Capture, handling and tagging of fish for telemetry studies

Dublin workshop, January 2015

Niels Jepsen,
DTU Aqua
Twenty years of experience with surgical implants and external tagging of:

- Atlantic salmon *Salmo salar* (14-102 cm)
- Common bream *Abramis brama* (40–55 cm)
- European eel *Anguilla anguilla* (35-65 cm)
- Chinook salmon *Oncorhynchus tshawytscha* (17–26 cm)
- Perch *Perca fluviatilis* (16-34 cm)
- Pike *Esox lucius* (17–85 cm)
- Pikeperch *Stizostedion lucioperca* (35– 80 cm)
- Roach *Rutilus rutilus* (12–26 cm)
- Trout *Salmo trutta* (stationary and sea-run 14– 90 cm)
Outline

• Capture, handling and anaesthesia

• Tagging procedures, surgical and external, not PIT

• Evaluations of treatment effects

• Discussion about the importance of asepsis

• Ethics/animal welfare
Capture of experimental fish

- Hatchery
- Trapping
- E-fishing
- Netting
- Angling
Capture of experimental fish

Trapping
Rotary screw traps
Trapping

- Most relevant for migrating fish
- Work demanding
- Must be attended daily
- Fish may become stressed - *holding stress for smolts!*
- Injury, scale loss
- Sampling bias?
Electro-fishing
Electro-fishing

- Limited to relatively small waters
- Best at medium-conductivity
- Not very labour-intensive
- Only short-term stressing of fish
- May injure especially larger fish
- Sampling bias?
Capture of fish with seine net
Commercial salmon fishing with seine net
Pound-nets
USFWS capture of sturgeon in gillnet
Netting

- Can be very tough on the fish
- Not very time or labour-intensive
- Purse-seines and pound-nets better than gill-nets
- Long-term stressing of fish
- Sampling bias?
Angling
Angling

• Short term stress
• Best for medium size fish
• Labor-intensive – but fun!
• Can be the most gentle way of capture
• Sampling bias?
Capture of experimental fish

Experience, studies?
200 lb. Gulf sturgeon
Weighing juvenile gulf sturgeon
Handling

• Limit to a minimum
• Touch the fish with wet hands
• Temperature plays a crucial role
• Large fish are a major challenge
• Is it OK to use nets? Use rubber!
• Use of sedatives ?
• Avoid holding wild fish in captivity for longer periods
Handling of experimental fish

Experience, studies?
Anaesthesia

- Immobilise fish
- Reduce pain and stress
- Easy to handle and distribute
- Low toxicity
Anesthetics: considerations

• Most anesthesia by a dip or bath treatment in a static bath or with flowing water
• **Correct dosage and choice of anesthetic depends on**
  • Degree of anesthetisation required
  • The *species*, size, and condition of fish
  • Water temperature and water hardness (pH)
Use net or hands?
## Stages of Anesthesia

<table>
<thead>
<tr>
<th>Stage</th>
<th>Descriptor</th>
<th>Behavioral Response of Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Reactive to external stimuli; opercular rate and muscle tone normal</td>
</tr>
<tr>
<td>1</td>
<td>Light sedation</td>
<td>Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal</td>
</tr>
<tr>
<td>2</td>
<td>Deep Sedation</td>
<td>Total loss of reactivity to all but strong external stimuli; opercular rate slightly decreased; equilibrium normal</td>
</tr>
<tr>
<td>3</td>
<td>Partial loss of equilibrium</td>
<td>Partial loss of muscle tone; swimming erratic; increased opercular rate</td>
</tr>
<tr>
<td>4</td>
<td>Total loss of equilibrium</td>
<td>Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes</td>
</tr>
<tr>
<td>5</td>
<td>Loss of reflex reactivity</td>
<td>Total loss of reactivity; loss of all reflexes</td>
</tr>
<tr>
<td>6</td>
<td>Medullary collapse</td>
<td>Opercular movements cease; cardiac arrest usually follows quickly</td>
</tr>
</tbody>
</table>
Some Common Fish Anesthetics

- Tricane methane sulphonate (MS222)
- Benzocaine
- Carbon dioxide
- Clove oil (eugenol)
- 2-phenoxy ethanol
- Metomidate
Dosages for fish anesthesia

• Dosages are rules of thumb. Always test before using.
• Stress to fish under anesthesia is dependent on temperature, pH, turbidity, and oxygen levels.
• Excessive amounts or exposure lead to stress and eventually death
• Water taken from original capture site should be used for anesthesia.
MS 222

• The only fish anaesthetic agent licensed by US FDA for finfish intended for human consumption and ornamental fish

• USFDA requires a 21 day waiting period before treated fish can be released to the wild or used for human consumption

• Absorbed and excreted by gills

• Dissolves directly in water
Carbon dioxide

- Primarily used to sedate fish during transport or to allow handling of large numbers of fish
- Difficult to control concentration
- Gas released more rapidly under conditions of low pH
- Consideration must be given to maintaining adequate oxygen concentration in same water
- Can cause spasms, thus injuring fish
- Currently, no regulatory USFDA action
Clove Oil- a safe anesthetic?

- A naturally occurring compound but not currently approved by the US FDA as a fish anesthetic
- Widely used by European fish biologists
- Very little known on its effects on fish physiology, immune response, or olfactory ability
- Highly effective, even in low doses
- Provides a much calmer induction to anaesthesia
- Longer recovery time, as much as 10 x MS222
Denmark:

Only Benzocaine and MS 222 are legal

- but withdrawal time???
Some anaesthetic references

- Anaesthetic options for Fish
  - [www.ivis.org/advances/Anesthesia_Gleed/bowser/chapter_frm.asp?LA=1](http://www.ivis.org/advances/Anesthesia_Gleed/bowser/chapter_frm.asp?LA=1)
Anaesthesia

Experience, studies?
Fish Tagging Considerations

• **Morphology and behaviour of fish**
  • Habitat for different stages of life history
  • Location in water column - pelagic or benthic
  • Fish morphology/body shape, stage of sexual maturity
From small ones
- To big ones
Unpredictable behaviour

Roach going underground
Gravid female pike
Nature of the system

• Radio-telemetry best for shallow (1-25 feet), low conductivity (<300 µm) fresh, and turbulent water
• Amount and type of vegetation
• Size of habitat/ecosystem and mobility of the fish
• Tracking method (air, boat, vehicle, and fixed station)
Selection of Tags

• Physical Considerations
  • Size of transmitter (length/width) related to shape of fish
  • Weight (air/water)
  • Tag weight can affect fish’s buoyancy, equilibrium, swimming performance, feeding, and expulsion rates
Transmitter Options

- Pulse width (spaces), pulse rate (ppm)
- Mortality (stationary interval)
- Duty cycle (on/off interval)
- Temperature (different ppm for range)
- Depth (archive transmitter)
- Coded/standard
- Sensors
- Satellite
Choice of tag, antenna length and material
Internal Implants

• Surgical implant w/ internal coiled antenna (loop or helical coil) app. 70 % range reduction

• Surgical implant w/ external antenna

• Stomach/oesophageal implant

• Oviduct implant
Internal Transmitters (A) Cloacal Implant, (B) Internal Pectoral Girdle Harness (C) Esophageal Implant; and (D) Body Implant w/ Internal Coil Antenna
Implant surgery considerations

• Type and amount of anaesthetic
• Choice of tag and antenna type
• Size and position of incision
• Suture material and type of suture
• Water temperature
Mortality rates with 3 species of fish internally tagged with radio transmitters

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>75-78 Deg Fahrenheit</th>
<th>63-69 Deg Fahrenheit</th>
<th>41-52 Deg Fahrenheit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill</td>
<td>100%</td>
<td>100%</td>
<td>33%</td>
</tr>
<tr>
<td>Black Crappie</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>White Crappie</td>
<td>100%</td>
<td>50%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Fish placed in a V shaped trough (A) or cradle (B) before surgery.
Support pillow
http://lan.sagepub.com/content/34/4/430.full.pdf
Typical field surgery setup
Surgical implants

• Location and size of incision and position and length of transmitter antenna

• Make incision just large enough to pass the transmitter into the body cavity

• Location of incision different for different fish species and stages of sexual maturity
Aerating gills before surgery
Position and size of incision
Inserting transmitter with internal coiled antenna into gulf sturgeon
Tagging eel with ATS-coiled antenna tag
Inserting transmitter with external trailing antenna in common bream
Inserting transmitter into walleye
Hollow needle used to position transmitter antenna
Implant body cavity surgery

- Needle type and size appropriate for fish
- Closure of incision (several methods – or not)
- Suture material
Absorbable sutures

- Sterile strand prepared from collagen derived from healthy mammals or a synthetic polymer
- Surgical gut and Ethicon products most common are Vicryl, Monocryl, PDS II, and Panacryl
- Differ by tensile strength and absorption rate

Dissolves too slowly!
Non-absorbable sutures

- Strands of material suitably resistant to the action of living mammalian tissue
- Surgical stainless steel and Ethicon products most common are Perma-hand silk, Ethilon nylon, Nurolon nylon, Mersilene Polyester, Ethibond Excel, Prolene polypropylene, and Pronova
Reference for knots, needles, and sutures

  49 pages of knot tying and recommendations of needles and absorbable and non-absorbable suture materials
Suture choices

- Type of suture depends on fish species and stage of life
- Availability of suture material
- Personal preference - some use silk

Most studies prove monofilament to perform best

Recent study on sturgeon (Miller et al. 2014) showed: PDS II suture material, regardless of the pattern, resulted in incision closure and less inflammation in a majority of fish (22 of 27) within 14 days.

PDS = Absorbable Polydioxanone

VICRYL RAPIDE™ (polyglactin) Suture

Bi-directional knotless (barbed) suture
Illustration of continual stitches

INTERNAL
Suturing gulf sturgeon
Radio tagged salmon smolt
Separate sutures on gulf sturgeon
Staples used to close incision in paddlefish
Transmitter attached to pelvic girdle bone
Close up of separate sutures
Expulsion of transmitters
Antenna with thin flexible wires with a plastic coating

Stiff antenna can cause tissue damage
Potential problems with implants

- Infection, inflammation or tissue necrosis at the antenna exit wound
- Rates of tag shedding and ways of implant exit depend on fish condition, tag weight, and environment
- Three ways implant can exit, through the: incision, intact part of body wall, or intestine
- Recommend laboratory/aquaculture experiments before field study to determine potential expulsion rates.
Encapsulation of transmitter
Pikeperch one year after tagging
Cloacal/oviduct insertion

- For fish if the oviduct opens into the body cavity commonly used for northern pike and musky
- Transmitter pushed through urogenital pore & into body cavity
- Antenna trails out urogenital pore
- Used to locate spawning grounds
- Transmitter is (maybe) expelled when fish lays eggs
Tying off transmitter antenna from oviduct
Stomach/esophageal transmitters

• Inserted through mouth into stomach
• Could pose difficulty in insertion through pharynx or result in regurgitation
• Methods-- plunger tube or ingested bait
• Antenna comes out through mouth or passed out gill cavity
• Least invasive attachment method, only used for fish that will not feed again e.g. Pacific salmon migrating upstream to spawn
Illustration of insertion of esophageal transmitter from mouth to stomach with transmitter antenna emerging from gills.
Inserting oesophageal transmitter with plunger
Surgical implants

Experience, studies?
External attachments

• **Location of attachment**
  • Dorsal mount (most common)
  • Saddle mount (gar– saddle fits on back one pin attachment– not common)
  • Dorsal fin mount (crappie– one case)
  • Very small tags may be mounted on fins or on T-bar tags.
External Transmitters (E) Dorsal Mount, (F) Saddle Mount, (G) Dorsal Fin Mount
• Easy, no anaesthesia
• Short-term studies
• Drag, entanglement
External dorsal radio transmitter (black) on gulf sturgeon
External attachment considerations

Advantages

• Quicker and easier than surgery
• Can be used on spawning and feeding fish
• Necessary for most DST-tags
• Often done without anaesthesia
External attachment consideration

- Disadvantages
  - Tag loss
  - High centre of gravity
  - Snag on vegetation
  - Increased drag/ altered swimming behaviour
  - Skin/mucus abrasions
  - Susceptible to predation
Dorsal fin mount— a special type of external transmitter used on crappie
Close up of dorsal fin mount
External tagging

Experience, studies?
Evaluation of methods

- Laboratory studies
- Recapture of tagged fish
- Compare performance of tagged fish with “normal”
Temporal publication trends in studies that evaluate tagging effects or refine procedures for implantation of electronic tags in fish (n=108). From Cooke et al. 2011.
Patterns in the duration of studies used to evaluate tagging effects or refine procedures for the intracoelomic implantation of electronic tags in fish. From Cooke et al. 2011.
What are we tracking ???
Physiological response to tagging

We used hatchery chinook smolts, 360 in total.

Control, gastric inserted & surgical implanted

Three parameters were measured: cortisol, glucose & lactate
Risk of predation
Impacts:
- Capture
- Handling
- Anaesthesia
- Tagging

Effects:
- Stress
- Mortality
- Changed behaviour
- Reduced growth

Increased risk of predation?
Can predation act as indicator for tagging/handling effects?

Results from pond experiments performed at DIFRES Silkeborg 2005 - 2006
4 ponds with predators were used for the trials.

A total of 480 preyfish were used, half of these were control and half were Treatment.

**Anesthesia:** Pike as predators, rainbow trout prey (9-15 cm), Clove oil and 2-phenoxy, 10 replicates for each agent.

**Transport:** (stress): pikeperch as predators, brown trout (9-16 cm), 20 replicates.

**Tagging:** (surgical implant): pikeperch as predators, brown trout (13-17.5 cm), 20 replicates.
Four identical 4x4x0.8 m ponds (8500 L)
Predators: pike and pikeperch
• Mimicking normal anaesthesia procedure
• Testing 2-phenoxy and clove oil
• Rainbow trout vs Pike
• Prey released 15 minutes after anaesthesia
Anaesthesia Trials

Overall % % predation

2-phenox. (n = 40)
Control (n = 40)
Clove oil (n = 40)
Control (n = 40)

(6) (4) (8) (5)
• Mimicking transport of fish for stocking

• 30 minutes of stress

• Wild brown trout v. pikeperch
Overall % Predation

Transported (n = 80) (39)

Control (n = 80) (35)

Transport - stress
• Normal surgical implant procedure
• Fish released 24 hours post surgery
• Wild brown trout v. pikeperch
Overall % Predation

- Radio Tagged (n = 77): (50)
- Control (n = 78): (43)

Tagging – surgical implant
Radio Tagged vs. Kontrol Fish

<table>
<thead>
<tr>
<th>Predation</th>
<th>Radio Tagged</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. May</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. May</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. May</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. May</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. May</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experimental

Conclusion

Treatment seems not to influence predation, but….

Counterintuitive, but match field results

The pursuing of this issue is important
Evaluation of tagging effects in the field

Lille-Å: 188 wild brown trout were captured by E-fishing
3 different treatments, and released back in the river
Recaptured (blind E-fishing) 6 months later and evaluated
CW-tagging for individual ID
No significant effect of treatment
Asepsis – is it important?
From *D. Mulcahy, 2003, ILAR Journal:*

”The surgical implantation of a nonsterile transmitter into a fish is an inhumane act and should not be performed”
<table>
<thead>
<tr>
<th>Treatment</th>
<th>N-tagged</th>
<th>Mortalities</th>
<th>Specific length growth (Var)</th>
<th>Healing (Median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean-A</td>
<td>25</td>
<td>3</td>
<td>0.04 (0.0155)</td>
<td>1.88</td>
</tr>
<tr>
<td>Dirty-A</td>
<td>25</td>
<td>4</td>
<td>0.06 (0.0054)</td>
<td>1.76</td>
</tr>
<tr>
<td>Clean-NA</td>
<td>25</td>
<td>1</td>
<td>0.09 (0.0065)</td>
<td>1.79</td>
</tr>
<tr>
<td>Dirty-NA</td>
<td>25</td>
<td>2</td>
<td>0.07 (0.0139)</td>
<td>1.71</td>
</tr>
</tbody>
</table>

Healing: 1 is perfect, 4 is bad.

No effect of using real dirty tagging v. very clean

Few studies tested this, but found similar results


Summary

• For all attachments gentle capture and handling is crucial
• The perfect tag and tagging method does not exist

Future focus on:

• Suture material
• Legalize anaesthesia
• Asepsis
• Tag/BM ratios
• Predation
• ??
### Comparison of transmitter attachment methods

<table>
<thead>
<tr>
<th>Factor</th>
<th>External</th>
<th>Stomach</th>
<th>Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Installation time</td>
<td>moderate</td>
<td>quick</td>
<td>slow</td>
</tr>
<tr>
<td>Difficulty</td>
<td>moderate</td>
<td>low</td>
<td>highest</td>
</tr>
<tr>
<td>Recovery time</td>
<td>moderate</td>
<td>quick</td>
<td>longest</td>
</tr>
<tr>
<td>Balance Problems</td>
<td>greatest</td>
<td>least</td>
<td>least</td>
</tr>
<tr>
<td>Transmitter Size</td>
<td>Large</td>
<td>Dependent on morphology</td>
<td>Dependent on morphology</td>
</tr>
<tr>
<td>Infection potential</td>
<td>moderate</td>
<td>least</td>
<td>highest</td>
</tr>
<tr>
<td>Snagging potential</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Drag/resistance</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>
## Comparison of transmitter attachment methods

<table>
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<th>Factor</th>
<th>External</th>
<th>Stomach</th>
<th>Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrasions</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>no</td>
<td>sometimes</td>
<td>no</td>
</tr>
<tr>
<td>Feeding interference</td>
<td>lowest</td>
<td>highest</td>
<td>lowest</td>
</tr>
<tr>
<td>Spawning interference</td>
<td>lowest</td>
<td>lowest</td>
<td>highest</td>
</tr>
<tr>
<td>Transmitter sensors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>external</td>
<td>easy</td>
<td>harder</td>
<td>harder</td>
</tr>
<tr>
<td>internal</td>
<td>harder</td>
<td>harder</td>
<td>easier</td>
</tr>
<tr>
<td>Transmitter expulsion</td>
<td>no</td>
<td>no</td>
<td>sometimes</td>
</tr>
</tbody>
</table>
Most important:

Use common sense and -
TEST your methods!!!
Animal welfare

If you wouldn’t do this to a dog, why do it to a fish?

PETA  FishingHurts.com
The five freedoms

• Freedom from hunger, thirst and malnutrition;
• Freedom from physical and physiological discomfort;
• Freedom from injury, disease and functional impairment;
• Freedom to express normal behaviour and social interactions;
• Freedom from fear and chronic stress.
The 3 R’s

• Reduce (tag-size, number of fish, handling time, stress)
• Refine (capture, handling and tagging methods)
• Replace (???)
• + The 4th R: Relevance
From D. Mulcahy, 2003, ILAR Journal:

"The surgical implantation of a nonsterile transmitter into a fish is an inhumane act and should not be performed"

"The surgical implantation of a transmitter into the coelom of a fish without anaesthesia is an inhumane act and should not be performed"
Forward:

- More exchange of experience, also negative
- More interdisciplinary work (Biology/Veterinary)
- Better options for funding for methodological studies
- Harmonization of guidelines, not rules
- Different regulations for studies of wild fish, with the purpose of species conservation and habitat rehabilitation.

We should actively take part in the debate about rules and regulations and about animal welfare in general!
Guidelines and other resources on the care and use of fish in research

Revision of Appendix A of the European Convention for the protection of animals used for scientific purposes. This revised version contains species-specific guidelines for a number of fish species.

The Canadian Council on Animal Care has produced a draft of comprehensive guidelines on the care and use of fish in research.

Guidelines for the Care and Use of Fish in Research by L.J. DeTolla et al. published in the ILAR Journal

Fish Research and the Institutional Animal Care and Use Committee by R.G. Borski & R.J. Hodson, North Carolina State University

Guidelines for the Use of Fishes in Research produced by the American Fisheries Society

Guidelines for reporting the results of experiments using fish

List of worldwide fish research organisations compiled by the Aquaculture Centre at the University of Guelph, Ontario, Canada

The Experimental Fish: online training for aquatic animals users by the Canadian Aquaculture Institute

Do fish feel pain?, a book by V.A. Braithwaite

The Journal Fish and Fisheries, which includes an article entitled "An Evaluation of current perspectives on consciousness and pain in fishes"

The Use of Fish Cells in Ecotoxicology: a report from an ECVAM workshop into the potentials for replacing the use of live fish

Guidelines for the operation of institutional animal care and use committees: IACUC's

The Care and Use of Amphibians, Reptiles and Fish in Research by SCAW (Scientists for Animal Welfare)