Charles Darwin University, Animal Ethics Committee
Ellengowan Drive, Darwin, Northern Territory, 0909

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Standard Operating Procedure:

RIEL SOP 03.2021 Surgical Implantation of telemetric transmitters into teleost fish

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CHARLES DARWIN UNIVERSITY AUSTRALIA

Standard Operating Procedure for the surgical implantation of telemetric transmitters into teleost fishes

Introduction

Telemetry has become one of the most widely applied methodologies for studying the behaviour of fishes in the wild. Acoustic telemetry, in particular, has become increasingly utilised in recent years following the development of cost-effective, highly reliable transmitters and passive receivers, with hundreds of studies undertaken across the globe (http://vemco.com/map/). As the use of telemetry becomes more frequent in fisheries research, there is an increasing need for consistency in techniques for tagging fish with telemetric transmitters (Cooke *et al.* 2011). Extensive research has demonstrated that internal implantation into the peritoneal cavity has minimal effects on the behaviour and physiology of fishes, whereas external attachment methods can be problematic (e.g., biofouling, snagging, attraction of predators; Kyne and Pillans 2014).

This document aims to establish best practice Standard Operating Procedures (SOP) for the surgical implantation of telemetric transmitters into teleost (bony) fishes for staff and students at CDU. The SOP describes the sequence of events involved in the surgical implantation of transmitters and can be used by researchers in the development of animal ethics applications to ensure consistency and high standards of animal care.

In line with existing guidelines for the surgical implantation of telemetric transmitters into sharks and rays (Kyne and Pillans 2014), the SOP for teleost fishes recognises specific considerations that apply when tagging large fish (≥50 cm total length). These include: increased induction and recovery times associated with chemical anaesthetisation of large fish; the relatively small size of the incision and transmitter in large fish; the logistical difficulties of using very large holding containers for post-surgery recovery of large fish in the field; and the longer total time in distress for large fish resulting from the above factors. In recognition of these issues, the SOP recommends specific anaesthetisation procedures depending on fish size and species as outlined below. The surgical procedure for implantation of transmitters is identical for all sizes and species.

Anaesthetisation

This SOP recommends different approaches to anaesthesia based upon the size of the fish, and whether the species of concern exhibits tonic immobility (loss of body movement induced by being placed in an upside-down position). Because anaesthesia of the fish with AquiS

depends on the diffusion of AquiS from the water into the blood via the gills, larger fish >50cm long (with a large volume of distribution [Vd]) require a longer time to reach a therapeutic blood level as compared to a fish <50cm long at the same concentration. This is compounded by the fact that AquiS is lipid soluble and is likely that lipid components in the blood and adipose tissue act as a depot for this drug, increasing the time required for induction in larger fish. Recovery is also longer in larger fishes due to increased time for drug elimination.

This recommendation is based on consideration of the need to balance the potential infliction of pain in fish during the procedure versus the increase in total time in distress (i.e., total handling time) associated with the use of chemical anaesthesia:

- Anaesthetisation is necessary for transmitter implantation in small fish (<50 cm total length) of all teleost species.
- Anaesthetisation is necessary, for any sized fish in species that do not exhibit tonic immobility, for the purpose of surgery.
- There is no requirement for anaesthetisation of fish ≥50 cm total length in species that exhibit tonic immobility (e.g. barramundi).
- Use of local anaesthesia (e.g. lignocaine) is not recommended due to regulatory uncertainties regarding the use of chemicals in fish that may later be consumed as food.

To anaesthetise fish for transmitter implantation, they should be placed into a large plastic container (minimum: 60 cm length x 40 cm width x 35 cm depth) containing Aqui-S solution made up using water sourced from the location of capture. The active ingredient in Aqui-S, *isoeugenol*, occurs naturally in clove oil. It is the only chemical anaesthetic approved for use on fish with a zero withholding period in Australia.

Air should be bubbled through the Aqui-S solution via a diffuser to ensure it remains well oxygenated. The fish should be visually monitored at all times during anaesthesia, taking particular note of body movement and opercular movements. The concentration of Aqui-S in the container should be sufficient to induce stage III anaesthesia (loss of gross body movements, cessation of opercular movements) within 3-5 minutes (1.5 ml per 50 L). Once stage III anaesthesia has been reached, the fish should be immediately removed from the Aqui-S solution and placed into a soft foam cradle for transmitter implantation. The time taken to

reach stage III anaesthesia should be recorded for each fish. After use, Aqui-S solution should be diluted by a factor of >5:1 before disposal on site.

Handling and transfer of non-anaesthetised fish

Fish that are not subject to anaesthetisation should be handled using a non-abrasive landing net and placed immediately into a foam surgical cradle or a large plastic container containing fresh water (water from the location the fish is captured) until commencement of the procedure. Non-anaesthetised fish should not be held for more than 3 minutes prior to commencement of the procedure. If the fish is captured on a support boat, it should be placed in a container containing fresh water and immediately transferred to the surgery boat. All boats involved in fish collection should remain within 500 m of each other, preferably in two-way radio contact, to ensure that fish are transferred as quickly as possible. In this way, transfer to the surgery boat will always occur within 5 minutes of capture.

Tagging procedure

The tagging procedure is identical for anaesthetised and non-anaesthetised fish. Fish should be placed carefully in an upside-down position into a soft foam holding cradle lined with clean, absorbent cloth wetted with local water to avoid loss of protective slime. Straps should be placed across the fish to hold it in position and ensure that it does not fall from the cradle during the procedure (see Figure 1).

An assistant should irrigate the gills using fresh water throughout the procedure to facilitate uptake of dissolved oxygen. This can be done by pouring fresh water directly onto the gills via the branchial aperture (gill opening) using a small plastic container. Alternatively, water may be pumped over the gills using a portable pump fitted with a tap to regulate flow.

To reduce the risk of infection to the fish, the person conducting the procedure should wear new surgical gloves during each procedure. All surgical procedures should be carried out with sterile disposable scalpels and suture packs. Forceps and needle holders should be sterilized between surgeries by submerging them in alcohol and then Hibitane disinfectant (100 ml per L). Transmitters should be placed in Hibitane for 15 minutes prior to the procedure and rinsed thoroughly with sterile saline prior to implantation.

Once the fish is placed in the V-shaped foam cradle, the implantation site (located approx. 10 cm anterior to the anal vent and slightly offset from the midline) should be swabbed with Betadine. An incision of 15-30 mm length (depending on transmitter size) should be made in an anterior-posterior orientation into the peritoneal cavity using a No. 11 scalpel (or equivalent). This incision may require the removal of several scales.

The disinfected and rinsed transmitter (not exceeding 2% of body weight) should be inserted gently into the peritoneal cavity through the incision and the incision closed with a single layer closure using three interrupted sutures placed into the musculature 3-4 mm beneath the skin (Ethicon 2.0 metric, absorbable monofilament, 26 mm swaged needle). The incision should then be covered with Powder Gel Fish Bandage and Betadine applied to the area using a small syringe. Use of Powder Gel prevents the Betadine washing off and can provide up to a day of topical protection from bacterial and fungal infection. The entire tagging procedure should take 3-4 minutes to complete.

After each procedure, the equipment should be prepared for the next procedure to avoid delay when the next fish is collected. This preparation includes changing the scalpel blade, placing suture in the needle holders, replacing swabs, placing surgical equipment in disinfectant and replacing water in the holding/recovery tank.

Figure 1: Barramundi on soft foam surgical cradle, showing holding straps, absorbent wetted cloth, assistant pouring fresh water into branchial chamber, and completed procedure with 3 interrupted sutures.

Recovery and release of anaesthetised fish

Following transmitter implantation, anaesthetised fish should be transferred to a holding tank and held individually in a container (minimum: 60 cm length x 40 cm width x 35 cm depth) of aerated water sourced from the point of capture. The fish should be visually monitored at all times during recovery from anaesthesia, taking particular note of body posture and opercular movements. Once fish have regained normal upright posture and gill ventilation for at least 3 minutes, they should be released as close as practical to the point of capture using a non-abrasive net. The time taken to recover from anaesthesia should be recorded for each fish.

Recovery and release of non-anaesthetised fish

Recovery in non-anaesthetised fish should be immediate. Following transmitter implantation, non-anaesthetised fish should be placed into a large, non-abrasive net and held in the water (over the side of the boat or at the water's edge). The behaviour of the fish should be observed visually to ensure recovery from the procedure (normal upright posture and gill ventilation). Once recovery is established, the fish should be released as close as practical to the point of capture.

Euthanasia of injured fish

In the event that an individual fish is injured and that injury is perceived to be minor, the fish should be released a soon as possible without being subjected to any procedures (i.e. tagging). If an injury is significant to the point of preventing survival of the individual, or if a fish fails to recover from the procedure, it should be euthanised via anaesthetic overdose followed by Iki Jime (a metal spike through the brain). Fish should be left in the solution (175 mg/L, as per Aqui-S usage guidelines) for at least 20 minutes after equilibrium has been lost and gill movement has entirely ceased to ensure that death has occurred. After 20 minutes of anaesthetic overdose, Iki Jime (a metal spike through the brain) should be undertaken to ensure that the fish has been euthanised. Iki Jime is widely recognised as a humane way of killing fish.

References

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