Fish in research and telemetry tagging methods

The following is based on the papers, where most references can be found:

Wargo Rub, A.M., Jepsen, N., Liedtke, T.L., Moser, M. L. & Weber, E. P. S. (2014). Surgical Tagging and Telemetry Methods in Fisheries Research: Promoting Veterinary and Research Collaboration. *American Journal of Veterinary Research*. **75**: 402-416.

Cooke, S.J., Wilson, A.D.M. Elvidge, C.K. Lennox, R.J. Jepsen, **N**. Colotelo, A.H. & Brown, R.S. (2015). Ten practical realities for Institutional Animal Care and Use Committees when evaluating protocols dealing with fish in the field. *Reviews in Fish Biology and Fisheries*.

Jepsen, N., Thorstad, E.B., Havn, T. & Lucas, M. (2015). The use of external electronic tags on fish – an evaluation of tagging effects. *Animal Biotelemetry*.

Photos and graphics can be seen in a Powerpoint presentation:

https://norecopa.no/media/7586/jepsen-taggging.pdf

Wild fish as experimental animals

With increasing anthropogenic stressors on wild fish populations, it is imperative to study and monitor the impacts of human population growth and activity level on a variety of fish species. This is particularly relevant to fish given that aquatic ecosystems are among the most threatened and fish are among the most imperiled taxa. Wild fish deliver many important ecosystem services in addition to anthropocentric services such as being fished recreationally (i.e. catch-and-release) or harvested for consumption. As such, many stakeholders relate closely with fish through some level of consumptive or non-consumptive exploitation . Fish have immense cultural and spiritual value in diverse human communities and provide economic livelihoods and sustenance for some of the most impoverished peoples on the planet. Many fish populations are actively managed by natural resource agencies that monitor the people, habitats or the fishes themselves. Maintaining the diverse values that fishes have to humans, while also maintaining their welfare, is one amongst many powerful examples of, at times, competing interests. Nonetheless, it is frequently the case that what is beneficial for the welfare of individual fish is also beneficial for fish populations and connects fish welfare and animal care concerns to population levels and ecosystem services.

All researchers using wild fish in their studies should consider the 3 R's. Because the large majority of studies aim at gaining knowledge of fish performance/behavior under natural conditions, **replacement** is not very relevant. **Reducing** numbers is always an issue, not the least because of the price of tags, and close considerations are done to keep numbers as low as possible and still get statistically significant results. It should be noted that compared to conventional tagging studies on fish, where thousands of fish were regularly tagged, electronic tagging studies rarely include more than hundred individuals. There is, however, a large scope for **refining** the methods on capture, handling and tagging wild fish, thus reducing the impact in terms of stress, fear and suffering on each individual. This will be discussed in the following..

Capture and handling of wild fish

In most countries, the use of wild fish must only be done under permission from an experimental animal committee or similar. Capturing fish in the wild, to be used for biological research, requires the highest regard for the integrity of the fish in question. To achieve this, deep knowledge of the species and life-stage/size/sex is necessary, because the physio-chemical requirements and robustness to handling is highly variable amongst even related species. However, there are some general issues to always be considered when catching and handling fish to minimize the stress and suffering of the fish. This issue is further discussed below, but specifically for capturing a few important points will be mentioned here.



Trapping and electrofishing is the most commonly used methods for gentle capture of experimental fish.

A short intro to fish telemetry

Use of electronic transmitter and monitoring systems to track movements of aquatic organisms has increased continuously since the inception of these systems in the mid-1950s. Within two decades, telemetry techniques were firmly established and in common use worldwide. By 1975 researchers were tagging hundreds of individuals from 40 fish species. Since then, telemetry has been used to investigate the movements of tens of thousands of individual fish, in addition to aquatic mammals, reptiles, amphibians, and invertebrates.

Tracking tagged fish in a lake

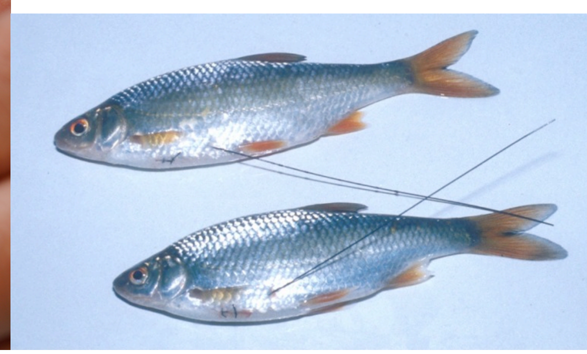
The earliest investigations used both radio and sonic (also known as acoustic) technologies. Both radio and sonic transmitters were comprised of electronic components and a battery potted in epoxy or encapsulated within a water-tight casing. Most radio transmitters also have a trailing antenna (sticking out of the fish) to increase transmission range.

A common attachment method for both types of transmitters involved external pins, wires, or sutures threaded through the dorsal musculature of the animal. Alternatively, the transmitter was inserted into the stomach (gastric implant). As telemetry was applied to more species, new external attachment methods were developed to accommodate a variety of body forms and habitats.

With the advent of long-lived, miniaturized acoustic transmitters in the 1970s, fish researchers turned to surgical implantation as a means of transmitter attachment. Implantation of transmitters directly into the body cavity had several advantages: it would eliminate the effects of drag experienced by fish with externally mounted equipment and would reduce tag loss associated with both external attachment and gastric implant (through regurgitation). Surgical implanting of radio tags is rather invasive and involves a trailing antenna where the antenna is passed from the body cavity out through the body wall to trail alongside the fish. A shielded needle technique was eventually devised to reduce the risk of damaging internal organs during this step and to improve post-operative recovery and healing. At this time, recommendations were also formulated for maximum transmitter weight relative to fish body weight, and the stage was set for rapid expansion of fish research that relied on telemetry.

Fundamental to telemetry studies is the underlying assumption that fish bearing transmitters behave in a manner that is representative of their untagged conspecifics. Early research efforts focused primarily on methods to reduce fish mortality after tagging and to increase the length of time that fish were capable of apparently normal movement. However, as the variety of transmitters increased and the scope of tagging studies expanded, the need increased for attachment techniques that minimized sublethal tagging effects. Many fish researchers embarked on studies to document the effects of transmitter attachment on a variety of fish functions (feeding, swimming capacity, buoyancy control, spawning behavior, susceptibility to predation, etc.).With these advances, fish researchers have gained new insight into both the capabilities and limitations of fish telemetry techniques.



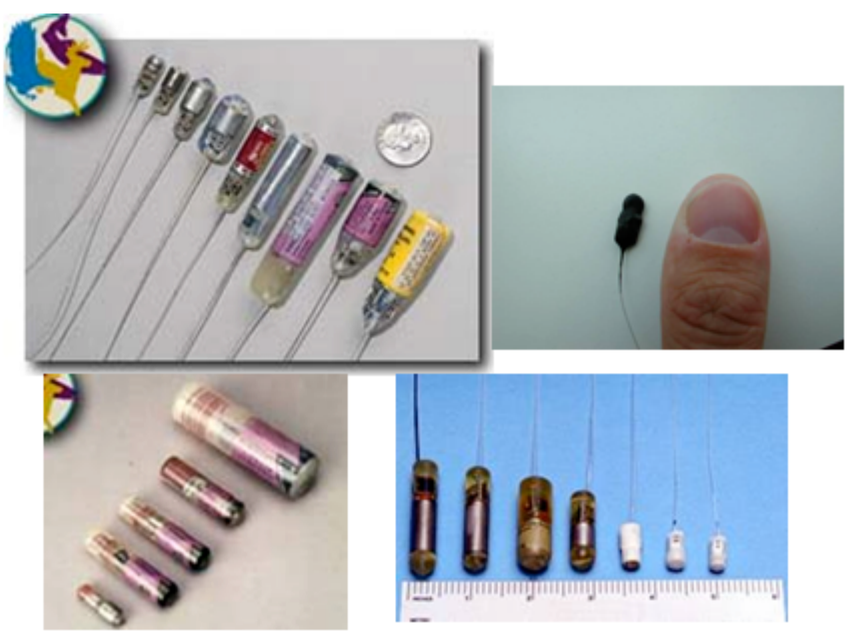

Typical field set-up for tagging of wild fish. Examples of external attachment and surgical implanting.

Contemporary Telemetry Studies

Today, fisheries researchers can choose from a wide variety of electronic tags to meet their monitoring needs, including radio-frequency ID tags, such as the passive integrated transponder (PIT) tag, and active radio or acoustic transmitters. A variety of sensors such as depth, temperature, and muscle activity can also be incorporated into or attached to telemetry transmitters. Thus, information can be archived for later retrieval or actively transmitted along with the tag's unique identification code. However, the fish must ultimately be recaptured to retrieve archived data.

Contemporary study designs range from basic monitoring of a few fish (10-30) over relatively short distances, to more complicated monitoring that may involve thousands of fish (10,000-30,000). These larger designs may evaluate performance over hundreds of kilometers and/or through multiple points along a migration corridor, such as fish passage structures, spillways, or turbines at dams.

Many telemetry studies are conducted on threatened or endangered species in an effort to provide resource managers with sufficient information to design and implement appropriate management actions. Telemetry allows researchers to collect data that is virtually impossible to obtain through other approaches, and holds the promise of precise measures of performance. As such, use of this technology is becoming more commonplace. Due to this widespread use, researchers are under increasing pressure to demonstrate that neither transmitters nor tagging protocols significantly affect the results of their studies or compromise the ethics on experimental animal welfare. Protocols for transmitter attachment in particular have come under increased scrutiny by government agencies, funding organizations and even professional journals, which stipulate that tagging methods must be demonstrably humane as well as scientifically sound.



Different types of electronic tags.

A 2004 survey of researchers actively using surgery for fish telemetry deployment indicated that they had learned transmitter implant techniques primarily from a combination of observation, various literature sources, and mentors. Less than 10% of respondents had received professional instruction from an educator or veterinarian through an academic or professional development course. Thirteen percent of respondents admitted that they had performed implant surgeries with no prior practice.

Fish Handling in Field Studies

Fish tagging are most often performed on the deck of a boat or at a makeshift tagging station on a dock or near the water's edge (see photo). Surgery locations are often remote and without amenities such as power and fresh water, thus presenting logistical challenges to the researcher. As such, the most important considerations regarding study design are often the most simple and related to fish handling rather than refined tagging technique.

Overall, the goal is to minimize handling, and the importance of basic planning to achieve this goal cannot be overstated. Correct handling can mean the difference between a successful tagging study and one that fails, regardless of the surgical procedure. Simply netting fish and removing them from the water has been shown to elevate stress levels measured as plasma levels of cortisol and glucose. Further actions such as chasing, netting, and crowding fish can exacerbate these responses. In many field studies, fish are tagged at the sampling site and are held only long enough to allow recovery from anesthesia prior to release. In these situations, researchers should always plan ahead to ensure that adequate containers and tank space for holding study fish prior to and after surgery, as well as to facilitate transfer through each stage of the procedure are available onsite. Some simple measures will help ensure that stress-inducing situations are minimized.

Frequent water changes are also an easy way to ensure consistent temperature. As a rule of thumb, water temperature in holding containers should be maintained within 2°C of source water temperature. Transfer of fish between holding tanks/containers should be kept to a minimum, and sanctuary nets should be used if possible. A sanctuary net contains a reservoir in the bottom, which retains water to ensure that netted fish remain submerged. The reservoir also protects fish skin and eyes from rubbing or bumping against webbing. Gently cupping fish in the palm of one's hands can also serve as an effective means of benign transfer. Researchers should be conscious of possible scale and mucus loss, continuing to handle fish gently when placing them onto measuring boards, balances, or surgery platforms. In some species, scales must be removed to facilitate surgery. During all procedures, the gills and skin should be kept moist.

Aseptic Techniques in fish surgery

For homeothermic animals, the practice of aseptic technique is considered a routine component of any surgical protocol. However, this is not always the case for fish telemetry implantation surgeries. Practicing aseptic technique and maintaining asepsis in an aquatic (and often remote) environment can be challenging for researchers. Furthermore, due to the sensitivity of fish integument to most disinfectants and sterilants, certain aspects of aseptic practice (such as applying chemical disinfectants to the incision site) can be counterproductive or even harmful. For these reasons, aseptic protocols have been inconsistent and less stringent for fish implantation surgeries conducted in the field than what one would expect for similar procedures conducted on homeotherms. There is a lack of evidence in the literature that pathogen transmission has actually occurred as a direct result of surgical tagging. Although infections in fish after tagging have been reported, these were described as secondary in nature rather than introduced through a breach in asepsis. Of the few surgical tagging studies that have been designed specifically to evaluate the infection risk associated with their methods, all have concluded that aseptic practices such as sterilizing transmitters and surgical instruments, were without merit for fish tagged under "normal" conditions.

The results from these have prompted some researchers to ask the question: Should requirements for asepsis be relaxed for fish implant surgeries under certain conditions? Considering the increase in telemetry work being conducted with endangered and threatened fish populations, refining tagging practices may be critical for successful long-term monitoring. Fortunately, despite the actual and perceived challenges presented by telemetry implant studies, there are general aspects of field based tagging procedures that are similar to those presented in a veterinary hospital. These include disinfection of the surgical environment, surgical instruments, and transmitters. Standard aseptic practices can be adapted under each of these headings to develop protocols for individual projects that are effective, and practical.

Surgical field and antibiotic prophylaxis

Most implant surgeries are conducted with the fish out of water and positioned in dorsal recumbence while anesthetic is applied over the gills. The area of the incision can be protected with a surgical drape or plastic wrap, provided it is kept moist. Draping is particularly useful for prolonged surgeries where the incision is relatively large and fish are kept out of water for longer than a few minutes. Plastic drapes are well suited for these longer surgeries, as they are both waterproof and easy to use in field settings. Researchers should keep anesthetic irrigation water localized near the head and opercula so that it does not flow into the incision. If the anesthetic is applied as a bath, care should be taken to ensure that the surgical platform is angled, with the incision kept well above the bath mixture.

A variety of disinfectants and antibiotics have been utilized for prophylaxis in fish surgery. However, none of these agents has been recommended for use in mitigating the effects of implant procedures. Disinfectants can irritate or damage skin, and both disinfectants and antibiotics may delay healing and enable or promote growth of opportunistic pathogens such as fungi. More tolerable agents such as iodine compounds have not been shown to be effective, either in promoting or preventing secondary infection after surgical incision.

Anesthesia

Often external tagging can be performed without anesthesia, but surgical implants are always performed on deeply sedated fish. Fish generally respond with a strong "flight" response when they are netted and handled. Therefore, some form of general anesthesia/sedation prior to handling is considered a best practice for surgical implant procedures on fish, particularly for small fish and juvenile life stages. In addition to reducing fish movement during the procedure, a general anesthetic will minimize handling stress resulting from physical restraint and will increase safety for both the fish and the researcher. From an animal welfare perspective, guidelines from the American Fisheries Society, the Canadian Council on Animal Care, and most US University IACUC committees currently recommend that surgical implant procedures be performed under general anesthesia whenever possible. However, whether or not fish cognitively perceive and suffer from painful stimuli is still being debated. If used, anesthetics should be administered to provide the lowest stage and lightest plane of anesthesia possible to safely conduct the specific procedure. For most procedures, this would be defined as the point at which there is loss of equilibrium and failure to respond to all levels of tactile stimuli experienced during the surgical procedure.

Chemical or bath-immersion is the most frequently practiced mode of inducing anesthesia. Once fish are anesthetized to a surgical anesthetic plane, they can be removed from the induction bath and easily transferred to a surgical platform. Telemetry implant surgeries are generally accomplished in under 2 minutes (not including anesthetic induction and recovery). Surgeries may be completed without administering further anesthetic, or fish can be maintained under anesthesia for the duration of the procedure. Maintenance anesthetic solution is perfused over the gills via gravity feed or pump or is administered by partial immersion in a second water bath.

Implantation and Wound Closure

Of all the aspects of the surgical process, incision closure techniques are arguably the most studied and described in the contemporary literature. However, at the same time, we believe they remain the least understood. Similar to any surgery conducted on homeotherms, the primary goal of wound closure after telemetry implant should be to close the body wall in a manner that will promote the most efficient healing. This will be achieved when disruption of tissue is kept to a minimum, tissue is maximally apposed, and closure materials are benign. Additional goals unique to telemetry research are to ensure transmitter retention and minimize impacts on fish behavior. Choice of wound closing method and suture material is not straight forward and require good knowledge of the species. There is a body of literature on this subject.

Evaluating the effects of capture, handling and tagging

Tagged fish must represent the natural behavior if results are to be useful for science or management. There is little value in observations about the survival, behavior, migration, or spawning activity of tagged fish that do not represent the untagged population. Yet despite this obvious constraint, there is little documentation showing that the performance of tagged fish in the field is similar to that of untagged individuals. For underwater organisms, such documentation is hard to produce. There are some solid field-based evaluations of survival after tagging for lake-dwelling fish but post-tagging performance of migratory fish is far more difficult to study. Even resident populations or particular life stages pose challenges for field examination of long-term survival and tag retention.

For these reasons, most studies specifically designed to examine the effects of handling and tagging have been conducted in captive or "closed" settings such as laboratories, hatcheries, and ponds. Although a laboratory study may be the best (or only) option for evaluating the effects of tagging, researchers should be aware that these types of studies can produce biased results. For example, fish may exhibit higher rates of survival and growth because they are no longer subjected to metabolic stressors of the natural environment, such as finding food, swimming against currents, avoiding predators, and migrating long distances. Thus laboratory conditions may underestimate the effects of tagging. On the other hand, fish in captivity may be subjected to stress from overcrowding, delayed migration, or poor water quality. These stressors can lead to delayed growth, secondary infections of the incision, increased risk of developing disease, or even death. To avoid these biases and improve telemetry tagging methods, more field-based evaluations of tagging effects are needed. Researchers should aim to incorporate diagnostic methods such as gross necropsy and histology into their study designs.

Recommendations

Millions of dollars are spent annually for telemetry technologies to study the performance of fish. These are high stakes that place researchers under increasing pressure to produce highly accurate and representative results using justifiable and repeatable tagging methodologies.

However, while most researchers recognize the value of adhering to veterinary principles and best practices when performing transmitter implant surgeries, they have not always received the training needed to understand and implement these practices. Improved telemetry methods and technical training will be achieved through a strong and consistent veterinary and research collaboration.