

Hypophysation: A technique for deployment of odour donor fish for control of the common carp (*Cyprinus carpio*).



## **Technical Report No. 5**

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***This report is part of a series of documents, which provide information and details of carp eradication efforts in lakes Sorell and Crescent as part of the Lakes Sorell and Crescent Carp Management Project.***

The aim of the project is to control the spread of carp within the state of Tasmania, with a view to their eradication.

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## **1. BACKGROUND**

The Inland Fisheries Service (IFS) has successfully contained carp within lakes Sorell and Crescent since 1995 when they were first identified in these waters. (Diggle et al. 2004). The Carp Management Programme (CMP) has significantly reduced the numbers of carp over this time using an integrated management approach. Multitude of fishing gears and techniques were used either in isolation or in combination to keep the carp number low in the lakes. This includes biotelemetry, gillnets, barrier nets, fyke nets, seine nets, traps, backpack electro-fishing, and boat electro-fishing (Diggle et al 2004).

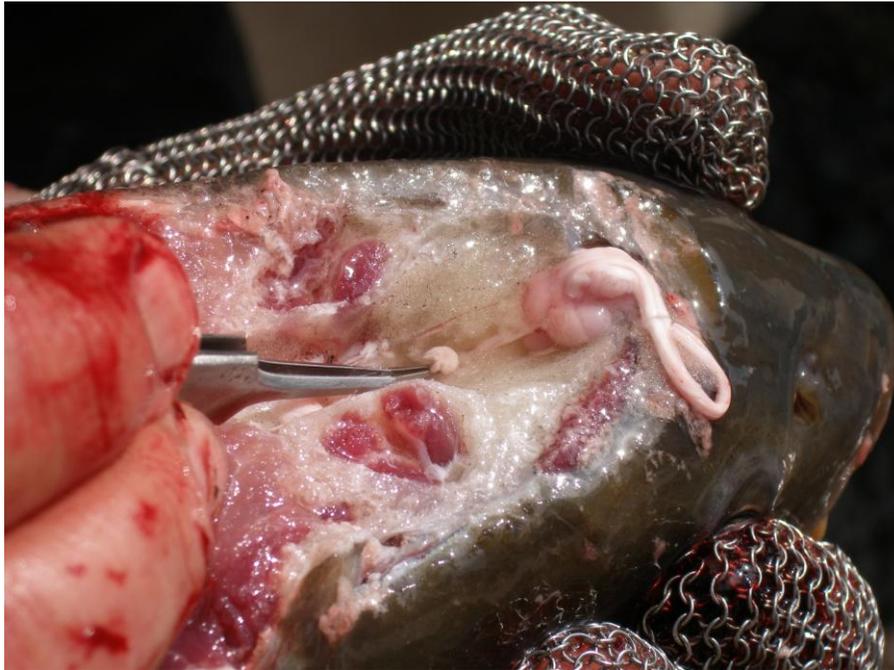
However it has been found that once the population drops, the tendencies for fish to aggregate in significant numbers (above 30) are limited, making removal of the last few remaining carp challenging (Diggle et al 2004). Therefore development and or recruitment of additional but environmentally benign techniques has been an ongoing pursuit of the CMP to assist carp eradication in Tasmania. Because the mature carp present the most risk and are difficult to locate in smaller populations, an approach that facilitated their attraction to and trapping at pre-designated sites was seen as a key strategy. To facilitate such attraction and trapping, a series of hypophysation trials were conducted during the course of spring and summer of 2005/06.

The hypophysation or injection of pituitary extract is one of the techniques employed to induce breed fish (e.g. Jhingran and Pullin 1985). This involves removing the hypophysis (pituitary gland) from a donor, its preparation and injection into another mature fish (female or male) to assist final maturation and spawning. We adopted this simple technique to induce a sexually mature carp in anticipation that such induction would lead to release of pheromones which when carried by water plumes would attract and trap feral mature carp at pre-designated sites. The trials conducted during 2005/06 were successful in attracting and trapping carp and hence the technique has become an integral part of the Integrated Pest Management Programme (IPM) at the lakes. Outlined below are the details of procedure that may be adapted to other carp control programmes in Australia or elsewhere where carp are deemed a pest.

## 2. MATERIALS AND METHODS

### 2.1. Removal of Pituitary Gland from Donor Fish

- Select sexually mature and unspent donor fish (male or female) as pituitary donors.
- Anaesthetise the donor fish using a suitable anaesthetic (e.g. phenoxy ethanol or AQUI-S) until deeply sedated (non-responsive to gentle squeeze on caudal region) and sacrifice.
- Decapitate the fish and remove the roof of the cranium by sawing in the anterior to posterior direction, well above the plane of the eyes to expose the brain.
- Wash and remove the fatty tissue away, hold the hind margin of the brain with a pair of fine tweezers, gently lift and fold the brain back on to itself (posterior to anterior direction) to expose the pituitary gland lying directly under the brain (Figure 1).



**Figure 1. A pair of fine tweezers pointing to the exposed pituitary gland.**

- Wash the surrounding tissue debris by rinsing with clean water before carefully removing the pituitary gland. It is important to ensure that the gland is not damaged

or perforated during collection (Figure 2), as gonadotropins (core inducing agent) may be lost easily during storage.



**Figure 2. Collection of pituitary gland**

### ***2.2. Storage of Pituitary Glands***

Pituitary glands that are not required for injection immediately can be stored for as long as 6 months using reagent grade acetone. For long-term storage first place the pituitary glands in airtight vials containing acetone for 24 hours and then replace the acetone with a fresh aliquot. These vials can be stored at room temperature or refrigerated until required (up to 6 months).

### ***2.3. Preparing and Injecting the Pituitary Extract***

- Just prior to induction of an odour donor fish, take out the stored pituitary glands from their respective vials and place on tissue paper to air dry. If working with fresh glands use them directly for extraction.

- Weigh the required amount of pituitary gland (based on the weight of the recipient fish: 4-6 or 10-16mg/kg body weight for male or female recipient respectively)
- Carefully transfer the weighed glands to a microvial (microfuge tube).
- Add sterile double distilled water (~200  $\mu$ l) to each vial and grind the tissue finely using a micro-pestle (Figure 3).



**Figure 3. Grinding pituitary gland in microvial**

- Following grinding more distilled water was added to achieve a concentration of approximately 40 mg/ml of pituitary extract and mixed thoroughly.
- The pituitary extract was then centrifuged at approximately 1-2K RPM for about a minute to separate the tissue debris from the extract (supernatant).
- A required amount of the clear supernatant was then drawn into a syringe (1-3cc / 21-25g 1.5-inch needle) for injection.
- The recipient mature fish (Figure 4) was then injected via the intramuscular route in the anterior caudal region (Figure 5).



**Figure 4. Example of healthy female recipient carp**



**Figure 5. Injection of supernatant in anterior caudal region**

#### ***2.4. Release of Induced Odour Donor Fish.***

Suitable sites with water plumes flowing through the holding pen (green bag, Figure 6) and traps (metal frame, Figure 6 middle) were selected for the trial. Holding pens were placed directly behind fish traps, such that pheromone (attractant) released by the odour donor fish would be carried through the traps and into the lake. Suitable barriers (e.g chicken wire mesh, foreground Figure 6) were installed to avoid inadvertent access of attracted fish to the donor fish.



**Figure 6. Picture showing a haul of feral carp trapped following deployment of odour donor carp. Typically the holding pen (green bag) is placed behind a fish trap (metal frame in the middle) held directly in the path of a water plume flowing into the lake. Note the chicken wire mesh barriers (foreground) used to avoid any of the attracted fish accessing the holding pen.**

#### **4. GENERAL COMMENTS**

The trials conducted by the CMP during spring and summer of 2005 indicates that adult radio-tagged male fish can move from as far as ~6 km overnight to sites where the odour donor fish are deployed. Significantly some of these attracted fish (radio-tagged) were

trapped along with a number of feral carp that were never captured before. The approach seems particularly useful when dealing with small number of fish in the lake. In our experience the natural environmental stimulations, including rise in water temperature is critical for the success of the experiments. Generally these sexual attractions and trapping will work best during the breeding season (Austral spring-summer) coinciding with rising lake level and on days when the water temperature is significantly higher ( $> 15^{\circ}\text{C}$ ). A good knowledge of water plumes will be of great assistance. If natural plumes are not available, one could use pumps to generate suitable plumes.

## **5. OTHER INDUCTION OPTIONS.**

Recent advances in induced breeding of fish have resulted in development of several commercially available inducing products, in liquid (e.g. Ovaprim) powder (e.g. Human chorionic gonadotrpín) or pellet (e.g. Ovapalnt) forms—all involving gonadotropin as the core inducing agent. One could use any of these depending on the need and ready accessibility, following the instructions of the manufacturer. Either male or female fish could be used as the odour donor, depending on the situation and need.

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