted for sterilisation, it was important to sex the fish unambiguously. However, the fish were just reaching puberty and it was difficult to sex them using secondary sexual characters. To circumvent this limitation we resorted to the use of a portable ultrasound machine. A panel showing sexing of carp using a portable ultrasound is shown in Figure 4. The developing testis could be easily located just based on size and its triangular (pointed) appearance at the anterior tip (Fig 4, inset). Females in the group generally had no discernable gonad.

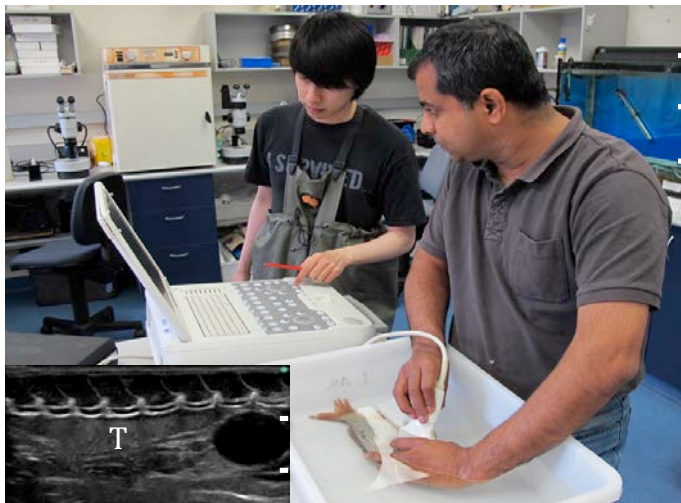


Figure 4. Sexing of carp using ultrasound. Note the developing testicular lobe with a pointed anterior end in the inset sonogram (bottom left; anterior to right). T, testis. Typically females at the same age had undetectable gonads.

A summary of the results of a pilot study on sexing the three-year-old carp (just coming into puberty) using ultrasound is presented in Table 1. The accuracy of predicting males was relatively high (95%), while that of the female was very low (54%). An example of a male wrongly predicted as female is shown in Figure 5. Typically such wrongly sexed males had underdeveloped gonads.

Table 1: Summary of sexing carp using ultrasound ( $n = 67$ ). Numbers in parenthesis indicate percentage accuracy of predicting the correct sex.

Predicted males	Actual males	Predicted females	Actual females
24	22	33	18
	(91%)		(54.5%)

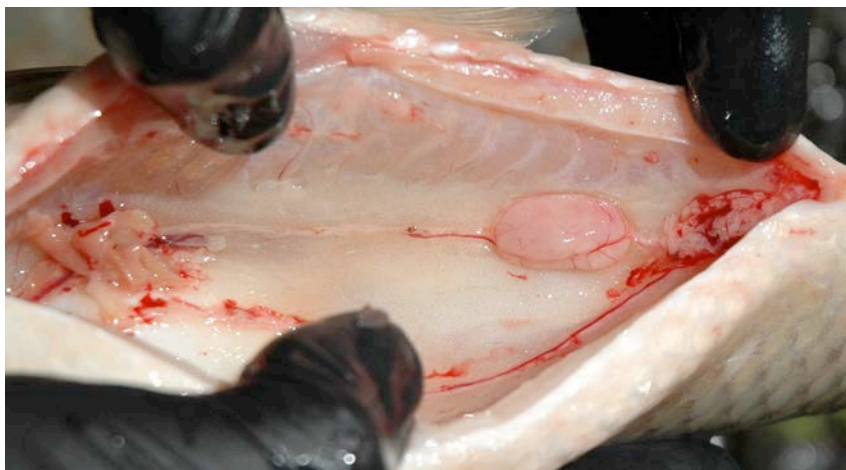


Figure 5. A typical underdeveloped testis, whose identity could not be predicted accurately using an ultrasound. In this particular example only the left testis was partially developed.



## Chemical sterilisation

Detailed results pertaining to chemical sterilisation experiments are presented in an honours thesis (Sangtian, 2013) and only a summary of these results is provided here. The results presented here are for the experimental period from Dec 2012 to March 2013.

## Growth indices

As presented in figures the average weight (Figure 6), length (Figure 7) and condition factor (Figure 8) of the treated fish were comparable to those of the controls at any sampling point. As expected there was significant difference between time points of the same group but not between the treatments at any given time point for the respective growth index.

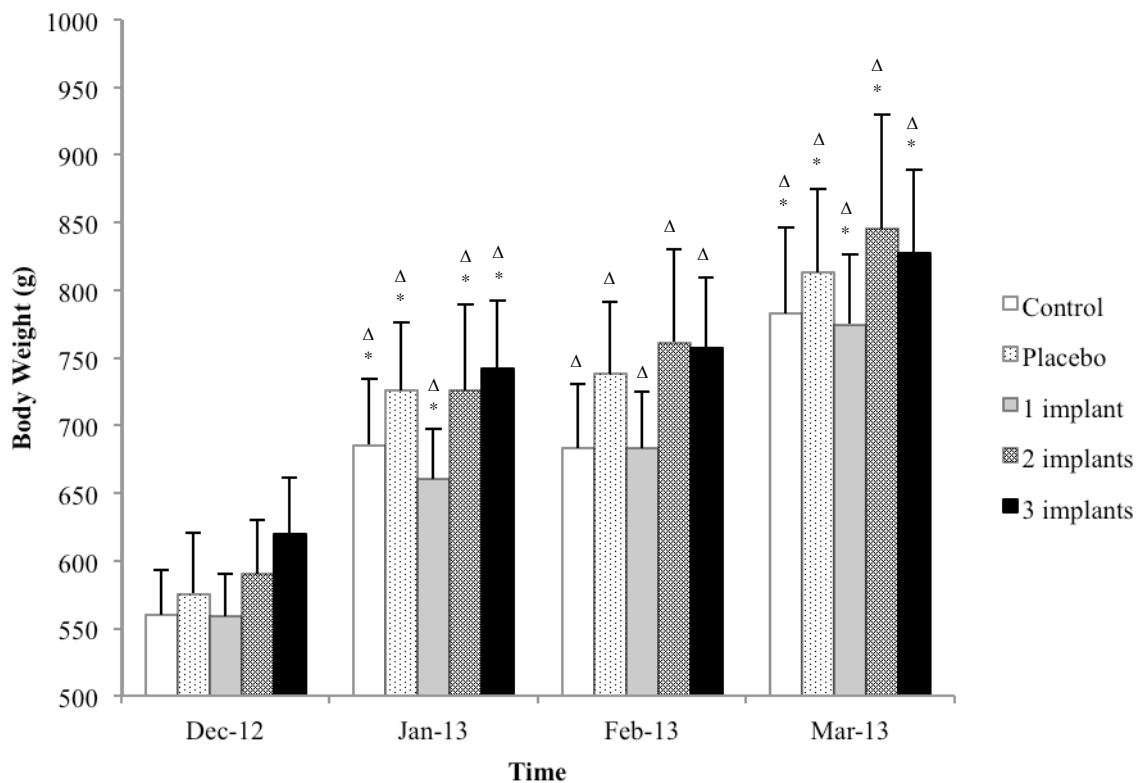


Figure 6. Changes in body weight of experimental common carp after Suprelorin<sup>®</sup> implantation (mean values  $\pm$  S.E.,  $n=16$ ). Asterisks present a significant difference from the previous time point for each treatment ( $P<0.05$ ). Delta ( $\Delta$ ) represents a significant difference for each treatment compared to first sampling (Dec-12) ( $P<0.05$ ).

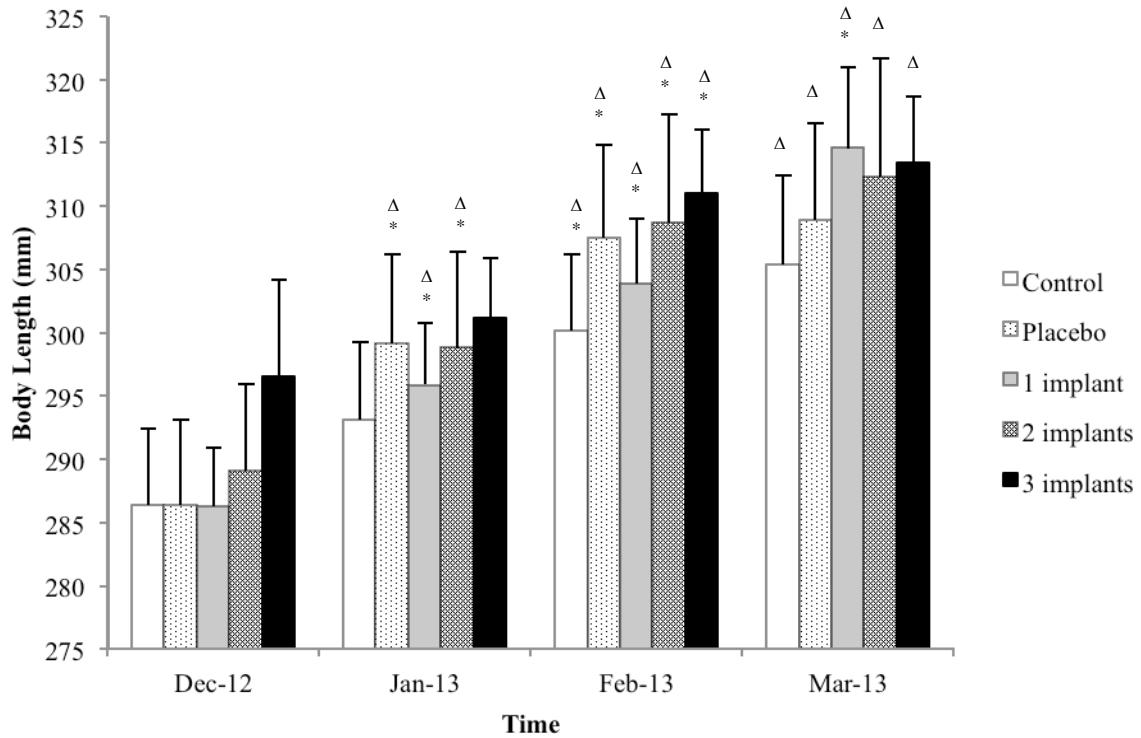


Figure 7. Changes in body length of experimental common carp after Suprelorin<sup>®</sup> implantation (mean values  $\pm$  S.E., n=16). Asterisks present a significant difference from the previous time point for each treatment ( $P < 0.05$ ). Delta ( $\Delta$ ) represents a significant difference for each treatment in comparison to first sampling (Dec-12) ( $P < 0.05$ ).

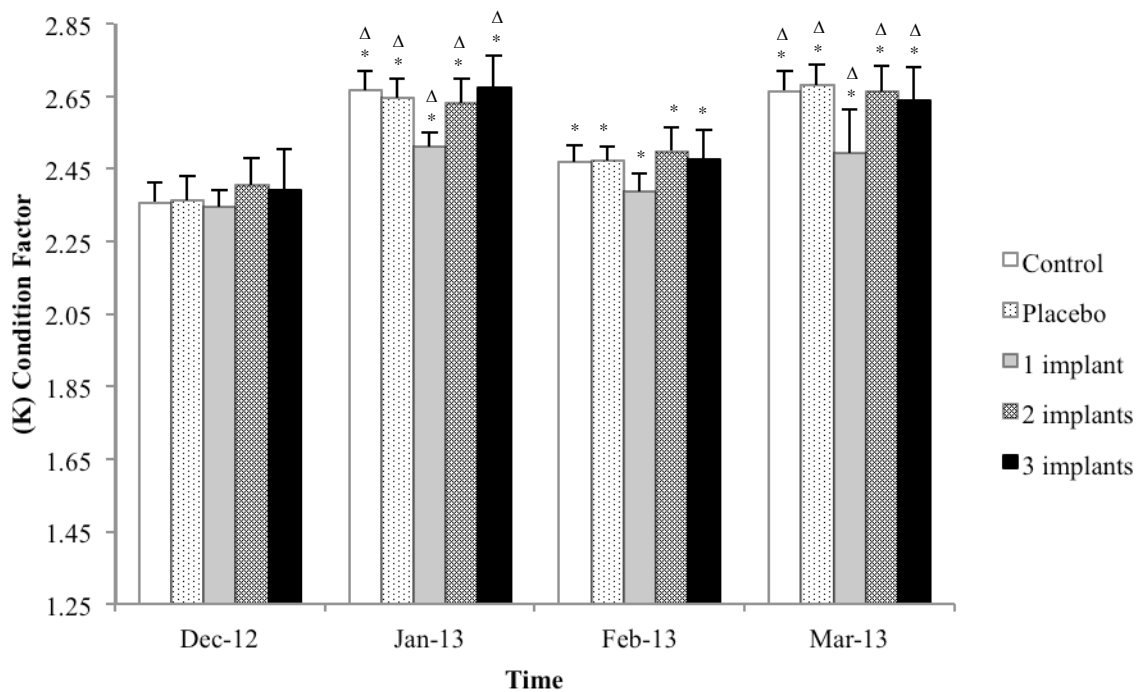


Figure 8. Changes in condition factor (K) of experimental common carp after Suprelorin<sup>®</sup> implantation (mean values  $\pm$  S.E., n=16). Asterisks present a significant difference from the previous time point for

each treatment ( $P < 0.05$ ). Delta ( $\Delta$ ) represents a significant difference for each treatment in comparison to first sampling (Dec-12) ( $P < 0.05$ ).

### Sperm expression and quality

Obviously, the sperm quality and quantity measurements were possible only from those individuals that expressed milt. At the beginning of the experiment (Dec 2012), none of the fish were expressing milt. However with progression of time some individuals in each of the treatment groups began to express milt (Figure 9). The proportion of individuals expressing milt was the lowest in those treated with two Suprelorin<sup>®</sup> implants (18.2 mg) and highest in placebo group.

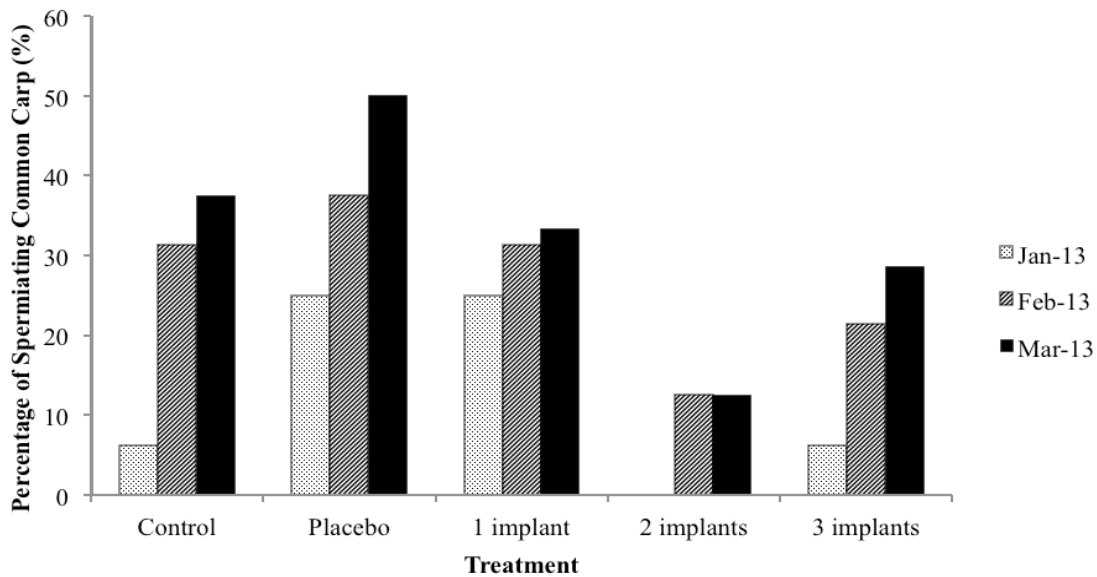


Figure 9. Percentage of spermating fish in each treatment groups (n=16/treatment).

The sperm count (spermatozoa/mL) and spermatocrit readings (proportion of packed volume of spermatozoa per volume of milt) are presented in Figures 10 and 11 respectively. Of the 80 fish, only ten produced milt when tested at the second sampling (January 2013) and this number rose to a 26 in March 2013. This was accompanied by corresponding rise in sperm count in the respective fish over time with few exceptions e.g. carp No. 29, 31, and 80 (Figure 10). The highest sperm count was from the common carp No. 19 that was implanted with 1 Suprelorin<sup>®</sup> pellet.

Of the spermating fish not all produced sufficient milt to conduct spermatocrit measurements. Nonetheless, the spermatocrit observations generally reflected the trend observed for sperm counts. The number fish with spermatocrit measurements increased from 2 in Jan to 14 and 19 in Feb and March 2013 respectively. Not surprisingly the carp no 19 (with 1 superlorin implant) had the highest spermatocrit reading at any given point, rising from about 42.5% in Jan to about 84.91 % in March (Figure 11).

A positive and significant co-relation between the sperm counts (density) and spermatocrit readings was obvious at both third ( $F_{1, 12} = 60.637, P < 0.001, R^2 = 0.8348$ , Figure 12a.) and fourth ( $F_{1, 17} = 69.704, P < 0.001, R^2 = 0.7938$ , Figure 10b) sampling points.

The curvilinear velocity (VCL) measurements were not significantly difference between treatments across the two sample periods where velocity data was available. Neither was an interaction between different sampling times (February and March 2013) and different treatments (ANOVA,  $F_{4, 73} = 0.493, P > 0.05$ ). Similarly, there was no difference in VCL between sampling time (ANOVA,  $F_{1, 73} = 2.521, P > 0.05$ ) and between different treatments (ANOVA,  $F_{4, 73} = 2.40, P > 0.05$ ) (Figure 13).

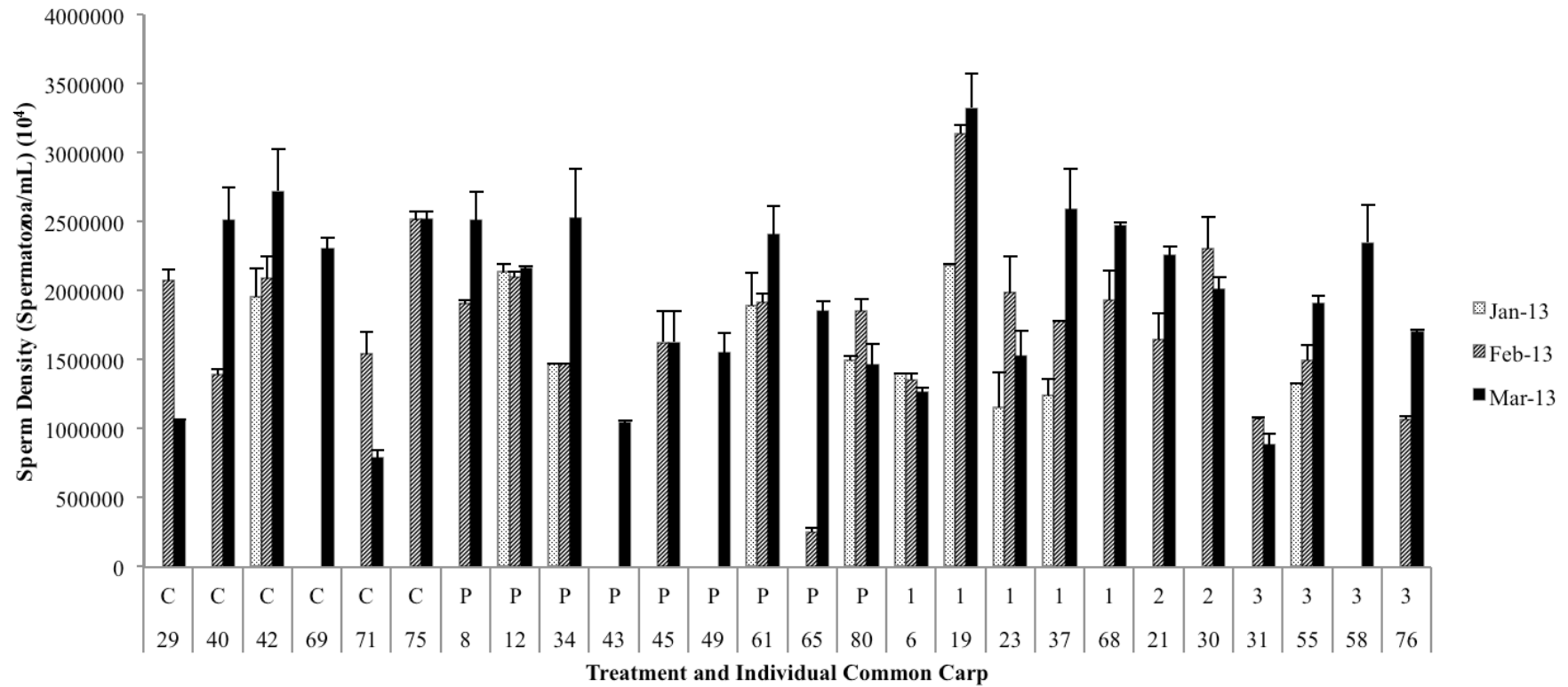


Figure 10. Sperm density of individually numbered spermiating carp from different treatments during the experimental period (January – March 2013). C, Control; P, Placebo; 1-3, 1-3 implanted fish respectively. No fish expressed sperm at the time of administering the Suprelorin® (December

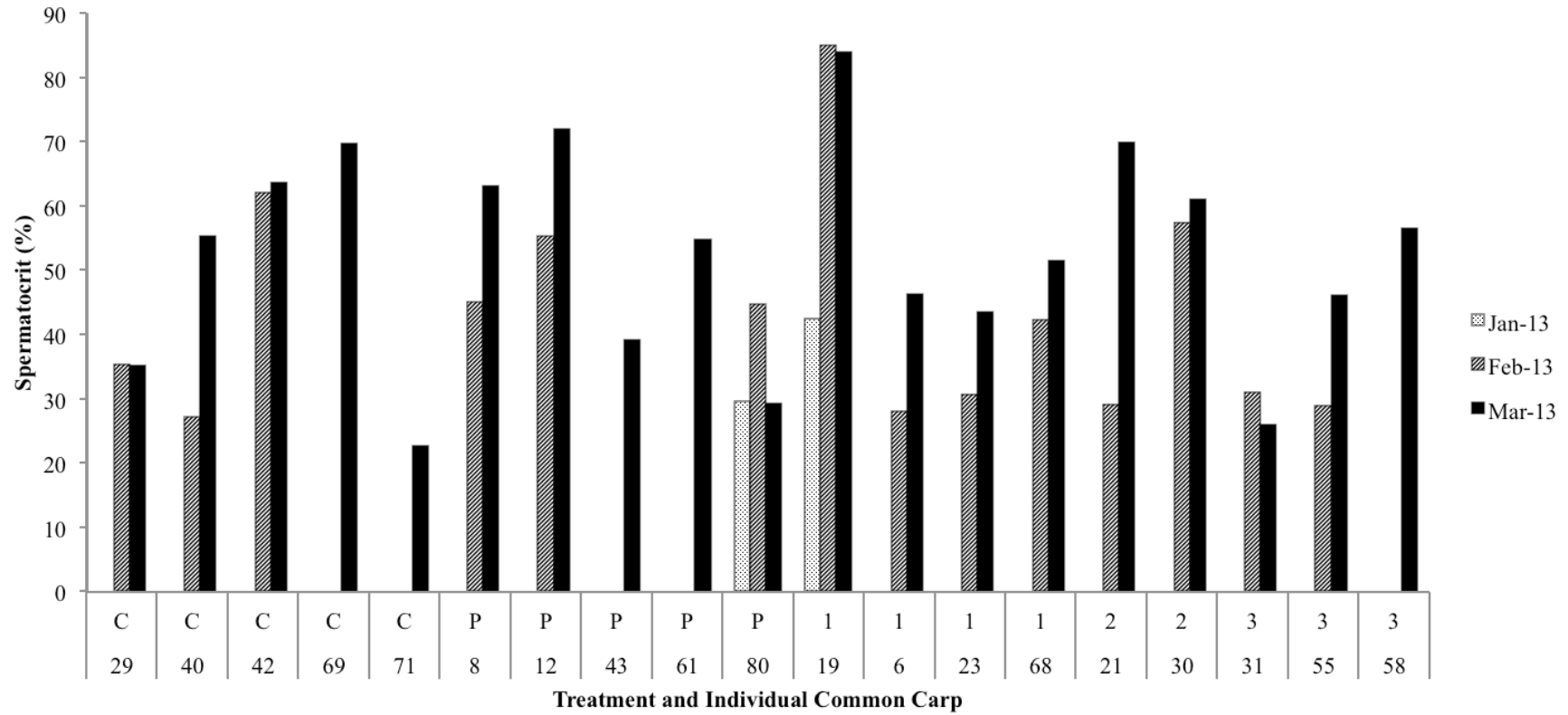
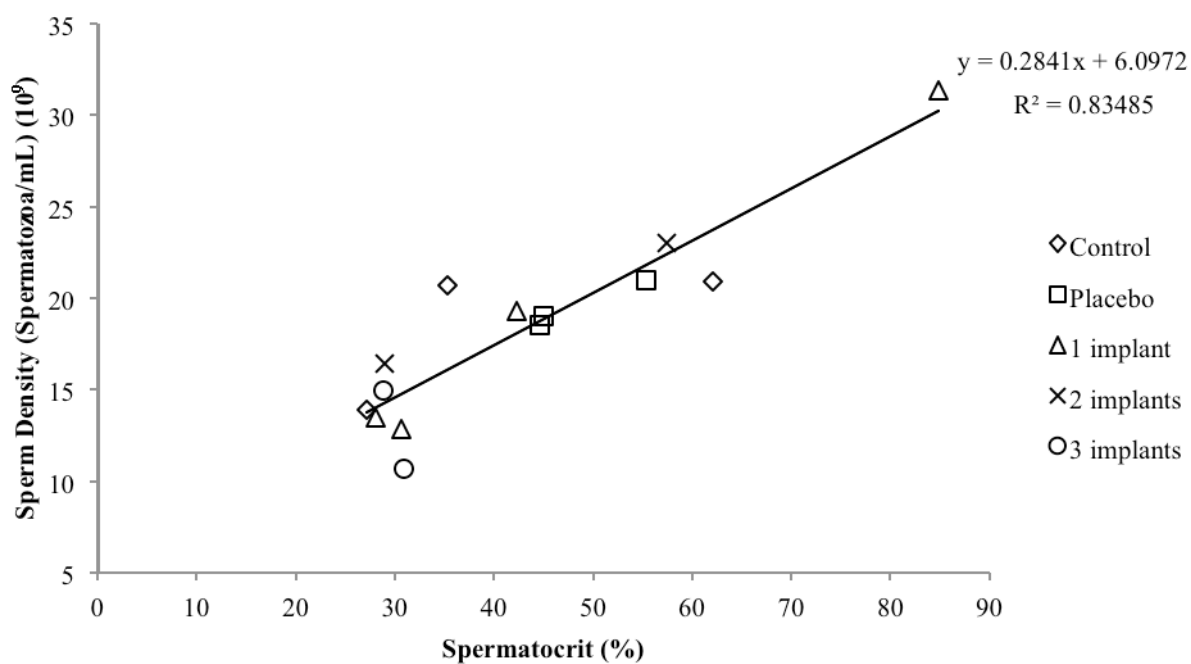


Figure 11. Spermatocrit values of individual male carp from different treatments during the experimental period (January – March 2013). Spermatocrit measurements were not possible in instance where insufficient milt was expressed. C, Control; P, Placebo; 1-3, 1-3 Superlorin® implanted fish respectively. None of the fish expressed sperm at the time of administering the Suprelorin® (December 2013).

a).



b).

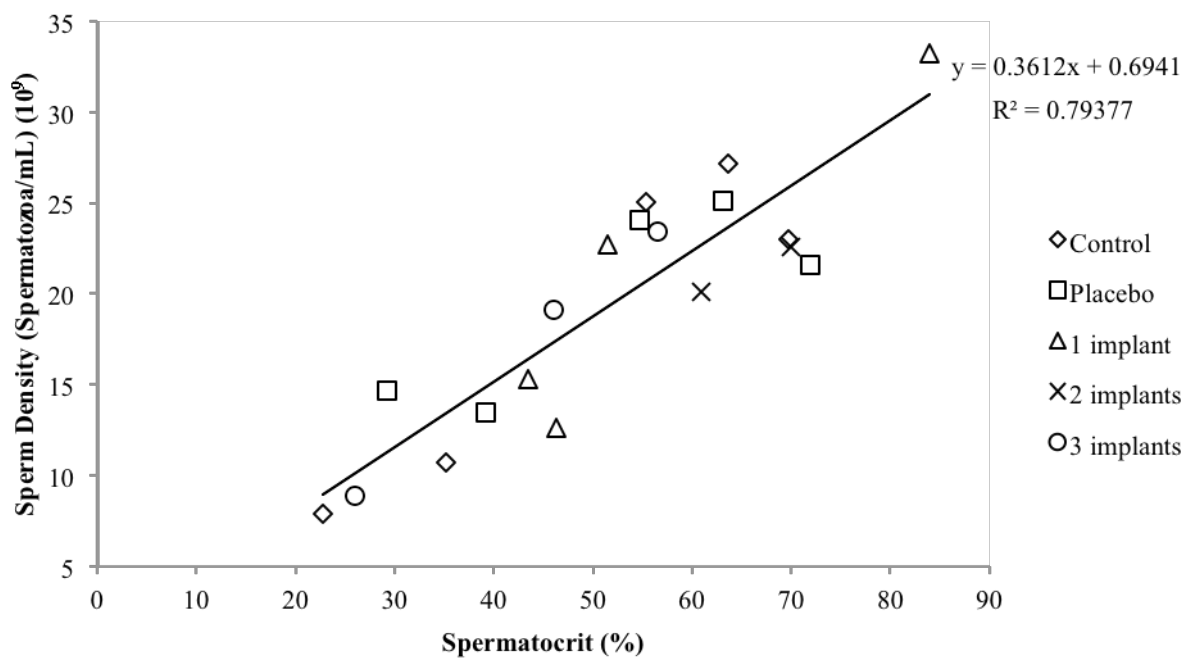


Figure 12. The relationship between spermatocrit (%) and spermatozoa density of experimental common carp in February 2013 (a.) and March 2013 (b.). The equations describe the linear relationship.

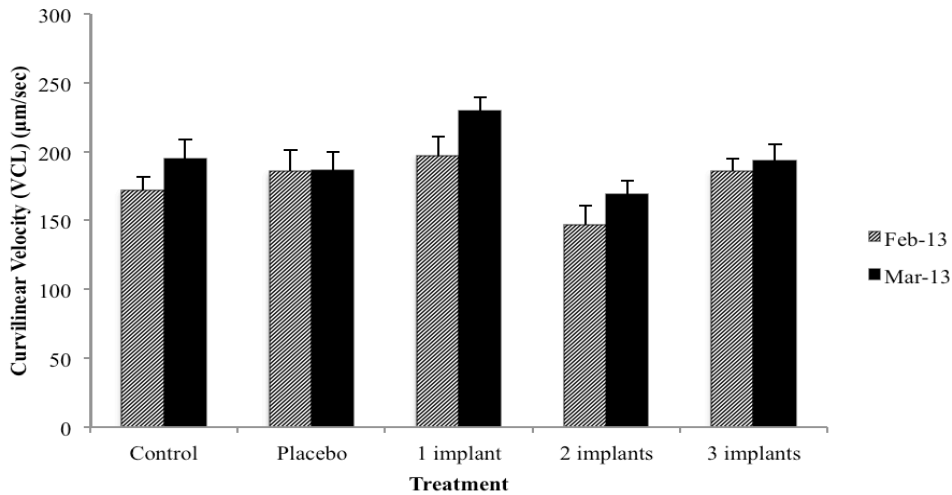


Figure 13. Curvilinear velocity of common carp spermatozoa from different treatments (mean  $\pm$  S.E.). No significant differences were observed either between treatments or sampling times ( $P > 0.05$ ).

### Hormone levels in control and treated fish

In general, there was an increase in the level of 11-keto Testosterone over time in all treatment groups (Figure 14). Interestingly, within each treatment groups, two subpopulations of individuals could be discerned; those with high (increasing over time) and those with low basal levels of 11-keto Testosterone.

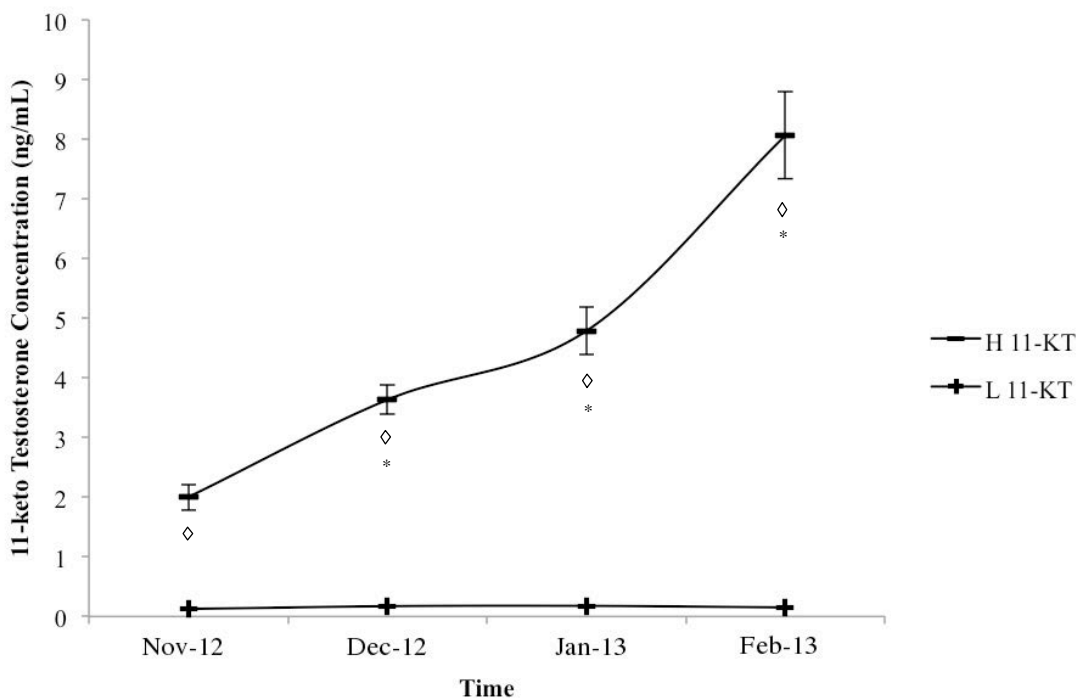


Figure 14. 11-keto testosterone concentration (mean values  $\pm$  S.E.) of all carp divided into two subpopulations; high 11-keto Testosterone levels and low 11-keto Testosterone levels. Means with an asterisk are significantly different from the previous time point ( $P < 0.05$ ). Diamonds indicated a significant difference between High 11-KT group (H 11-KT) and Low 11-KT group (L 11-KT) at each sampling time ( $P < 0.05$ ).

When the data on these high 11-KT (H 11-KT) and low 11-KT (L 11-KT) subpopulations were analysed, a significant difference in 11-keto concentrations between the H 11-KT and L 11-KT groups was evident (*Repeated Measures ANOVA*,  $F_{1.633, 122.486} = 34.836$ ,  $P < 0.001$ ). The 11-keto Testosterone level of the H 11-KT significantly increased over time (Figures 14–15), but those of the L 11-KT was very low and remained stable (Figure 14).

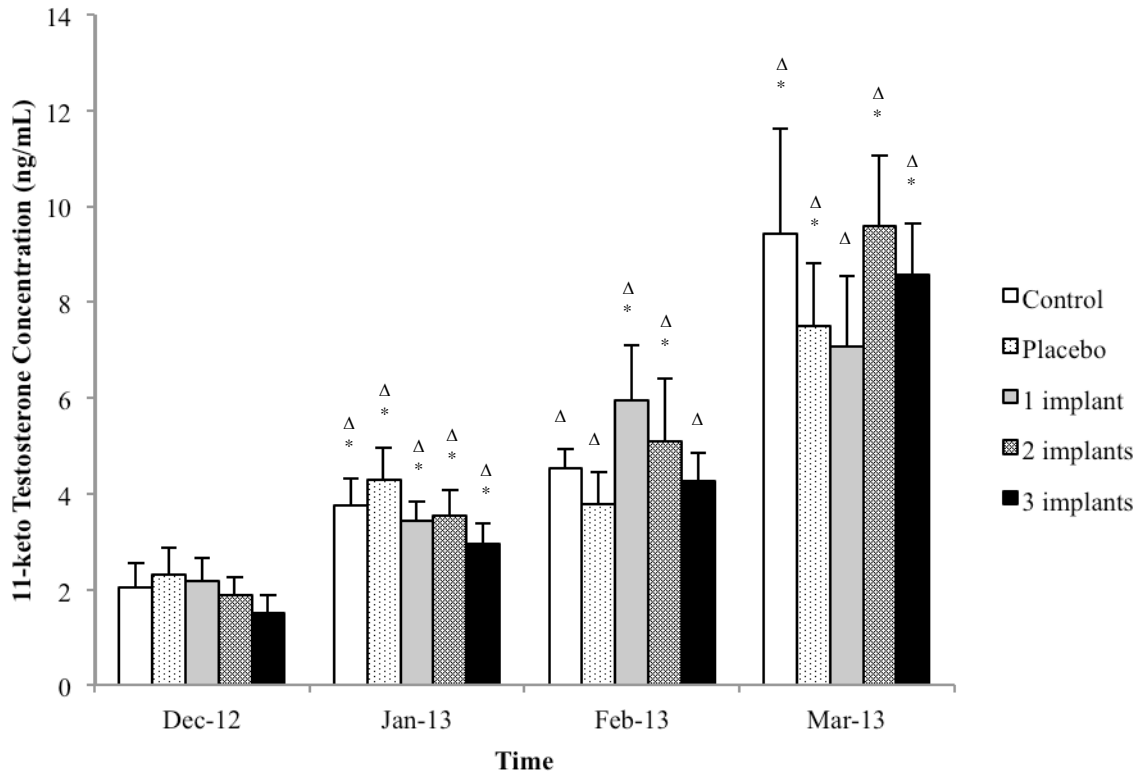


Figure 15. Changes in 11-Keto testosterone concentrations of experimental common carp from the high 11-KT level group (H 11-KT) after Suprelorin<sup>®</sup> implantation (mean values  $\pm$  S.E.). Asterisks indicate a significant difference from the previous time point for each treatment ( $P < 0.05$ ). A significant difference for each treatment in comparison to the first sampling (Dec-12) is shown by a delta  $P < 0.05$ .

### Testis morphology and histology

All control fish had normally developing testis from the anterior to the posterior margin of the body cavity (Figure 16A). In contrast several fish treated with Suprelorin<sup>®</sup> had abnormally developing testis. Two examples of such testis abnormalities in treated fish, which also did not express sperm, are presented in Figure 16B&C. In the first case (fish treated with 3 Suprelorin<sup>®</sup> implants) the testis was partially developed with only the middle section well developed with both anterior and posterior regions incompletely developed (Figure 16B). In the second case (fish treated with 1 Suprelorin<sup>®</sup> implant) morphological abnormality at the base of one lobe (Figure 16C, LHS) was observed while the other lobe was incompletely developed (Figure 16C, RHS).



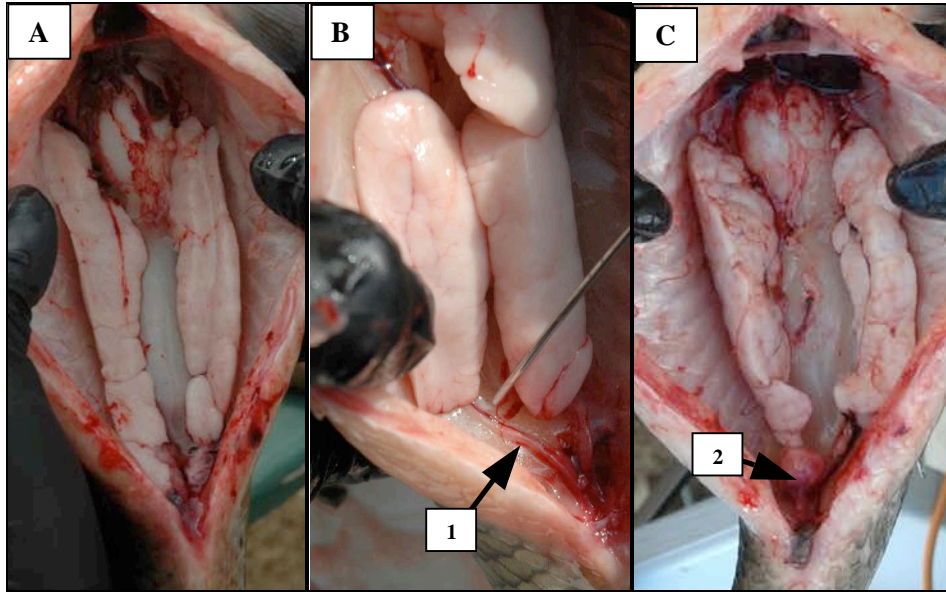


Figure 16. Gross morphology of control (A.) and two Suprelorin® treated carp (B. and C.). Note gonadal abnormalities in B and C. 1, underdeveloped posterior section of the testis; 2, abnormal non-glandular tissue growth.

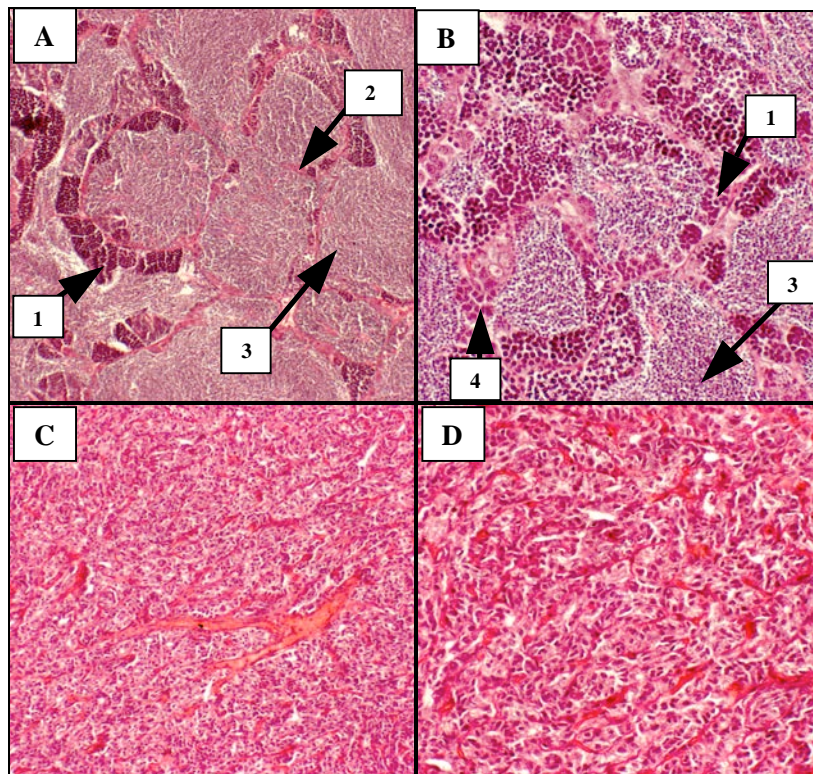


Figure 17. Histological section through normal (A-100X, B-200X) and through abnormal non-glandular growth presented in Figure 14C above (C-100X, D-200X); 1, spermatids; 2, lumens filled with Spermatozoa; 3, spermatozoa; 4. Spermatogenic cyst

The histological sections through most testis samples appeared normal, with the exception of a section through the abnormally developing region of testis from the fish treated with a single Suprelorin<sup>®</sup> implant. A micrograph through a control fish testis and that of the treated fish with the abnormally developing testis are presented in Figure 17A&B and 17C&D respectively. In control fish, the lumen (2) was filled with spermatozoa (3) and the lobules contained numerous spermatogenic cysts (4). In addition, spermatids (1) could be readily identified (Figures 17A&B). In contrast, the testis of the non-spermiating carp (with 1 implant of Suprelorin<sup>®</sup>) did not have a well-defined structure. The spermatozoa and spermatids were scattered and few and the lumen was poorly defined (Figure 17C&D).

## **Surgical Sterilisation**

Predominantly sutures (n =17) or clips (n=24) were used to tie the severed ends of the testis. Electro-cautery was attempted in only two individuals; in one both the testicular lobes were secured using electro-cautery and in the other, one lobe was elector-cauterised and the other lobe clipped. A panel showing the suturing and clipping procedures is presented in Figure 2.

A summary of all the surgically sterilised fish is presented in Table 2. In all 44 individuals were operated, with two succumbing to death within 7 days post surgery. Another four operated fish were sacrificed to make post surgery observations. The 38 surviving fish were assessed monthly for growth, sperm production (quality and quantity) and blood hormone levels. The animals showed excellent healing with the exception of two that succumbed to death, within 7 days post surgery. Only a small proportion of the animals were expressing sperm before the surgery (about 20.4 %). Seven of these nine-spermiating individuals before surgery did not discharge sperm post surgery, indicating a sterilization success rate of about 77%. Of those that did not spermiate prior to the surgery (n=27) eight (30%) had begun to express sperm on or before October 2013 (~10 months post surgery), indicating that they were not successfully sterilised. The remaining 19 fish (70%) have not expressed milt to date despite the injection of Ovaprim (0.1 ml/kg fish) to enhance sperm expression. In total 28 surgically sterilized fish were generated at an overall success rate of 73%.

Table 2. Summary of surgical sterilisation experiments.

Ligation method	No. Operated	No. Survival	*No. Sacrificed	No. Spermiating Pre surgery	§No. Confirmed sterile
Sutures	17	17	2	2 (11.7 %)	2 (100 %)
Clips	24	22	2	5 (20.8 %)	3 (60 %)
Sutures and Clip	2	2	0	0	–
Cautery	1	1	0	1 (100 %)	1 (100 %)
Cautery and Clip	1	1	0	1 (100 %)	1 (100 %)
<b>Total</b>	44	42 (95 %)	4	9 (20.4 %)	7 (77 %)

\*Sacrificed within a week post surgery; §Number that did not express milt post- but did so pre-surgery.

### Growth and condition of fish

Just before surgery (Feb-2013) the average weight of the control fish was significantly heavier than those of the surgically operated fish ( $P < 0.05$ ) but they remained comparable at subsequent time points (Figure 18). The average weight increased significantly only between Feb-2013 and March-2013 with no further increase, coinciding with onset of autumn. Indeed there was a decreasing trend in May and June 2013 albeit not significant. Interestingly, there was no significant difference in average length of the fish, between treatment groups or time points over the experimental period (Figure 19). Somewhat reflecting these observations the condition factor improved only between Feb and March 2013, and remained comparable over the next eight months (May 2013 to Jan 2014) of the experimental period (Figure 20).

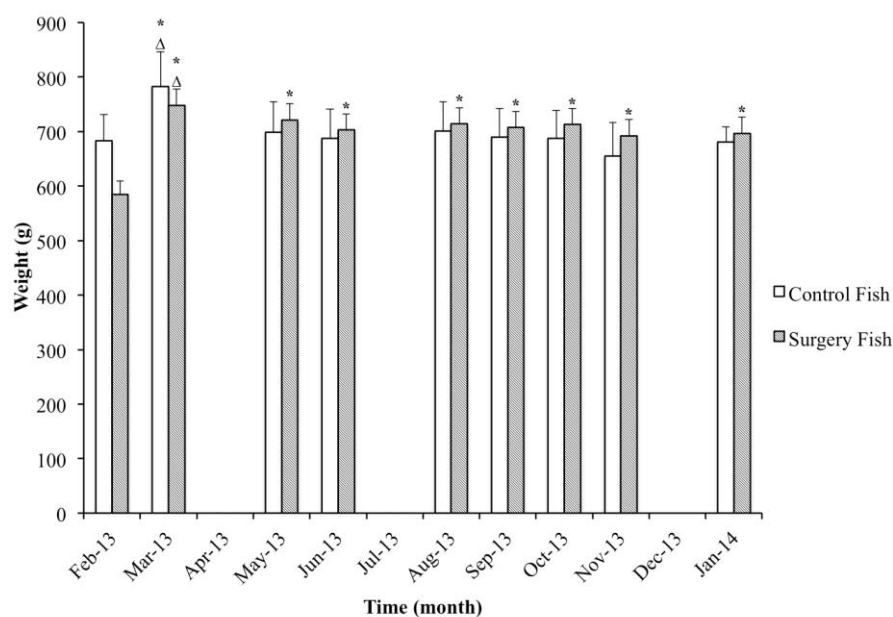


Figure 18: Changes in body weight of sterilised and control fish (mean values  $\pm$  S.E). Delta ( $\Delta$ ) represents a significant difference from the previous time point for each treatment ( $P < 0.05$ ). Asterisk represents a significant difference for each treatment compared to first sampling (Feb-13) ( $P < 0.05$ ).

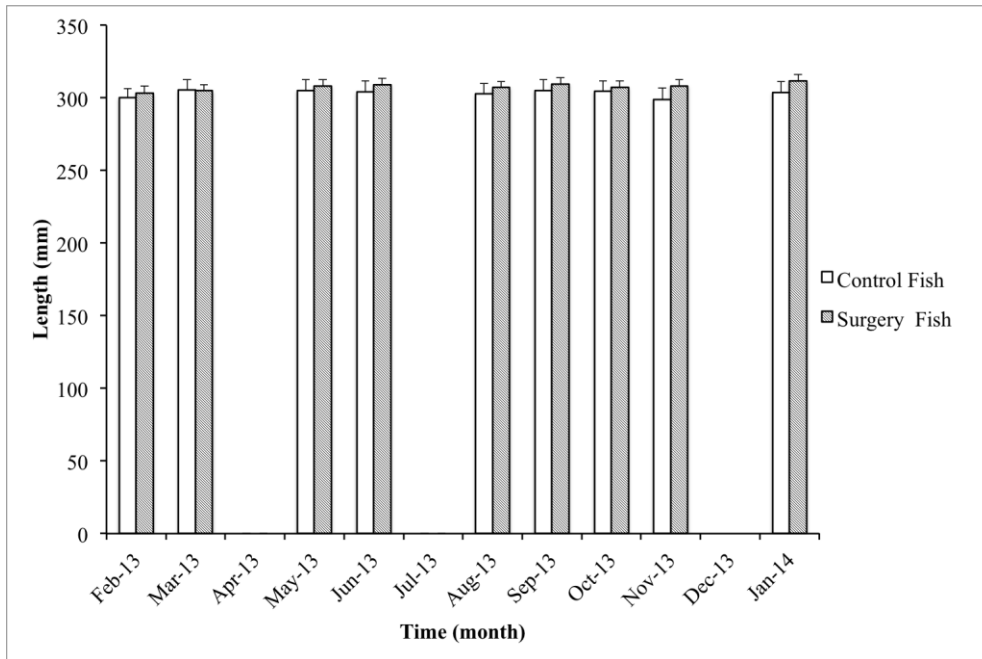


Figure 19: Changes in body length of sterilised and control fish (mean values  $\pm$  S.E). No significant differences were observed throughout the experimental period ( $P > 0.05$ ).

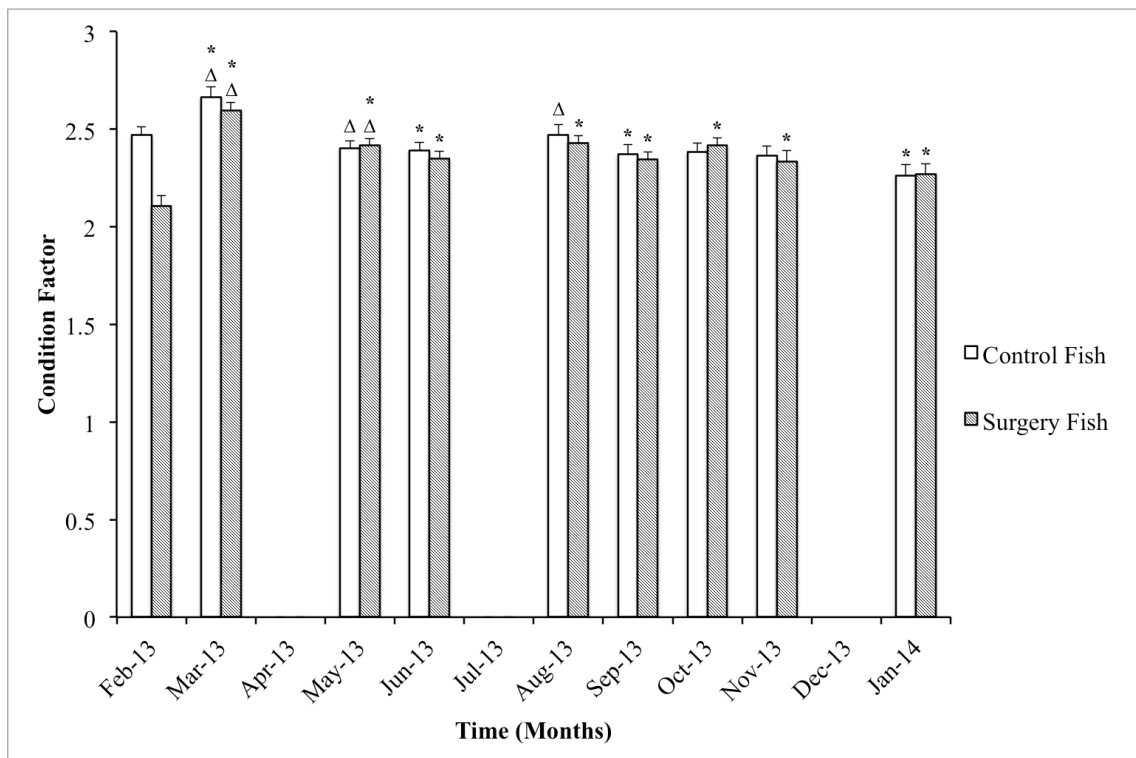


Figure 20: Changes in condition factor (K) of sterilised and control fish (mean values  $\pm$  S.E). Delta ( $\Delta$ ) represents a significant difference from the previous time point for each treatment ( $P < 0.05$ ). Asterisk represents a significant difference for each treatment in comparison to first sampling (Feb-13) ( $P < 0.05$ ).

## Circulating testosterone levels

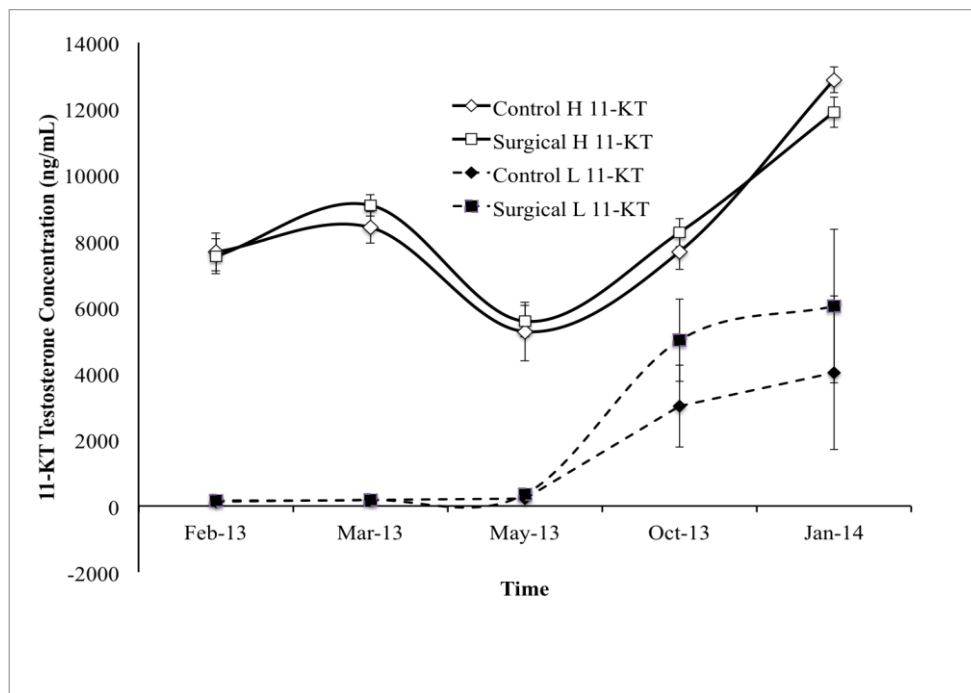


Figure 21. Changes in 11-Keto testosterone concentrations of surgically treated and control carp from the high (H 11-KT) and low (L 11-KT) 11-KT level groups (mean values  $\pm$  S.E.).

As in the case of chemical sterilisation experiments two subpopulations of males could be discerned; those with high (H 11-KT) and low (L 11-KT) basal levels of 11-keto Testosterone respectively (Figure 21). Within the H 11-KT group, there was a fluctuating trend; initially (March 2013) the 11-KT levels increased followed by a drop (May 2013) and once again increasing over the last two sampling points (Oct 2013 and Jan 2014). In contrast, the levels remained basal in the L 11KT group over the first three (Feb – May 2013) and only increased at the last two (Oct 2013 and Jan 2014) sampling points.

## Movement and distribution of radio-tagged carp

All radio-tagged fish were released at a single location known as the Dago Point on the southeast part of the lake (see Figure 1). A day after the release the fish were found dispersed throughout the lake with no apparent treatment influenced patterns (data not shown). During the observation period (Oct 2013 to Feb 2014) radio-tracking was conducted 98 times, with 1542 tracking events recorded. The observation revealed that the individual fish traversed to different parts of the lake; many moving between the eastern and western part of the lake. A pooled and individual Kernel-density distribution pattern of each of the groups is presented in Figures 22 and 23 respectively. As is evident the distributions were spread over different parts of the lakes, which often overlapping patterns. The Kernel-density estimation also indicates several highly utilised areas. Prominent, concentrations occurred in the bay immediate to the release point, shores of St Georges Island, and wetland habitat along the eastern part of the southwest bay. Within the broad distribution patterns, some were unique to each of the treatment groups.

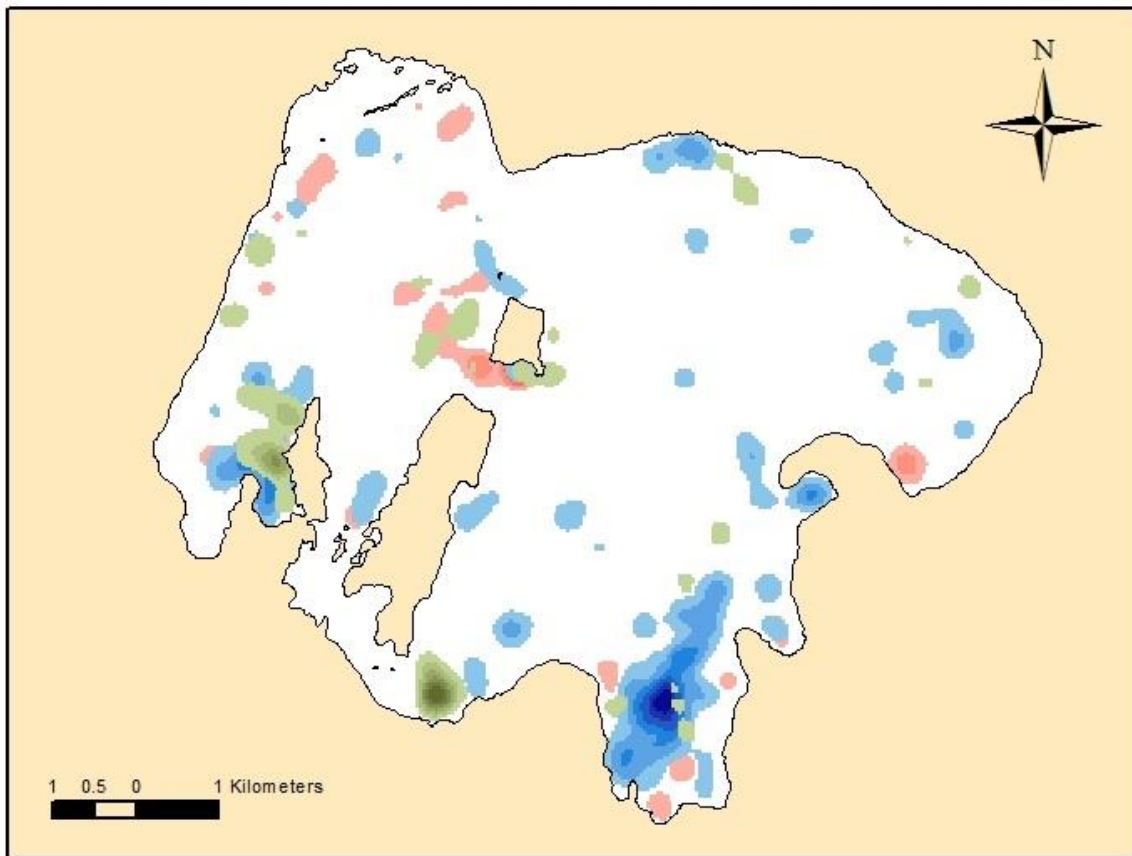


Figure 22: Kernel density maps showing distribution of control males (green), potential chemical sterilised male (red) and surgically sterilised male (blue) transmitter fish in Lake Sorell (Oct 2013-Feb 2014). Efficiency rating (the average proportion of tagged fish identified on a given day) for each group was 74, 72 and 86 % respectively.

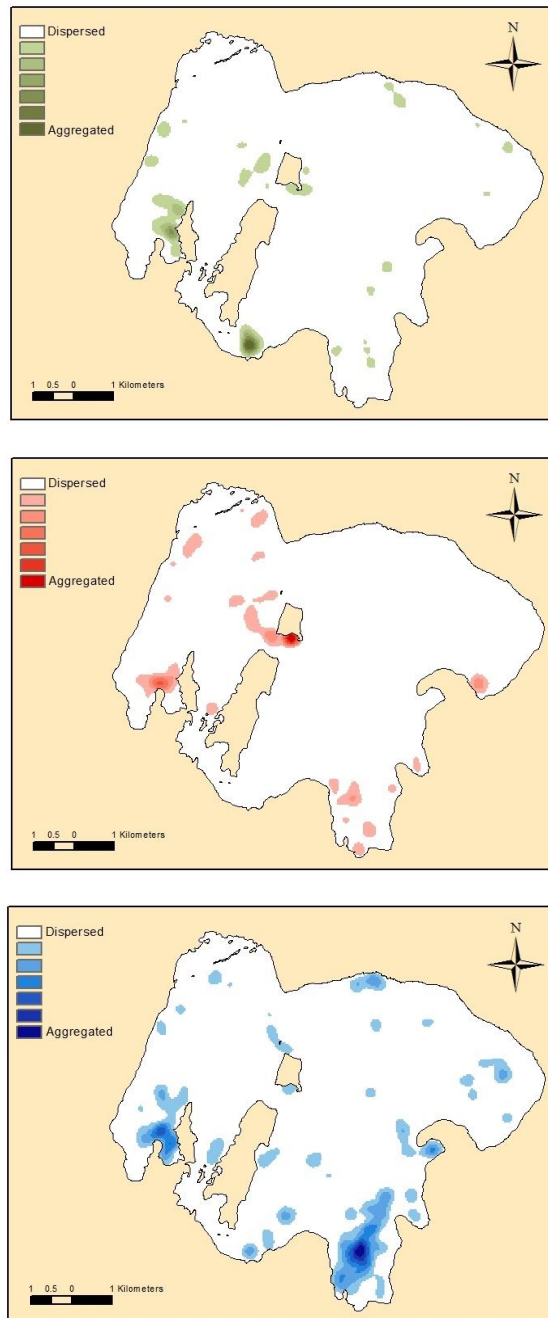


Figure 23: For clarity the pooled kernel density maps in Figure 21 above are shown separately for each group of fish. Top, control males (green); middle, chemically sterilised males (red); bottom, surgically sterilised males (blue). Efficiency rating (the average proportion of tagged fish identified on a given day) for each group was 74, 72 and 86% respectively.

### Capture of Judas and feral carp

When three or more radio-tagged fish were located within a 50 m radius, a targeted fishing effort was initiated involving a combination of electrofishing and gill netting. During the observation period (Oct 2013-Feb 2014) a total of 32 such aggregations were fished, returning a total catch of 405 feral carp. Over the same period (Oct 2023-Feb 2014) a total of 2029 carp were caught in the lake. Collectively the radio tagged fish led to the capture of about 20% of the total carp caught in the lake. This translated to catch per radio-tagged fish (CPRF) of 7.91 with contributions of, 2.71, 9.14 and 11CPRF by the chemically sterilised, surgically sterilised and control groups of Judas fish respectively.

Three Judas carp (coincidentally one individual from each treatment group) were also captured in fyke nets placed along the barrier nets protecting access to prime breeding habitat.

## Morphological observations on unsuccessfully sterilised carp

All surgically sterilised fish that continued to express milt (n= 11) were sacrificed and subject to morphological observations. Typically in these individuals the testis had repaired and re-established the connections to the urogenital sinus. A typical example is shown in Figure 24. Uniquely in one instance the testicular lobes were fused with the intestinal tract and the individual expressed milt via the anal pore (Figure 25). In yet another case one lobe was successfully sealed with the clip intact while the other had re-established the connection to urino-genital pore (Figure 26).

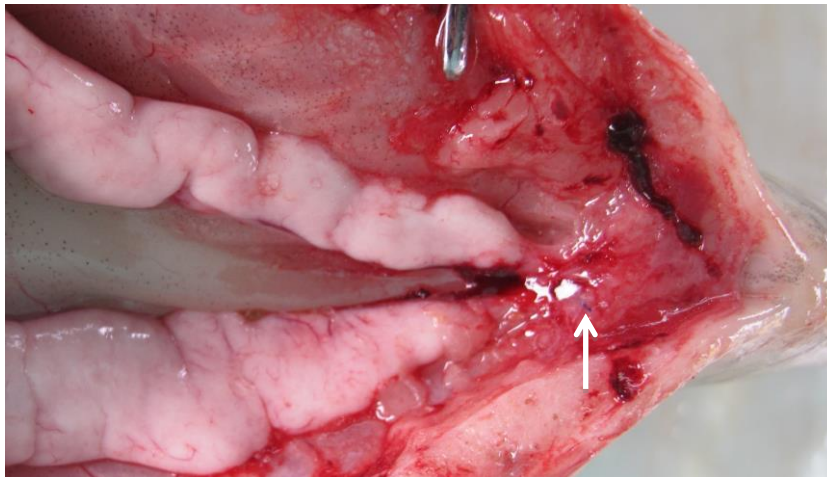


Figure 24. Photograph of a typical individual showing near complete repair of testis re-establishing connection to the urino-genital sinus in which surgical sterilisation had failed. Note the remains of suture used to tie the severed ends (arrow).

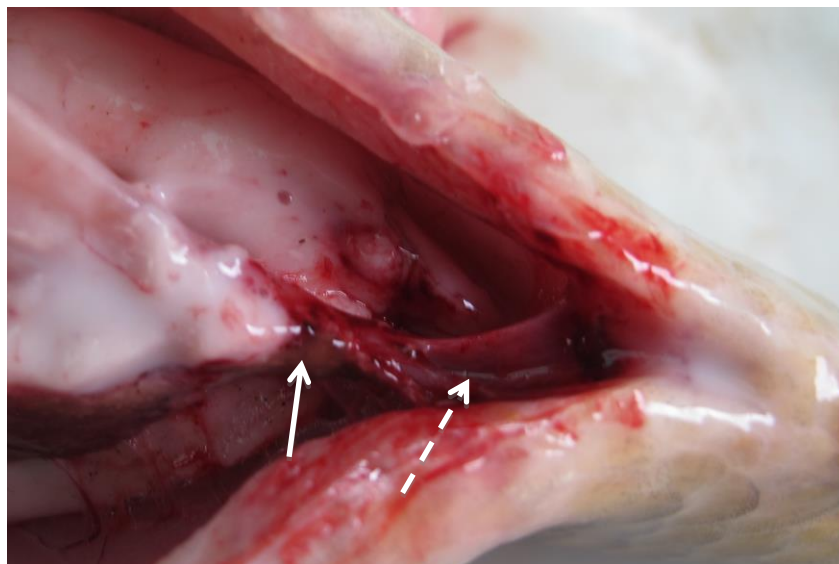


Figure 25. Photograph showing fusion of testicular lobe to the intestinal tract (solid arrow) in an individual that continued to express milt following surgery. Dashed arrow points to the stream of milt flowing via the intestinal tract.



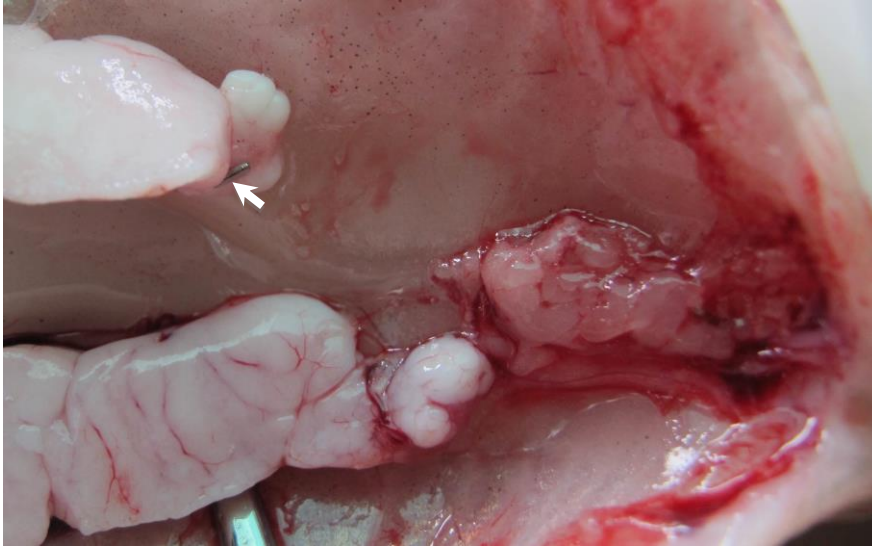


Figure 26. Photograph of an unsuccessfully sterilised individual in which one lobe (left) was successfully tied, whilst the other had established the connections. Note the clip in place (arrow).

# Discussion

While the Judas technique to capture feral carp has been successfully employed to eradicate carp in Lake Crescent, Tasmania, its use in the adjoining Lake Sorell had catastrophic consequences in 2009—contributing to mass recruitment events. This prompted the development of sterile fish with a view to deploy them as Judas fish that could continue to assist in capture of feral carp but would negate the risks associated with recruitment. With this objective in mind three different sterilisation approaches were explored—chemical, surgical and a non-surgical Essure technique—to sterilise carp. Discussed below are outcomes of the chemical and surgical sterilisation experiments, and the efficacy of the sterilised individuals to serve as Judas fish. The Essure technique was found to be less feasible in the circumstances and discontinued.

## Ultrasound facilitates reliable sexing of carp

In Tasmania the average body length of male and female common carp does not differ until 6 years of age as has also been reported elsewhere (Tempero et al., 2006), making it difficult to differentiate between males and females when selecting (pre-spermiating) fish for experiments. Moreover, the use of secondary-sexual characters was unreliable, particularly in case of sub-adults that were about to reach puberty. To circumvent this limitation we successfully adopted the use of ultrasound. Generally most males had relatively large gonad compared to females allowing selection of males for the experiment non-invasively. The high accuracy (91%) of the ultrasound to select/sex male carp—as demonstrated in the preliminary trial—was achieved by a conservative approach to cull/exclude any those that did not have discernable sized gonads. Consequently, this conservative approach contributed to the observed lower accuracy (54.5%) of sexing females. We believe that the accuracy of sexing/selecting males for both chemical and surgical sterilisation experiments was further increased, although this could not be determined in case of the former as many of the chemically treated individuals are still being retained for longer observations. If any indication, no fish was rejected on count of wrong sex during the surgical sterilisation experiments. However, sexing simply based on the size of the gonads could result in errors more so whilst adopting the technique to mixed cohort of populations, an aspect that needs further investigation. The consequences of misidentifying the sex could have different implication depending on the application. For example, in case of a surgery, it could cause undue delay to the operation, as those discovered as of wrong sex will have to be discarded partway through the surgery. In contrast, the consequence of wrong identification of sex in chemical sterilisation experiments could remain undetected for a long time, compromising the integrity of experiments.

## Effects of Superlorin® do not mimic mammalian observations.

Chemical sterilisation was explored as a rapid and more humane approach and involved administering Superlorin®, a commercially available GnRH agonist that has been known to cause temporary sterility in many mammals including canine (Trigg et al., 2006), feline (Risso et al. 2012) and porcine (Kauffold 2010) animals. The approach was pursued despite the knowledge that Superlorin® causes repression of testosterone in mammals and therefore could compromise reproductive behaviour of chemically sterilised Judas fish, most particularly for their ability to associate and signal breeding aggregations. However, they were expected to assist in locating general aggregations such as those driven by thermal and gustatory cues.

The drug (Superlorin®) itself does not appear to have any anabolic or catabolic effect on the carp as the mean weight and length, and the condition factors of the treatment groups including controls were comparable over the experimental period. There are no comparable piscine examples, however, similar observations on growth and general health of pigs (Kauffold 2010) and domestic cats (Risso et al. 2012) have been reported.

A higher proportion of spermiating individuals in the placebo controls may imply that the drug was able to repress sperm production in the treated individuals. Nonetheless, complete repression of sperm production in all the treated individuals was not achieved, despite using doses three times those recommended for most canine and feline domestic pets. Moreover those individuals that expressed milt did not show any sign of compromise in the quality of the sperm as determined by spermatocrit and sperm mobility assays. This outcome is in contrast to many previous studies in mammals including ferrets (Schoemaker et al., 2008), cats and queens (Munson et al., 2001; Rubion and Driancourt, 2009) where complete suppression of sperm expression was observed in all animals implanted with Suprelorin®. However, in dogs such repression was not observed in 10% of the treated animals (Trigg et al., 2006), suggesting that the efficacy of the drug may vary from animal to animal, depending on the physiological status of the animal at the time of administering the implant as well as the dose administered. In this study, the lowest proportion of spermiating fish were observed in fish treated with two implants (18.8 mg). It is therefore likely that two pellets of Suprelorin® are approximating to an optimal dose for sterilising male common carp. Nevertheless, detailed investigations using different doses and longer observations are required to fully elucidate the efficacy in repressing sperm production and hence reliable sterilisation.

Interestingly, the steroid hormone 11-KT measurements revealed the presence of two subpopulations of males—those that express high (H 11-KT) and low (L 11-KT) 11-KT. Coincidentally, all the spermiating individuals belonged to the H 11-KT group with none in the L 11-KT group. Based on the sacrifice of a small subset of individuals from both (H and L 11-KT) groups we are certain that these are natural grouping of males likely representing those that are beyond and under an as yet un defined threshold of maturity. The morphological status of the gonads in the two different groups could not be verified, as this would have required premature sacrifice. However, It is common for males of the same cohort to mature at different rates, often delayed by a year or two in the lakes (Diggle et al 2004; Donkers et al 2012). When these H 11-KT and L 11-KT groups were compared (between groups and over time) separately, only the former group showed an increase in the levels over time, but not between treatments. The rise in the 11-KT level in this group with the progression of the spawning season is consistent with the previous observations in this species (Saha et al., 2002) and turbot (*Scophthalmus maximus*) (Estevez et al., 1995), where 11-KT levels significantly increased towards the end of breeding season. However the observation that superlorin® treatment did not suppress 11-KT levels is in contrast to those that observed repression of testosterone levels in ferrets (Schoemaker et al., 2008), dogs (Triggs et al., 2006), pigs (Kauffold 2010) and cats including queens (Munson et al., 2001; Rubion and Driancourt, 2009; Risso et al. 2012). This may imply that the Superlorin® acts via an entirely different pathway to repress reproduction in fish or it may not suppress reproductive pathways at all in fish. However, the observation that a low number (only two fifths) of the H 11-KT individuals expressed milt following Superlorin® treatment may support the former hypothesis. It is also likely that the choice of 11-KT as a surrogate to measure the effects of Superlorin® is inadequate and more direct surrogates such as the luteinising hormone could serve as better reporter of the Suprelorin ® effects in fish.

A hypothesis that Superlorin® may be acting via an entirely different pathway in fish may be supported by the unusual morphological abnormalities observed in some of the treated individuals, where typically the posterior region of the testis was consistently affected blocking sperm expression, albeit showing slightly different abnormal patterns in different individuals. Such an outcome of uncompromised 11-KT activity and gorged gonads yet with blocked sperm expression in a proportion of the treated individuals was unexpected. Contrary to our prediction, the outcome presents a potential to deploy chemically sterilised Judas fish that are not compromised in reproductive behaviour, thus assisting in locating breeding as well as general aggregations of carp in the wild. However, a detailed and long term observations are warranted to elucidate as to why only some individuals may be prone to such sterility and what pathways may be triggering the observed gonadal abnormalities.

## Surgical Sterilisation does not impair physiological responses of carp.

Although gonadectomy or castration has been carried out in many species of fish most of these are limited to short term physiological and behavioural observation with little emphasis on long term survival and their use in fisheries management. Here we sought to develop a surgical approach that would result in reliable long-term sterility in the species, so that they could be successfully deployed as Judas carp to assist carp eradication programme in Tasmania. The challenge was to block sperm expression, whilst keeping most of the testis intact to minimise physiological and behavioural impairments.

Fish require special consideration during surgery. The incision must be kept out of water and yet the skin must be kept moist and gills continuously irrigated. Many detailed surgical apparatus and procedures to achieve this have been reported, but our adaptation of using a net cradle suspended in a plastic bin (Figure 1) and AQUI-S® (0.3 mg/l) as the anaesthetic agent provided a reliable means to carry out the surgery in field situations. This is supported by the observed high survival rate (95 %) compared to those previously reported in fish—generally about 50% with a rare exception of up to 87% (Bart and Dunham 1990). In addition to general (AQUI-S®) anaesthesia we recommend the use of local anaesthesia (0.5 % bupivacaine) at the site of surgery to override potential local pain responses (e.g. muscle twitch) or premature recovery during surgery.

While gonadectomy (surgical castration) can be readily achieved, an ability to retain most of the gonad and yet block sperm production can be quite challenging. This is particularly the case as carp demonstrate remarkable capacity for testicular regeneration. The repair including re-establishment of the testicular tracts observed in those individuals that were not successfully sterilised directly supports this (e.g. Figures 24–26). Similar capacity for high regeneration following complete gonadectomy in a closely related species of carp (grass carp *Ctenopharagadon idella*) has been reported (Underwood et al 1986). In this context, the observed sterilisation success rate of about 73% are quite significant. We attribute this success to the four-point (two on each lobe) microsurgery adopted, where in a tubular section of the testis was first secured (with either clip or suture) with the intervening section (2-3 cm) subsequently excised. However, in 27% of the individual the procedure was unsuccessful, suggesting that a significant improvement to the techniques can be made. In practice, the use of clips to secure the tubectomy wounds was the most feasible but it appears to be the least reliable when it comes to achieving sterility. This may be because the clips used were slightly larger for the tissue girth and hence did not pinch/secure the severed ends tightly in some individuals, allowing the free ends to regenerate despite a constriction at the site of the clips (see Figure 26). A slightly different scenario could be envisaged in those individuals where the severed ends were sutured. The regeneration here generally seemed to grow over the suture. Interestingly we note that the only individual in whom the tubectomy wounds were secured with electro-cautery was successfully sterilised. Conceivably, the electro-cautery seals the ends of the tissue making it less prone for regeneration. We therefore infer that use of electro-cautery could yield better results, albeit based on a single individual.

While the survival rate of the sterilised fish was high the fish did not grow well over the experimental period except during the first month following the surgery. The latter observation suggests that the surgery itself did not impair growth an aspect supported by a similar trend in growth of the control (non-operated) fish.

Similar to chemically treated fish we observed the presence of two subpopulations of males—those with high (H 11-KT) and low (L 11-KT) 11-KT levels. The observed comparable levels of 11-KT in sterilised H 11-KT and L 11-KT individuals with that of the corresponding H 11-KT and L 11-KT control groups suggests that the synthesis of the sex steroid was not affected by the surgical treatment. We therefore infer that the surgical procedures do not affect or impair the physiological and consequently behavioural traits of the sterilised fish and are likely to be similar to those of control fish.

## **Sterile Judas carp behave similar to controls and adult males in Lake Sorell.**

Carp has long been observed to prefer certain habitats and environments that are season specific, an aspect relatively well documented in lakes Sorell and Crescent (Taylor et al 2012). Consistent with these previous observations (Taylor et al 2012) the sterile Judas fish released during this study, immediately dispersed from the point of release, (some dispersing over 10 km) providing the first signs that the fish were not adversely affected by the surgery nor by the radio-implantation process. This was further confirmed by the long-term distribution patterns that show that the carp were distributed widely over the lake (Figure 22 and 23). The observed Kernel-density distributions are consistent with previous patterns (Taylor et al 2012) in the lake confirming that carp preferentially utilise shallow littoral habitat during summer months and often pushing into marshy habitat with abundant vegetation. The patterns also indicate that the sterile Judas carp displayed a tendency to aggregate on a shallow rocky shoal (AOI1: around ST Georges Island), another pattern that recapitulates the documented (Taylor et al 2012) spring-summer behaviour of carp in the lakes. Typically the distribution patterns of the control, and sterilised groups of fish overlapped, with some patterns unique to each of the group. The unique patterns are likely caused as a result of home range/territorial behaviour of individual fish and are unlikely to be influenced by the surgical sterilisation process. This is supported by over-lap of the observed unique patterns with those previously reported by Taylor et al (2012) in the lake. The patterns unique to individual fish have been attributed to resident or mobile behaviours of individual fish in the lake (Taylor et al 2012). The capture of three sterile Judas fish (coincidentally one from each group) in fyke nets (passive gear) placed along the barrier nets protecting access to spawning habitat is also consistent with the behaviour of adult males pushing into prime breeding habitat.

Collectively the observations suggest that the sterile Judas carp were able to readily assimilate into the lake population and behaved more or less similar to the control Judas fish, consistent with established behavioural patterns typical to spring-summer breeding season in the lake.

## **Sterile Judas fish contribute to capture of feral carp.**

The Judas technique has been a corner stone of the carp eradication program in the Tasmanian lakes, contributing to about 63 % of the fish captured in Lake Crescent since 1997 to their eventual eradication from the lake in 2007.

As predicted the sterile Judas carp aggregated (3 or more radio-tagged fish within 50 m radius) on multiple occasions further supporting the argument that they behave similar to any carp. The aggregations triggered targeted fishing events and assisted the capture of feral carp. The collective contribution of the Judas carp (control and sterile Judas carp) to the total carp captured during the study period is relatively low (20%) compared to those previously observed in Lake Crescent (~63%). This may simply reflect the short-term (5 months) nature of the current study compared to the lake Crescent observations (over 12 years). It is likely the proportional contribution of Judas fish to capture feral carp will increase as the population in the lake declines an inference consistent with the observation in Lake Crescent. Alternatively the reduced efficiency of the Judas carp may simply reflect the differences in fishing techniques employed. For example random over night gill net fishing was employed in the lake for the first time and coincided with this study period. Coincidentally the gill nets have been capturing bulk of the carp currently caught in the lake (~52%). However as the fish numbers in the lake decline the random gill net efficiency is likely to decline. The use of Judas fish becomes all the more important to lead to and capture the “smart carp” with learnt behaviour to evade capture by passive gears such as the gill net. Such gear avoidance behaviour by “smart carp” is well documented (Diggle et al 10130. In such circumstances the reliance on Judas fish to locate aggregation, particularly breeding aggregations and their timely removal becomes all the more important for eradication to be successful—as was the case in Lake Crescent (Diggle et al 2013).

# Conclusions.

This project developed and demonstrated the utility of sterile Judas carp to assist in targeted capture of feral carp at Lake Sorell Tasmania. The sterile Judas carp have become an integral part of the Tasmanian carp eradication program.

Although the Suprelorin<sup>®</sup> treatment (chemical sterilisation) did not result in repression of semen production in all the treated individuals, several remained milt free despite having high levels of 11-KT profiles and gorged gonads that are typical of control (milt expressing) males. The blocking of milt/sperm expression in a proportion of the treated individuals appears to be facilitated by gonad abnormalities typically at the posterior region of the testis. Contrary to our earlier expectation, such sterilised individuals with uncompromised sex steroid levels are likely to assist in locating reproductive as well as other general aggregation of carp in the lake. The observed gonadal deformities, may imply that the Suprelorin<sup>®</sup> may be acting via an as yet unknown pathway in fish that requires further investigation.

The tubectomy did not have adverse effect on growth, general health or physiological responses (hormone levels) of the treated individuals. The sterilisation success of over 70% sustained over a relatively long period (10 months) appears without precedence and is hence quite significant. Of the three approaches adopted to tie the severed tubular testis ends, clipping was the most practical, but resulted in slightly lower success rates. The electro cautery is conceivably more promising but requires specialised equipment. A combination of clipping and electro cautery is likely to increase sterilisation efficiencies further.

The movement and distribution patterns of radio-tagged fish suggest that the sterile Judas carp (both chemical and surgical) behave similar to controls, and are consistent with historical habitat choice preferences of carp typical to spring-summer season in the lake. The sterile Judas (coincidentally a representative each of chemically and surgically sterilised fish) fish were caught in fyke nets (passive gear) placed along the barrier nets protecting access to spawning habitat. Such capture susceptibility is consistent with the behaviour of adult males pushing into prime breeding habitat.

Collectively the results suggests that is possible to sterilise carp both chemically and surgically, in a manner that do not compromise their behaviour allowing continued deployment of Judas technique for management or eradication of carp populations, more significantly negating the risks of their contribution to spawning and recruitment.

# Implications

The project successfully developed and demonstrated the utility of sterile Judas fish, replacing the practice of employing reproductively active male Judas fish in the management and eradication of carp in Lake Sorell Tasmania. The sterile Judas fish appear to retain all traits associated with the Judas technique, whilst mitigating the risk of their contribution to inadvertent spawning.

The benefits of the technique and outcomes of this project to the Tasmanian carp management program have been immediate and multi-fold. In the first instance it has allowed the continued risk free deployment of the Judas carp technique for efficient fishing of the remaining carp from Lake Sorell and is expected to contribute to the eventual eradication. The monetary benefits of eradicating the carp from Tasmania are significant. Typically the management program itself costs about 800K/per annum and any spawning/recruitment event sets back the program by about 7 years. In this context, the practical value of the sterilised Judas fish is immense as they eliminate the risk associated with spawning. More broadly, the collective benefits to the lucrative recreational fisheries of Tasmania and native biodiversity are immense.

The surgical and technical procedures developed during the course of this project have already attracted national and international attention with a desire to adopt the approaches for controlling Koi carp in New Zealand and bighead carp in the USA. These techniques are easily transferrable and can be improved for application elsewhere including mainland Australia, where carp are a problem.

The project also contributed directly to the education outcomes through tinging of an honours student and two trainee veterinarians.

From the perspective of basic biology, the project provides the first evidence that Superloin® can cause sterility in fish but its contrasting mode of action compared to mammals, suggests an as yet unidentified pathways of its action in vertebrates.

# Recommendations

Based on the outcomes of the project we make the following recommendations:

- Should the intention be to adopt these tools elsewhere, we recommend that the sterilised animals be monitored for a sufficiently long time (over months) to verify the sterility.
- Combining the electro-cautery and clipping techniques could enhance the efficiency of surgical sterilisation further.
- Noting that chemical sterilisation resulted in sterile individuals without compromising the hormone levels, we recommend that it could have broader applications than previously thought—including assisting in locating breeding aggregations.
- The Tasmanian carp management program preferentially use the sterile Judas fish in the management and eradication of carp from the Tasmanian lakes.
- The sterile animals generated as part of this project but not yet deployed be maintained and monitored on a regular basis, so they can serve as a reserve pool for deployment in the future.

## Further development

The efficiencies of surgical sterilisation are likely to increase significantly by integrating electro-cautery and clipping. Similarly the unexpected outcome of chemical sterilisation without compromising hormone levels offers greater promise as a more powerful and humane approach for sterilisation of carp. We recommend that both these approaches be refined further.

Although we attempted Essure® as a non-surgical sterilisation approach, it was discontinued owing to difficulties associated with its administration—largely due to lack of access to relatively expensive endoscopic imaging tools. It however, remains a very potent tool and has the potential to circumvent the need for longterm post surgery observations.



# Extension and Adoption

Even before the commencement of the project the concept was presented at the national carp management meeting in Melbourne and the two annual (2011 and 2012) Tasmanian carp review meetings.

Following inception, the project progress was communicated and updated regularly through the quarterly report of the Tasmanian carp control program, which is circulated electronically both nationally and internationally (over 100 subscribers). A formal presentation of the progress was provided at the subsequent annual carp review meetings (2013 and 2014) in the presence of local and national stakeholders, notably the then state fisheries minister. A public poster/display presentation was also made at the 2013 annual IFS open weekend in Liaweene, Tasmania (18–19 May), which attracted in excess of 4000 visitors over two days. A similar display providing an update of the project was undertaken at the 2014 annual IFS open weekend (17-18 May 2014).

The project also attracted an invited article in The Australian Veterinary Journal “Sterile Judas carp set to betray their own in Tasmania 2014. 92:N16-17.”

An oral presentation titled “Sterile Judas carp—surgical sterilisation does not impair growth, endocrine and behavioural response of male carp” was presented at the Tenth International Symposium on Reproductive Physiology of Fish (Olhao, Portugal 25-31 May 2014).

Most significantly, the sterile Judas fish generated by the project have become an integral part of the Tasmanian carp management program.

## Project coverage

The project received media coverage on multiple occasions and a list of which is summarised below.

No.	Date	Title	Type
1	10/10/2013	Sterile carp receive radio transmitters in ongoing fight to eradicate <a href="http://www.ifs.tas.gov.au/news/sterile-carp-receive-radio-transmitters-in-ongoing-fight-to-eradicate">http://www.ifs.tas.gov.au/news/sterile-carp-receive-radio-transmitters-in-ongoing-fight-to-eradicate</a>	Media release
2	16/10/2013	Sterile Judas carp released into Lake Sorell <a href="http://www.ifs.tas.gov.au/news/sterile-judas-carp-released-into-lake-sorell">http://www.ifs.tas.gov.au/news/sterile-judas-carp-released-into-lake-sorell</a>	Media release
3	26/11/2013	Judas carp set to eradicate pests in Tasmania (In: Get Farming Australia) <a href="http://www.getfarming.com.au/pages/farming/articles_view.php?fid=9200020131126120348">http://www.getfarming.com.au/pages/farming/articles_view.php?fid=9200020131126120348</a>	News Article
4	4/11/2013	Female carp targeted in Lake Sorell eradication campaign <a href="http://www.abc.net.au/news/2013-11-04/female-carp-targetted-in-lake-sorell-eradication-campaign/5066704?section=tas">http://www.abc.net.au/news/2013-11-04/female-carp-targetted-in-lake-sorell-eradication-campaign/5066704?section=tas</a>	ABC TV News
5	5/11/2013	Carp eradication campaign in Lake Sorell Link shared by Minnesota Invasive Species Centre	Facebook

# Project materials developed

1. Sangtian, M. 2013. Efficacy of GnRH agonist deslorelin (Suprelorin<sup>®</sup>) to sterilise common carp *Cyprinus carpio* (Linnaeus, 1758). A thesis submitted to the University of Tasmania. pp 74.
2. Patil J.G. 2014. Sterile Judas carp set to betray their own in Tasmania. Australian Veterinary Journal. 92: N16–17.
3. Patil J.G. Purser G.J and Nicholson A. (in review). Sterile Judas carp: surgical sterilisation does not impair growth, endocrine and behavioural responses of male carp. General and Comparative Endocrinology.

# Appendices

List of researchers and project staff

Name	Role	
Jawahar G Patil	Principal investigator	
John Purser	Co-investigator	
Andrew Nicholson	Consulting Veterinary Surgeon	
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