Capture, handling and tagging

of fish for telemetry studies

Dublin workshop, January 2015

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Twenty years of experience with surgical implants and external tagging of:

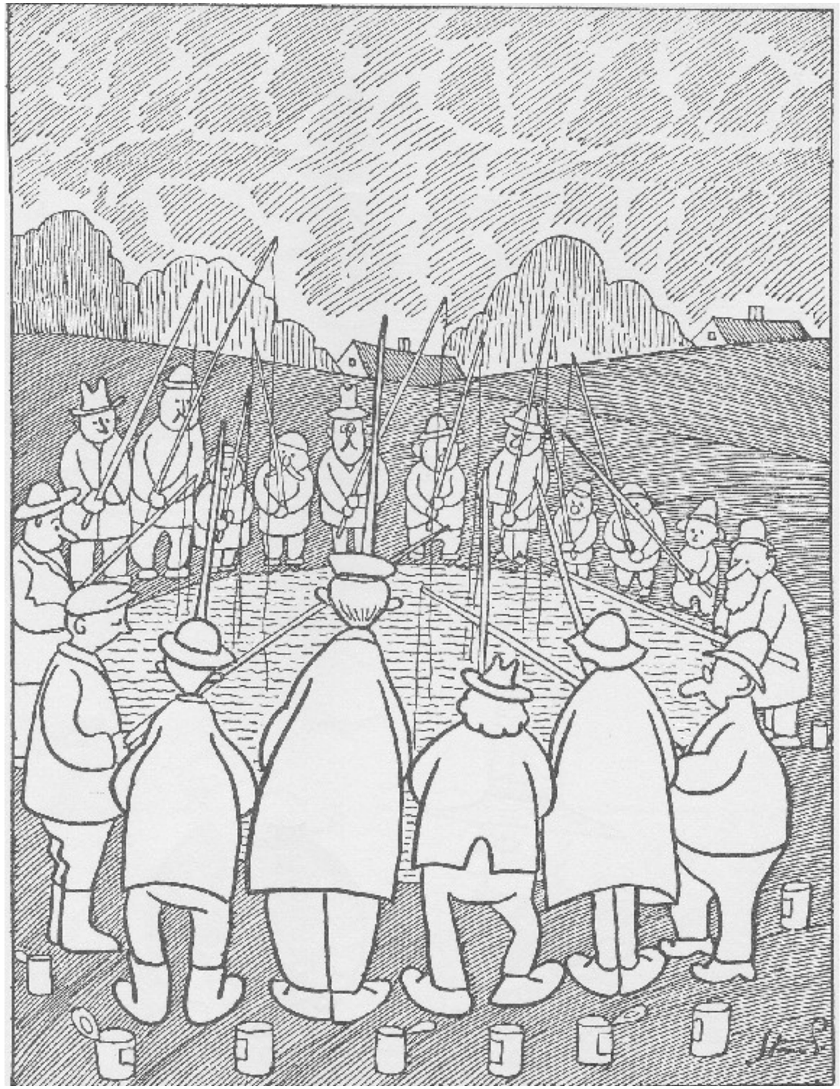
- Atlantic salmon *Salmo salar* (14-102 cm)
- Common bream *Abramis brama* (40–55 cm)
- European eel *Anguilla anguilla* (35-65 cm)
- Chinook salmon *Oncorhynchus tshawytscha* (17–26 cm)
- Perch *Perca fluviatilis* (16-34 cm)
- Pike *Esox lucius* (17–85 cm)
- Pikeperch *Stizostedion lucioperca* (35– 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

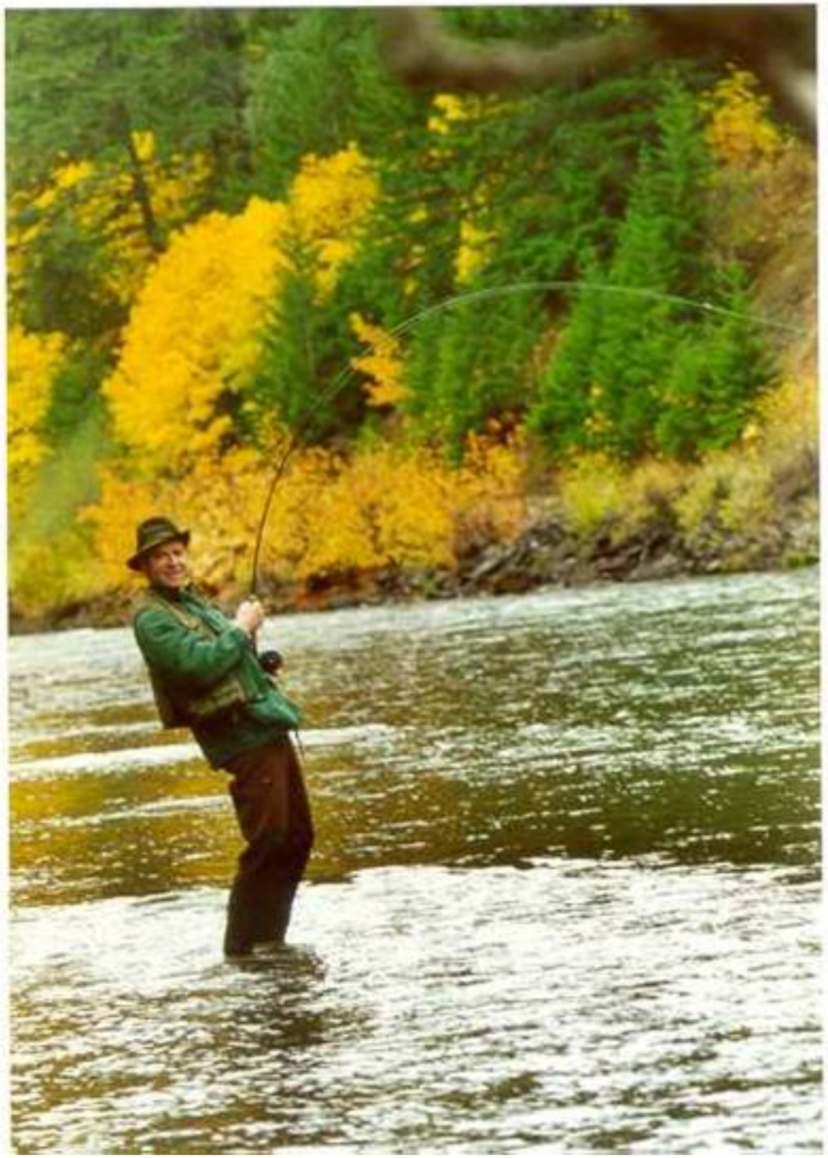

SEATTLE

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

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

http://www.ivis.org/Anesthesia_Gleed/bowser/chapter_frm.asp?LA=1

Some Common Fish Anesthetics

- **Tricane methane sulfonate** (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/Anesthesis_Gleed/bowser/chapter_frm.asp?LA=1

Anaesthesia

Experience, studies ?

Fish Tagging Considerations

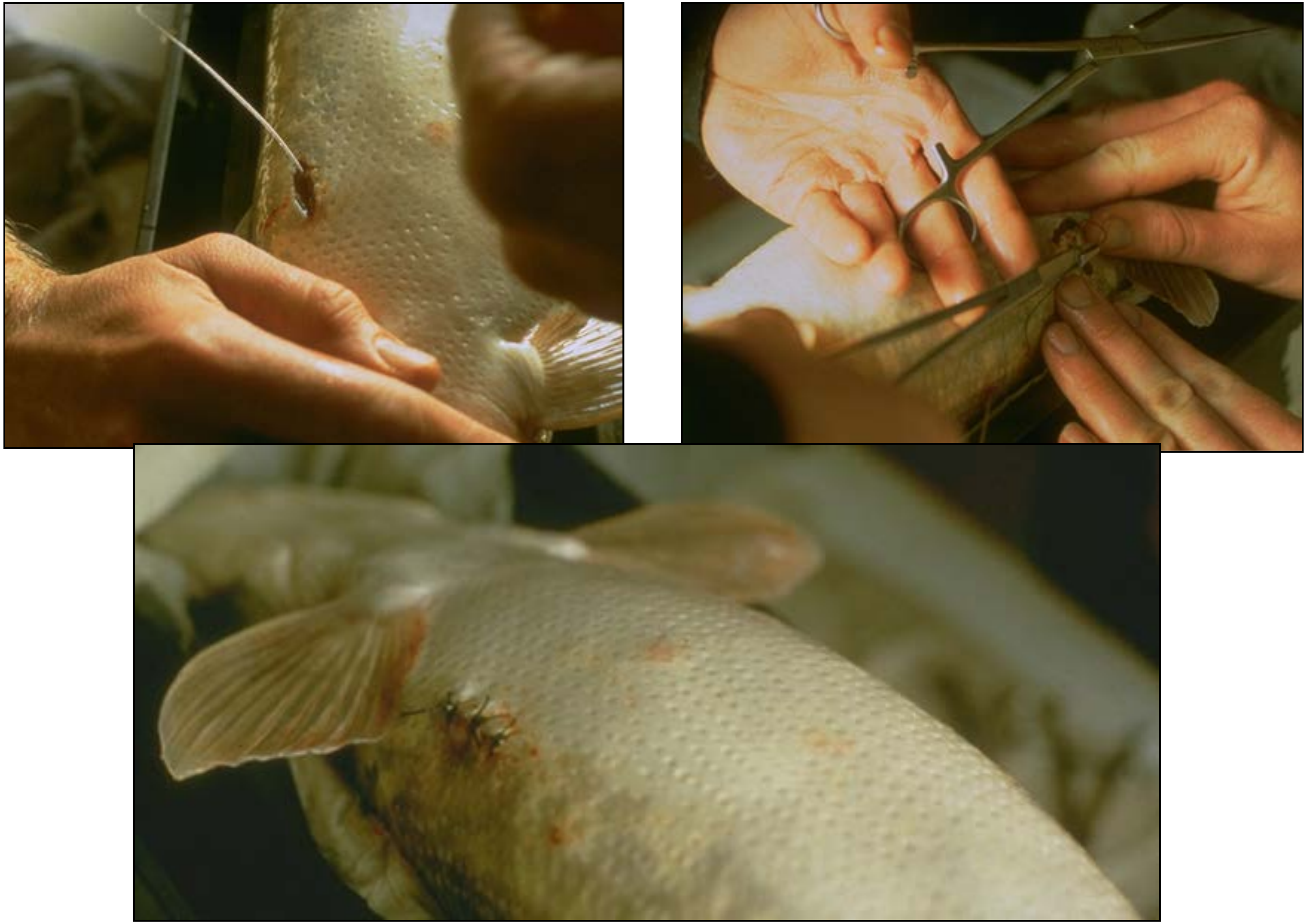
- **Morphology and behaviour of fish**
- Habitat for different stages of life history
- Location in water column- pelagic or benthic
- Fish morphology/body shape, stage of sexual maturity

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
- Sattelite

Choice of tag, antenna length and material

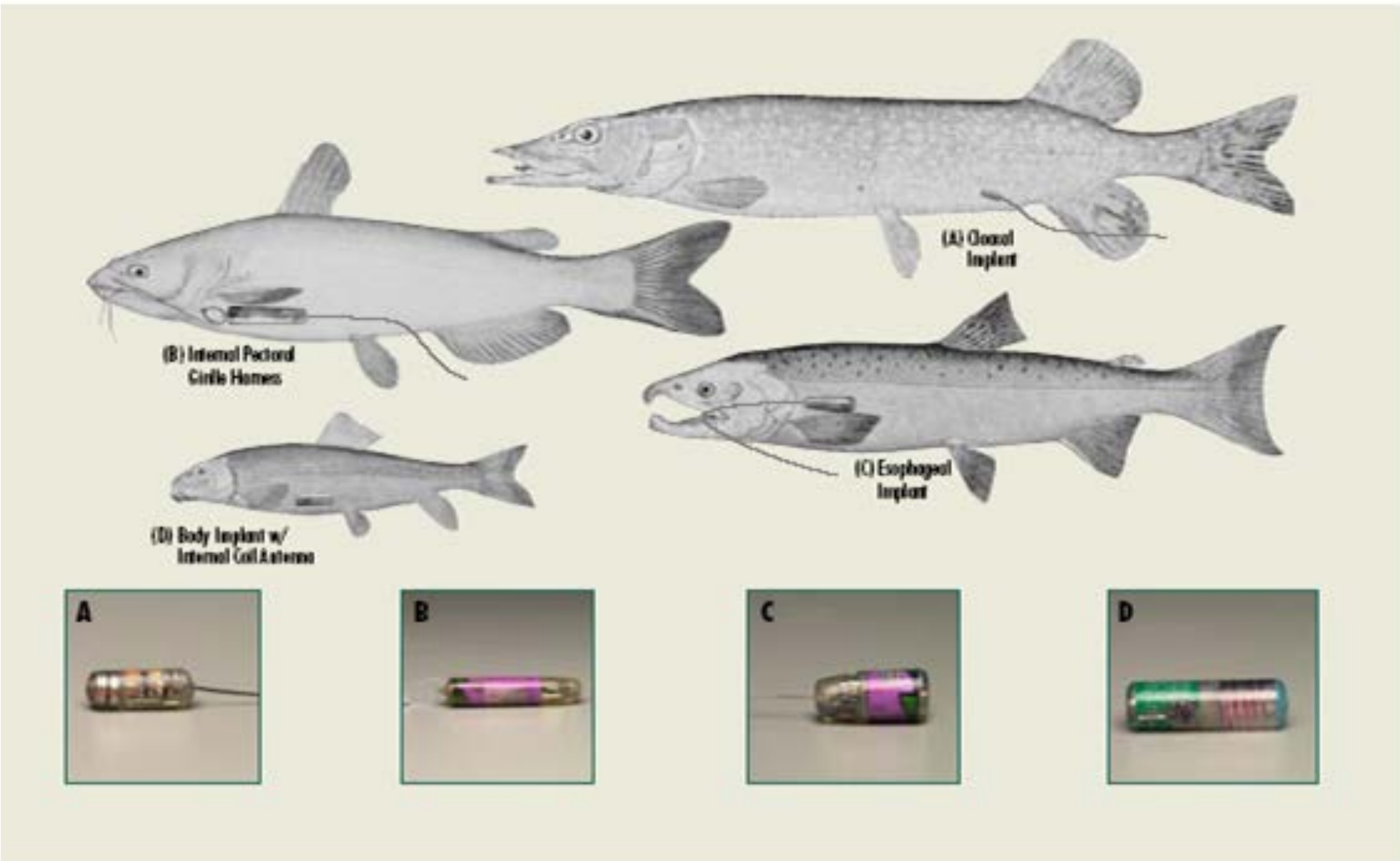
Internal Implants

- 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

Fish Species	75-78 Deg Fahrenheit	63-69 Deg Fahrenheit	41-52 Deg Fahrenheit
Bluegill	100%	100%	33%
Black Crappie	100%	0%	0%
White Crappie	100%	50%	0%

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

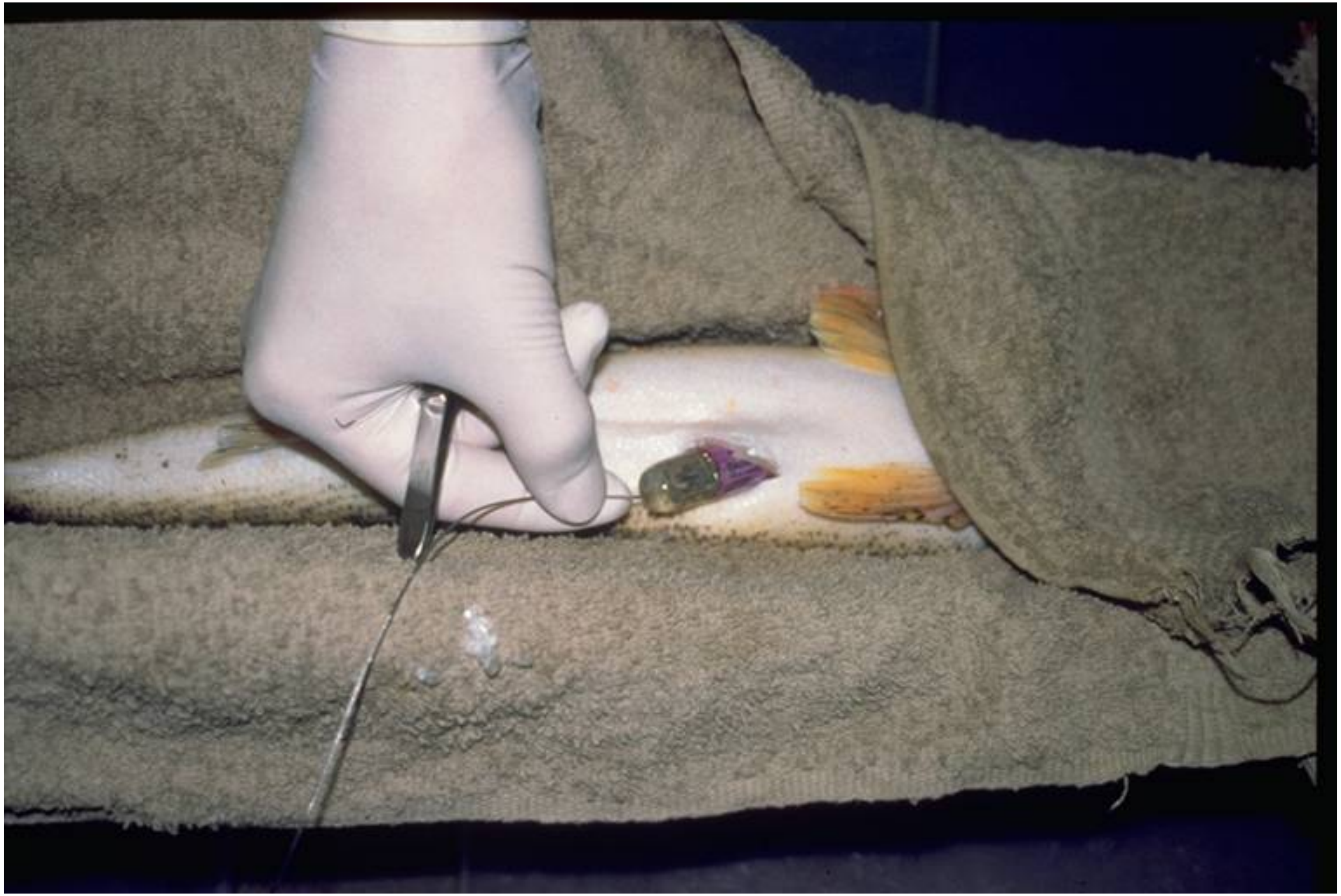
Aerating gills before surgery

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

- http://www.jnjateway.com/public/USENG/526ETHICON_Encyclopedia_of_knots.pdf
  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 ??

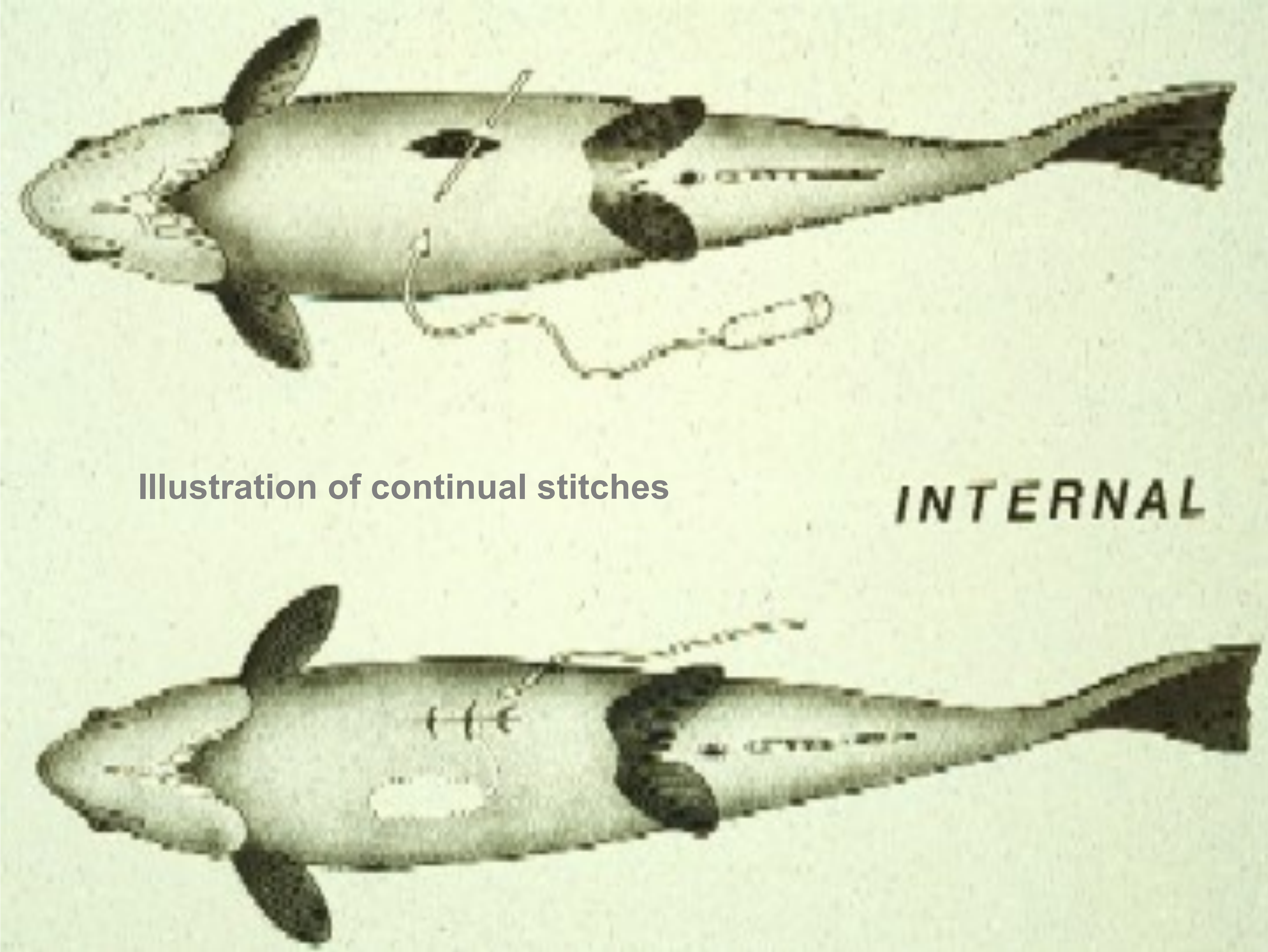
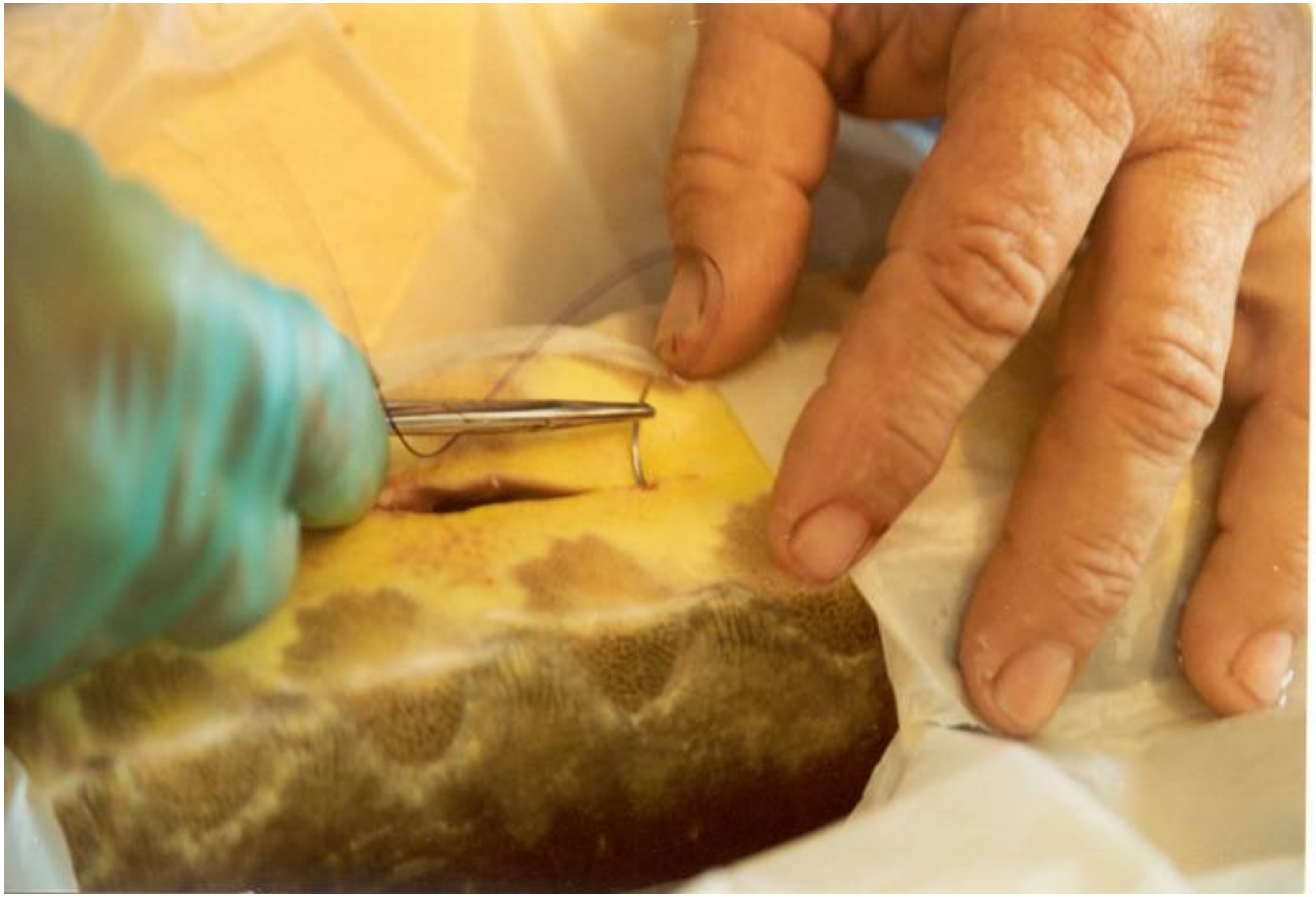
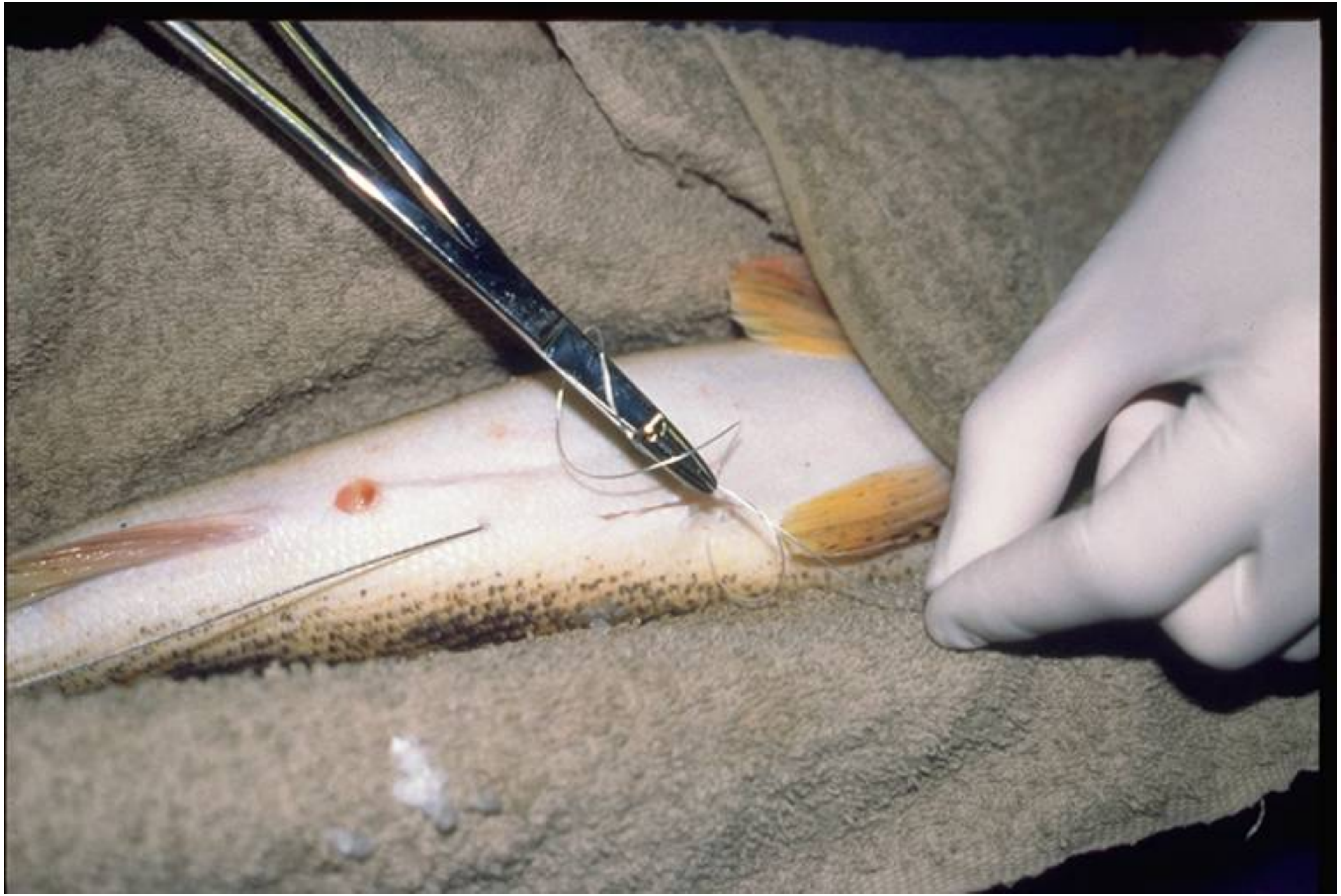


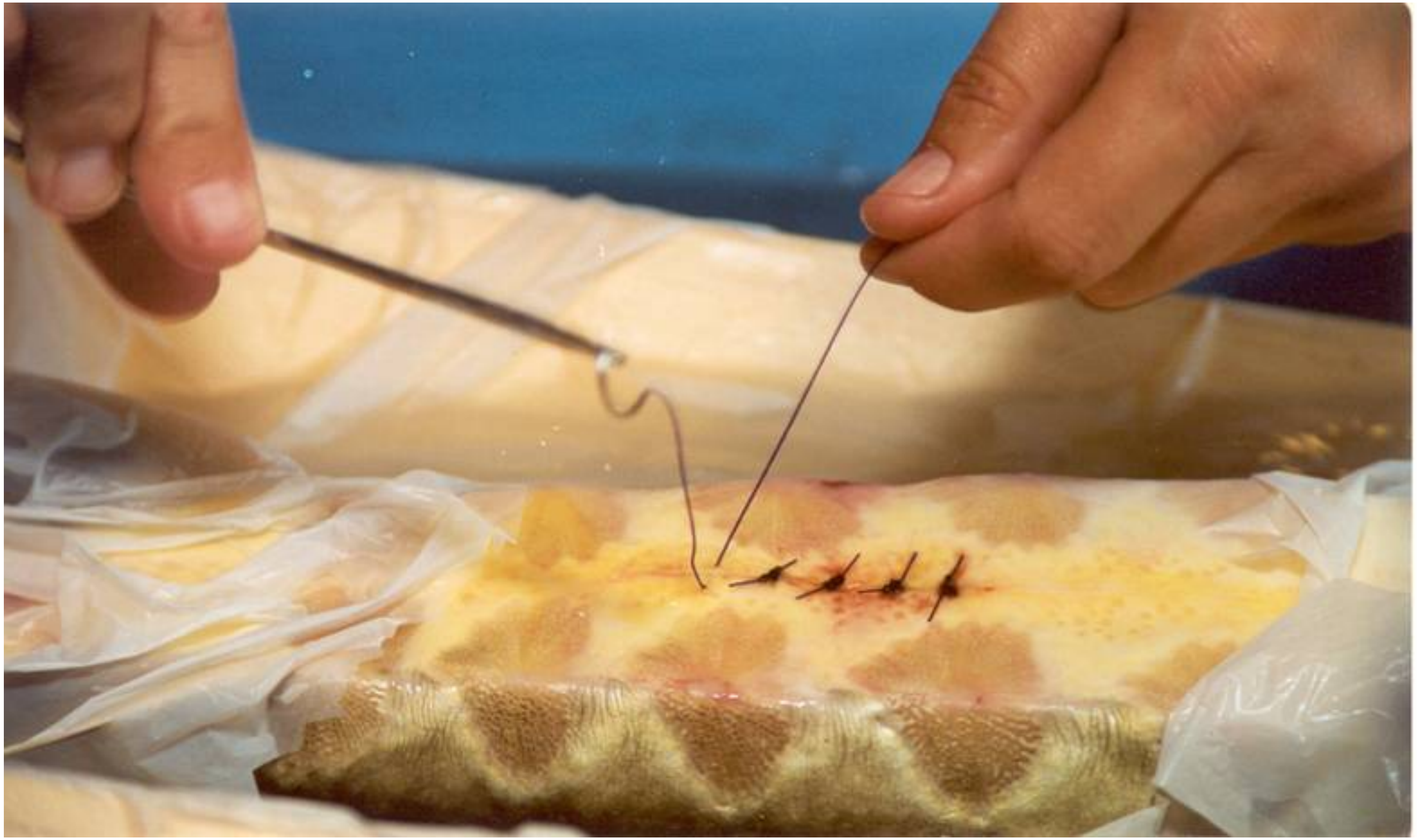
Illustration of continual stitches



Suturing gulf sturgeon

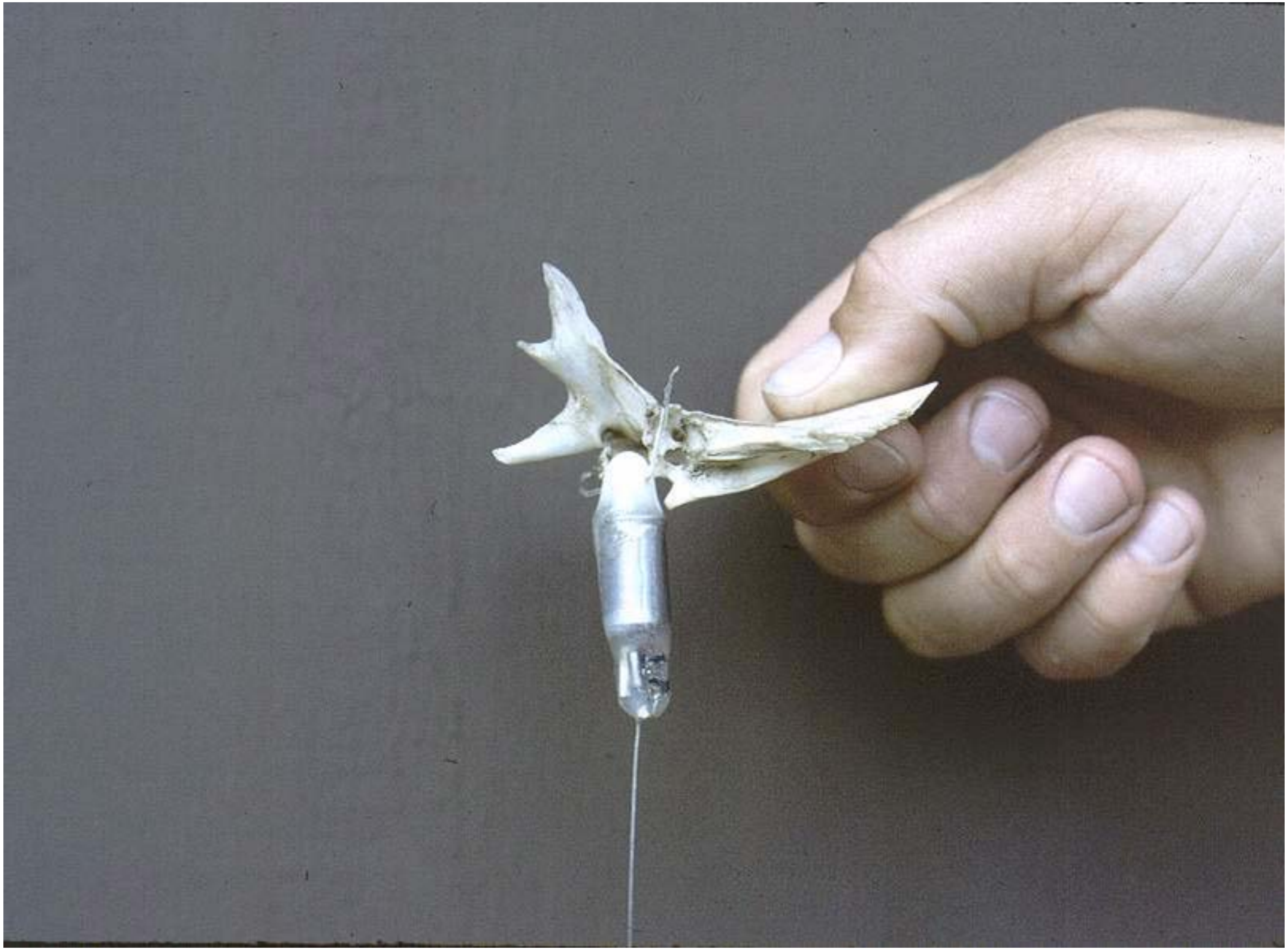


Radio tagged salmon smolt



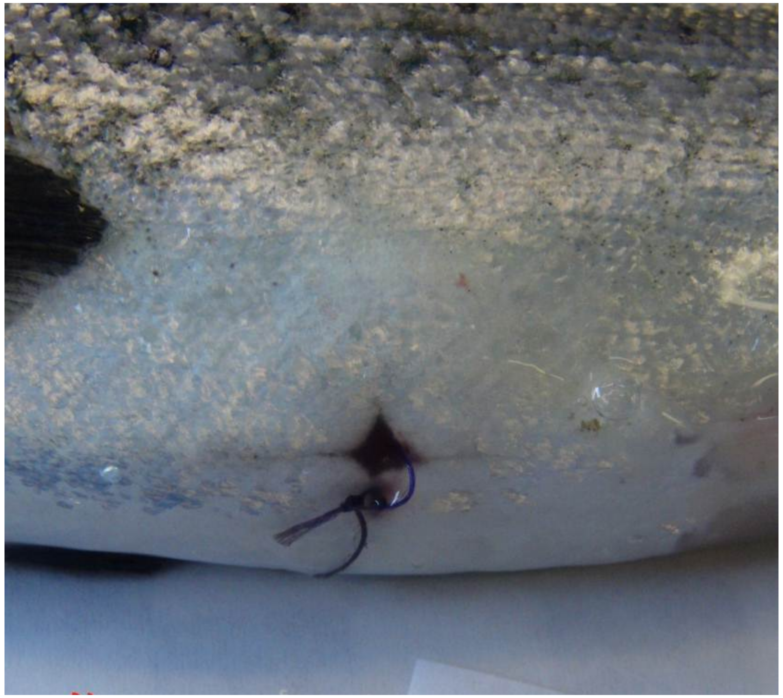
Separate sutures on gulf sturgeon

Staples used to close incision in paddlefish

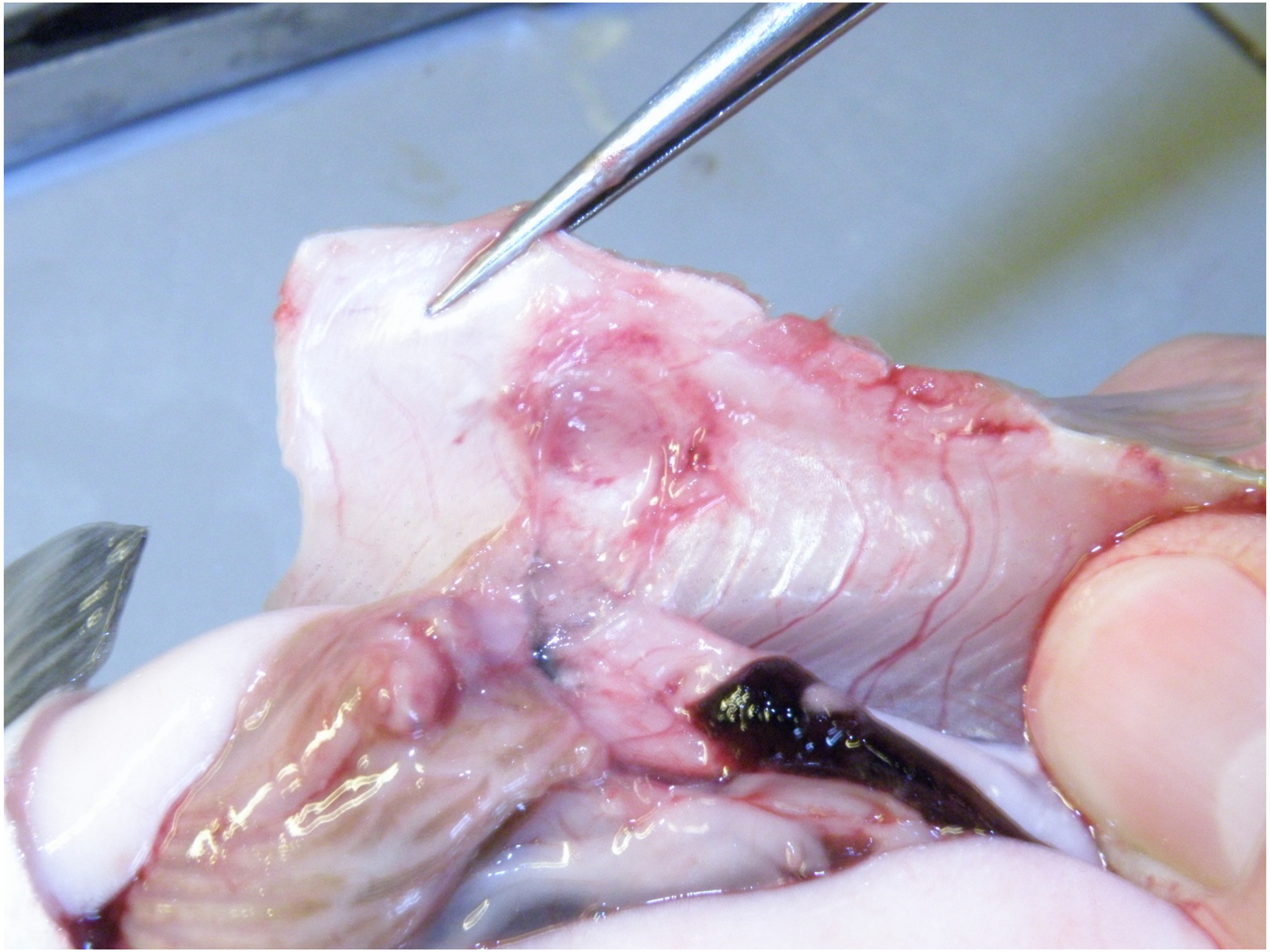


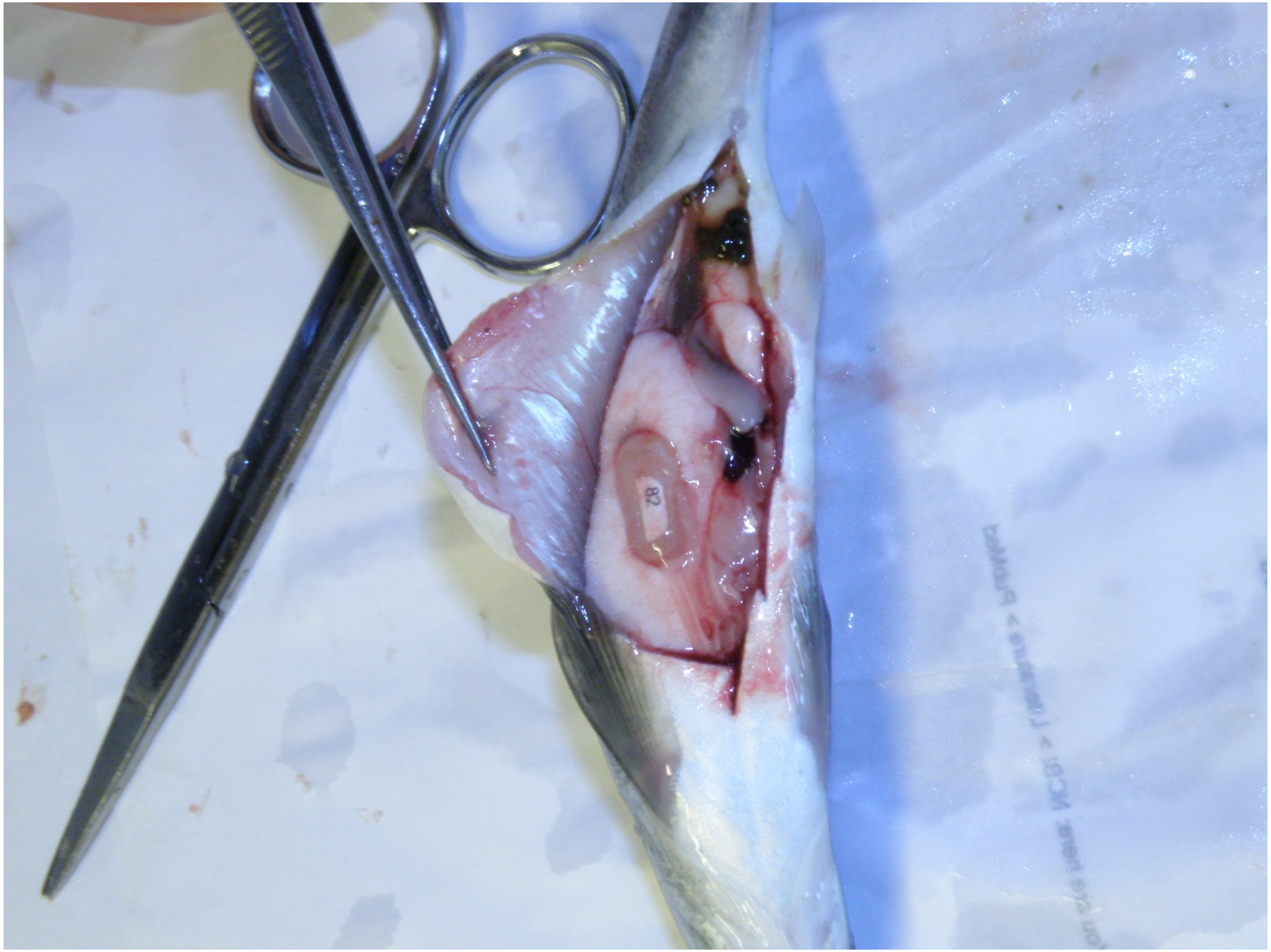
Transmitter attached to pelvic girdle bone


Close up of separate sutures



Expulsion of transmitters



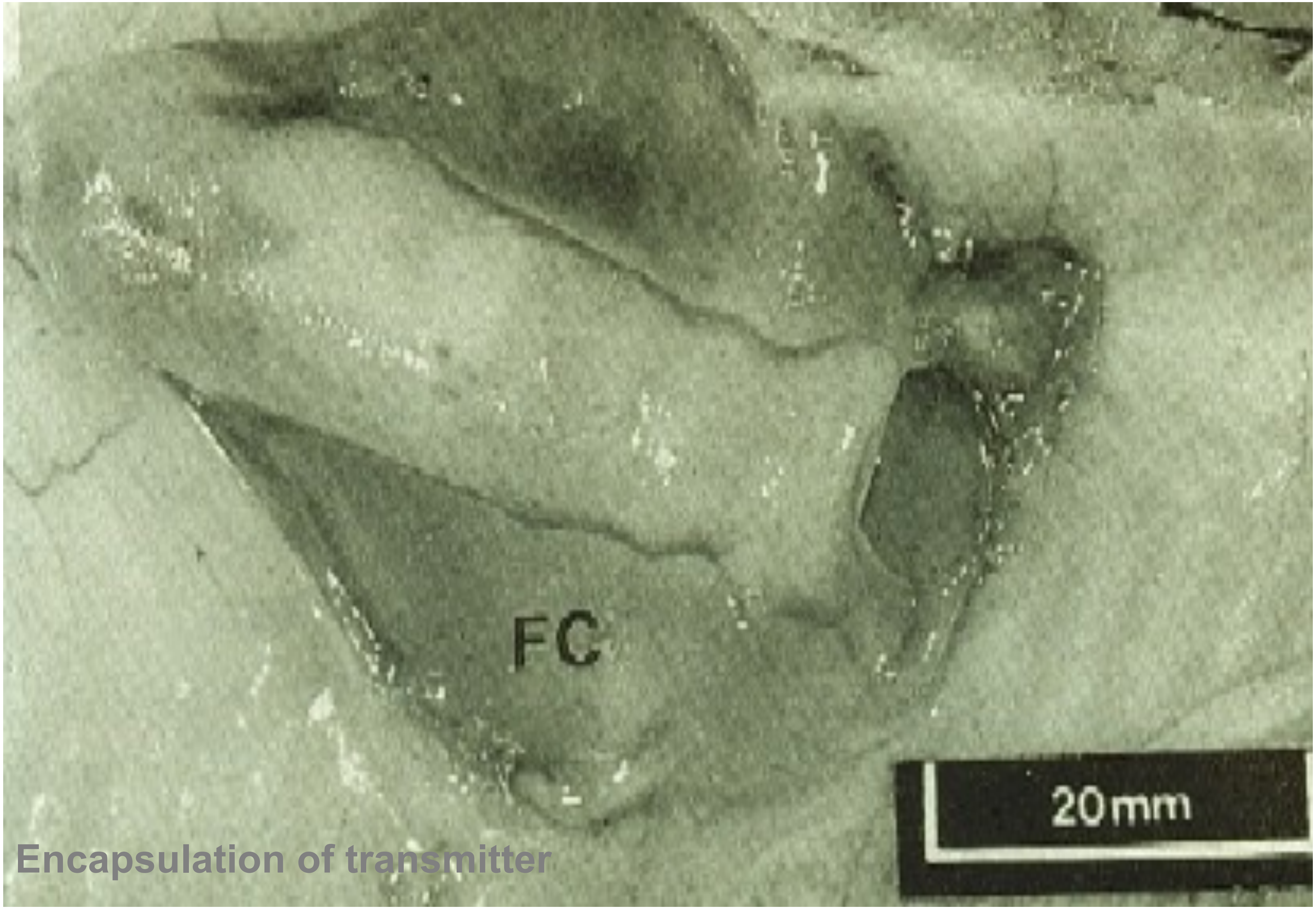


Stiff antenna can cause tissue damage

Antenna with thin flexible wires with a plastic coating

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

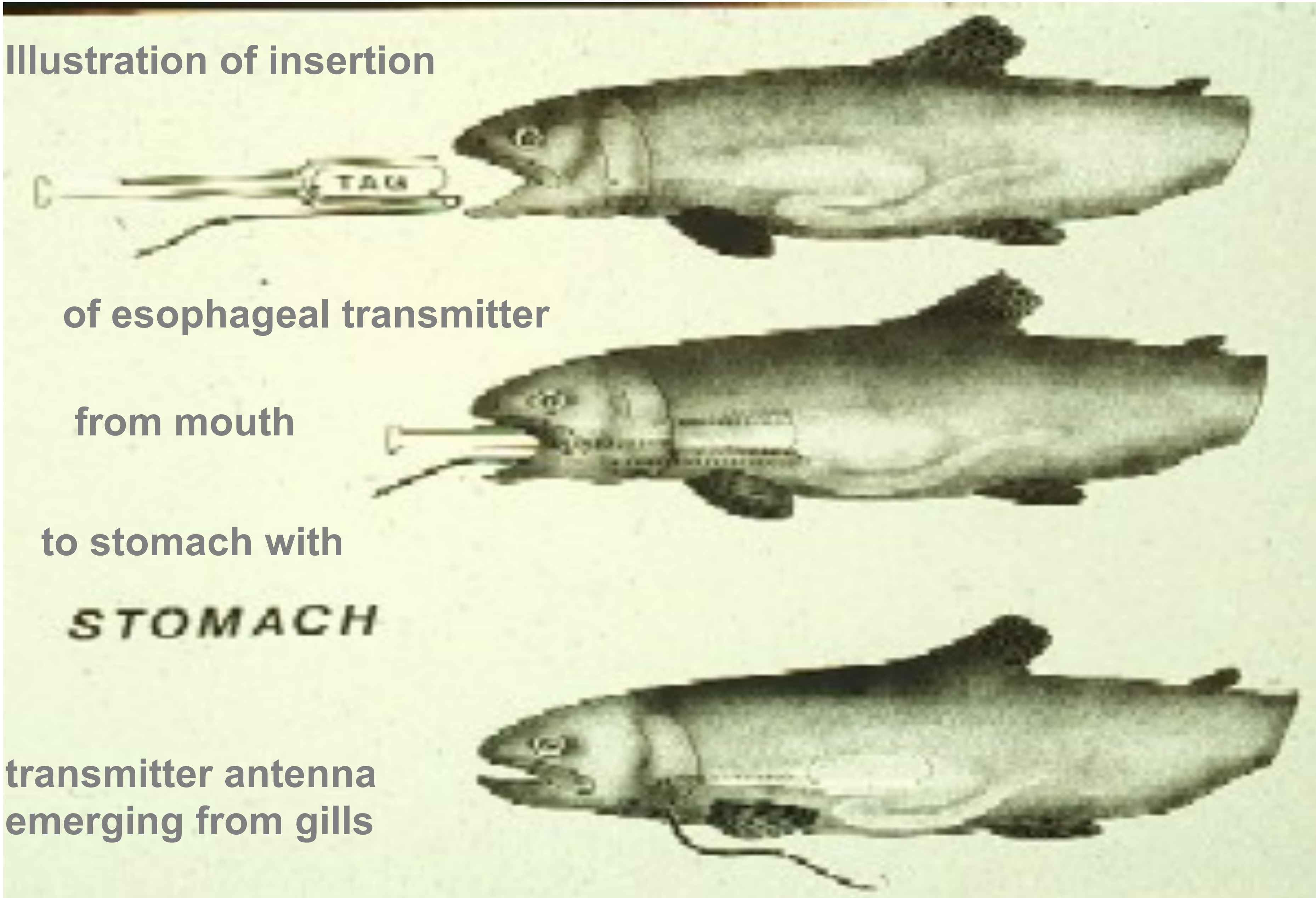
- For fish if the oviduct opens into the body cavity commonly used for northern pike and musky
- Transmitter pushed through urogenital pore & into body cavity
- Antenna trails out urogenital pore
- Used to locate spawning grounds
- Transmitter is (maybe) expelled when fish lays eggs

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



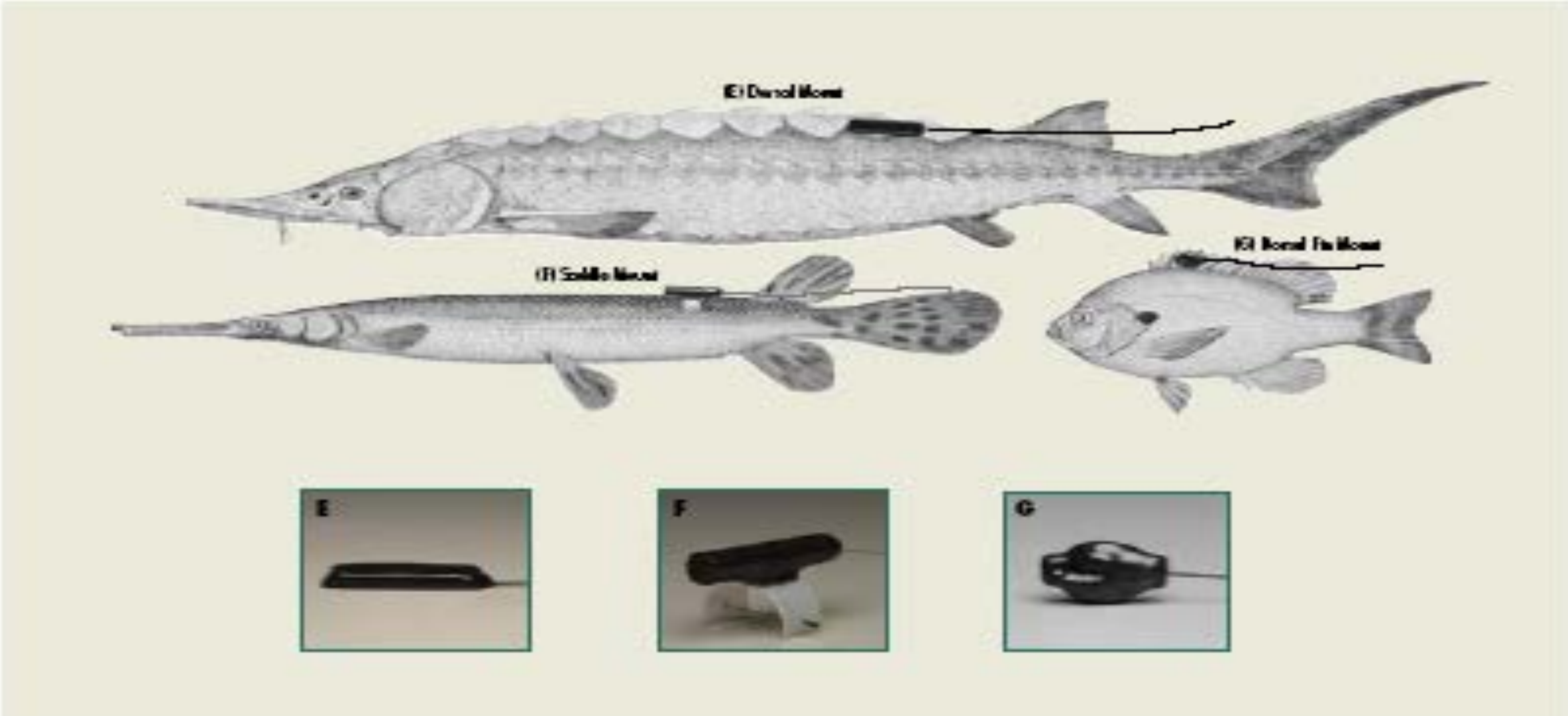
Inserting oesophageal transmitter with plunger

Surgical implants

Experience, studies ?

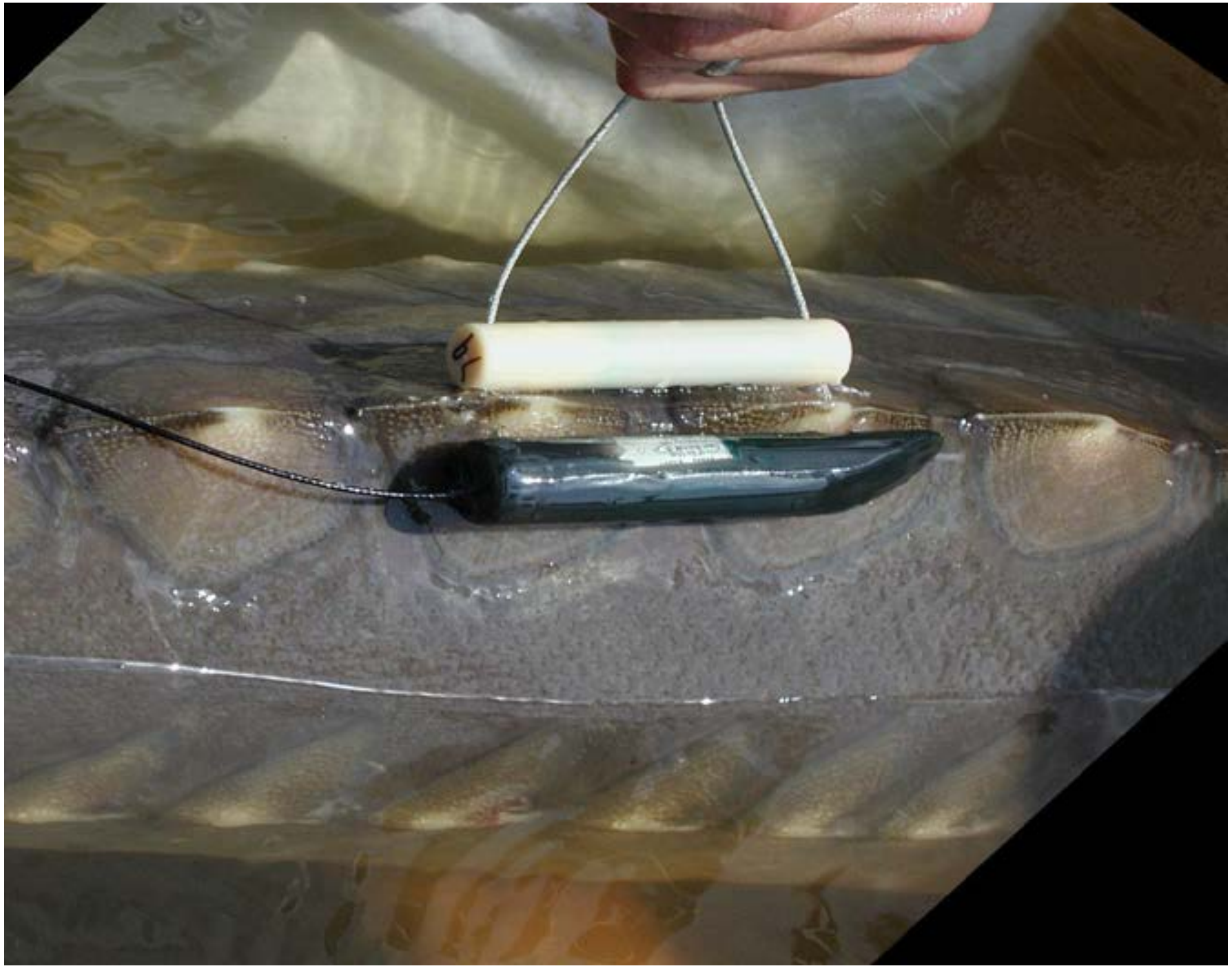
External attachments

- **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

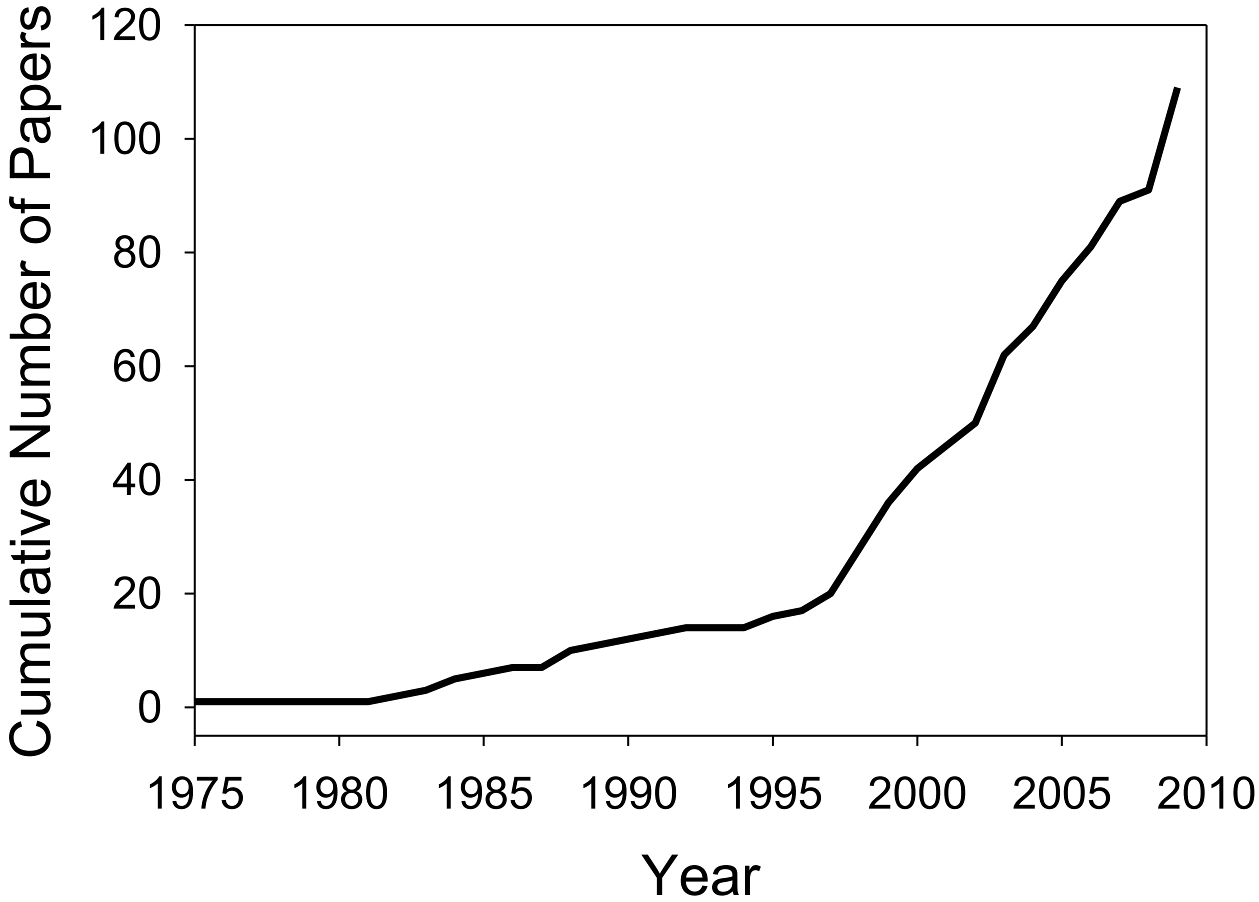
Close up of dorsal fin mount

External tagging

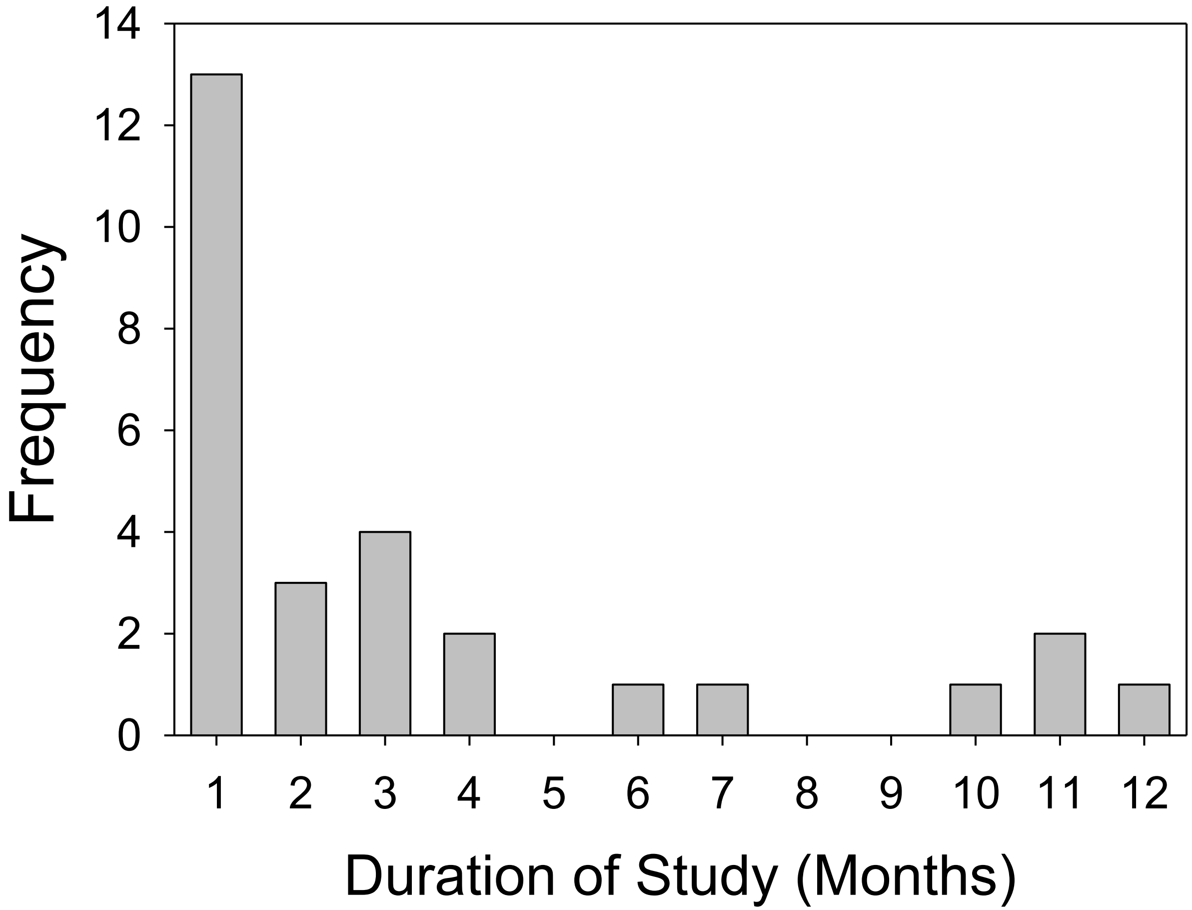
Experience, studies ?

Evaluation of methods

- Laboratory studies
- Recapture of tagged fish
- Compare performance of tagged fish with "normal"

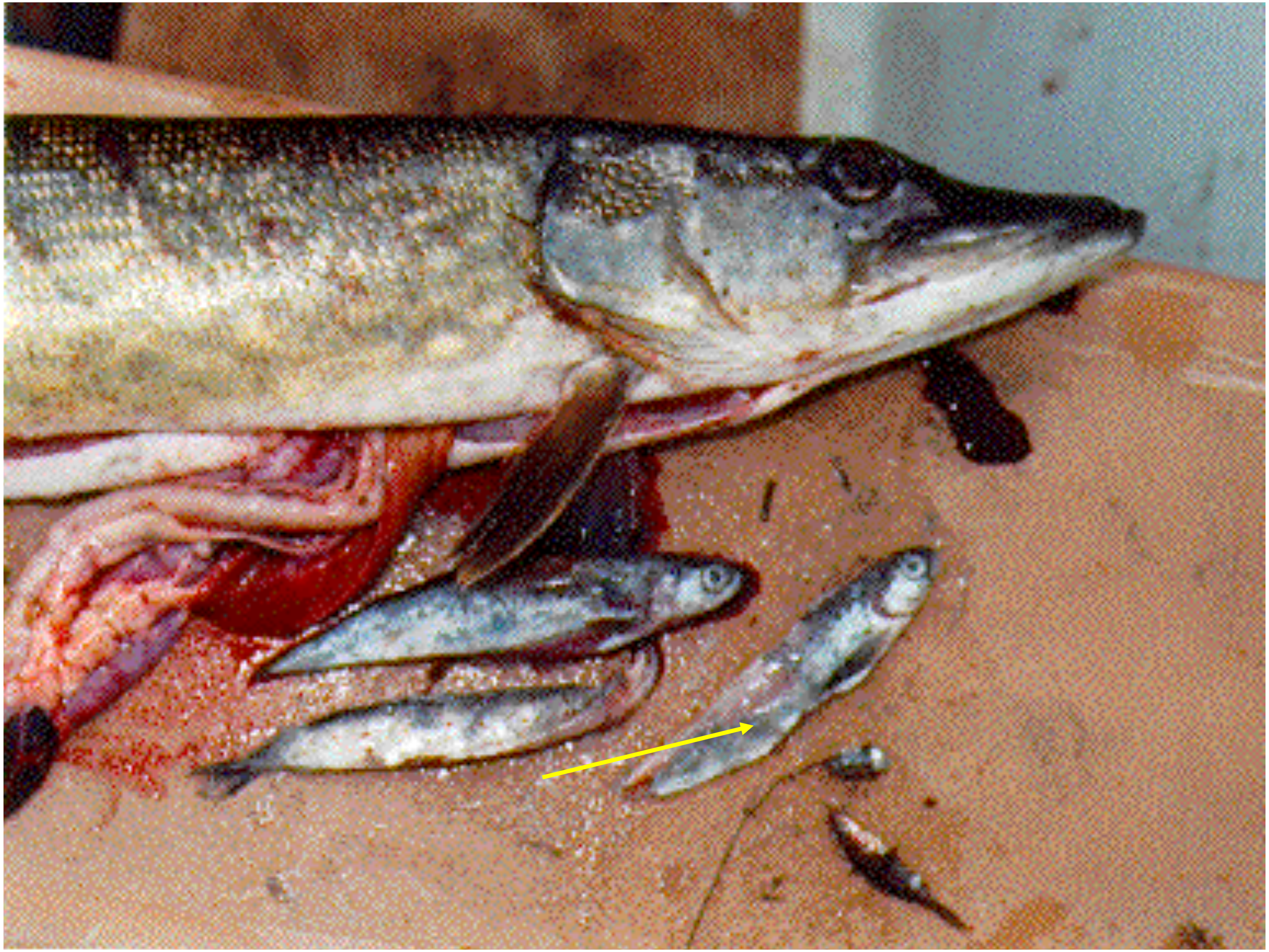


Temporal publication trends in studies that evaluate tagging effects or refine procedures for implantation of electronic tags in fish (n=108). From Cooke et al. 2011.



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 ???

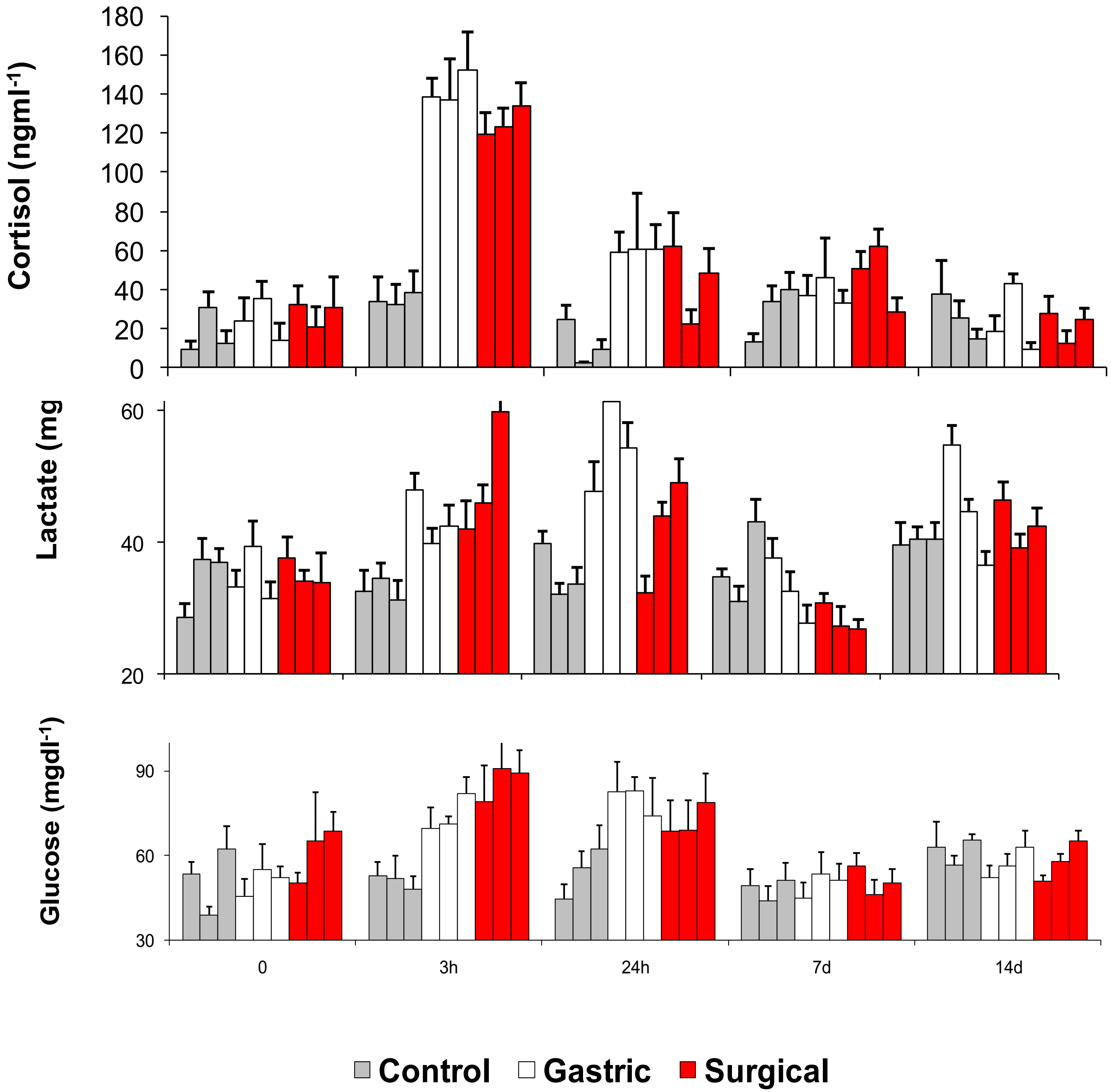


Physiological response to tagging

We used hatchery chinook smolts, 360 in total.

Control, gastric inserted & surgical implanted

Three parameters were measured: cortisol, glucose & lactate


Cortisol (ngml-1)
180
160
140
120
100
80
60
40
20
0
Lactate (mg
60
40
20
Glucose (mgdl-1)
90
60
30
0
3h
24h
7d
14d
Control
Gastric
Surgical

Risk of predation

Impacts:

Capture

Handling

Anaesthesia

Tagging

Effects:

Stress

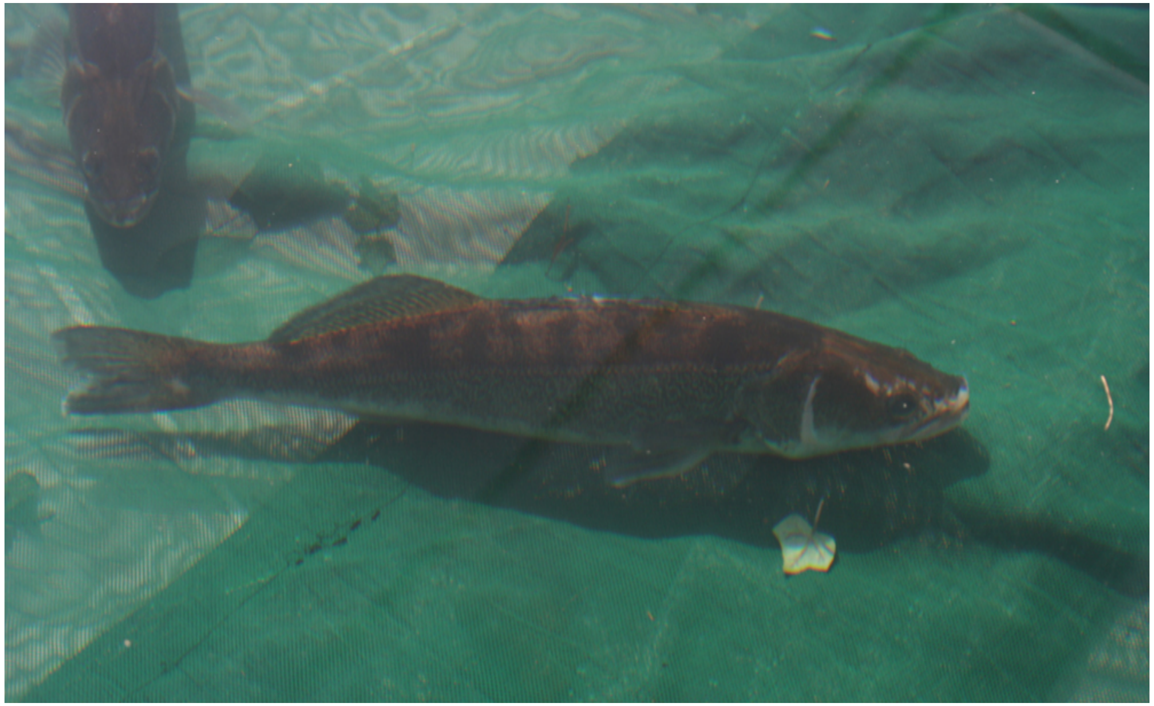
Mortality

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.

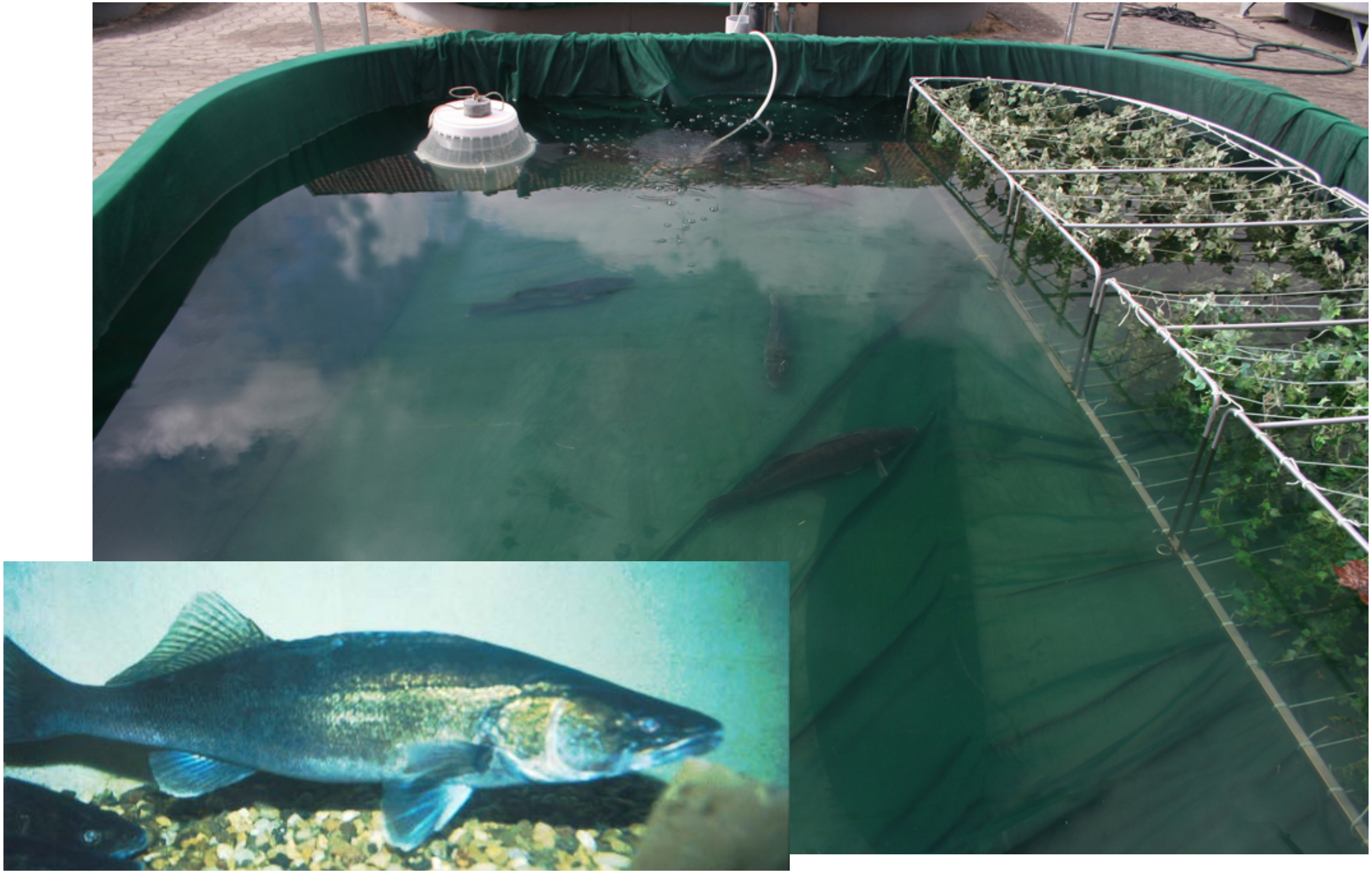
A total of 480 preyfish were used, half of these were control and half were Treatment.

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.

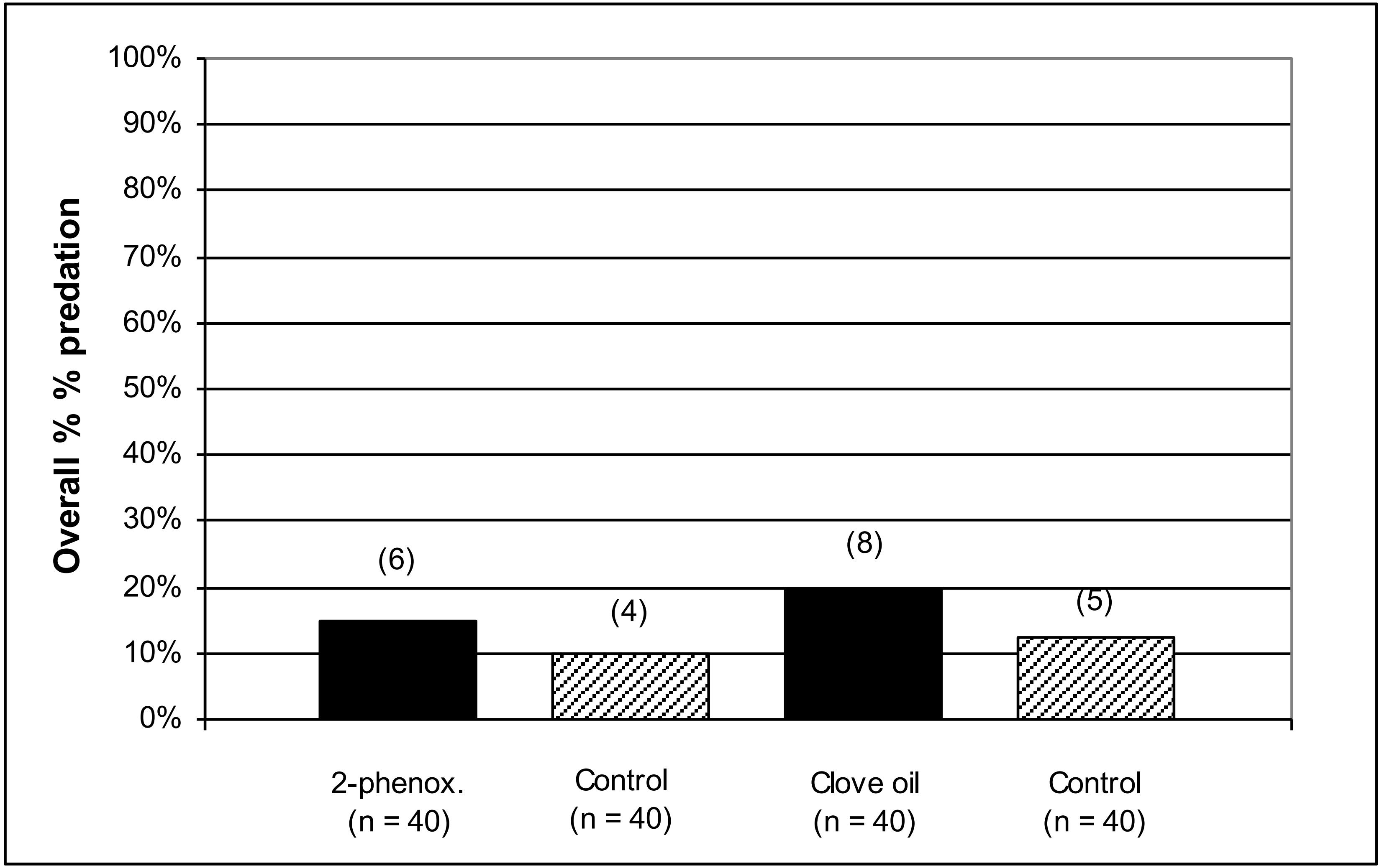
Tagging: (surgical implant): pikeperch as predators, brown trout (13-17.5 cm), 20 replicates.

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

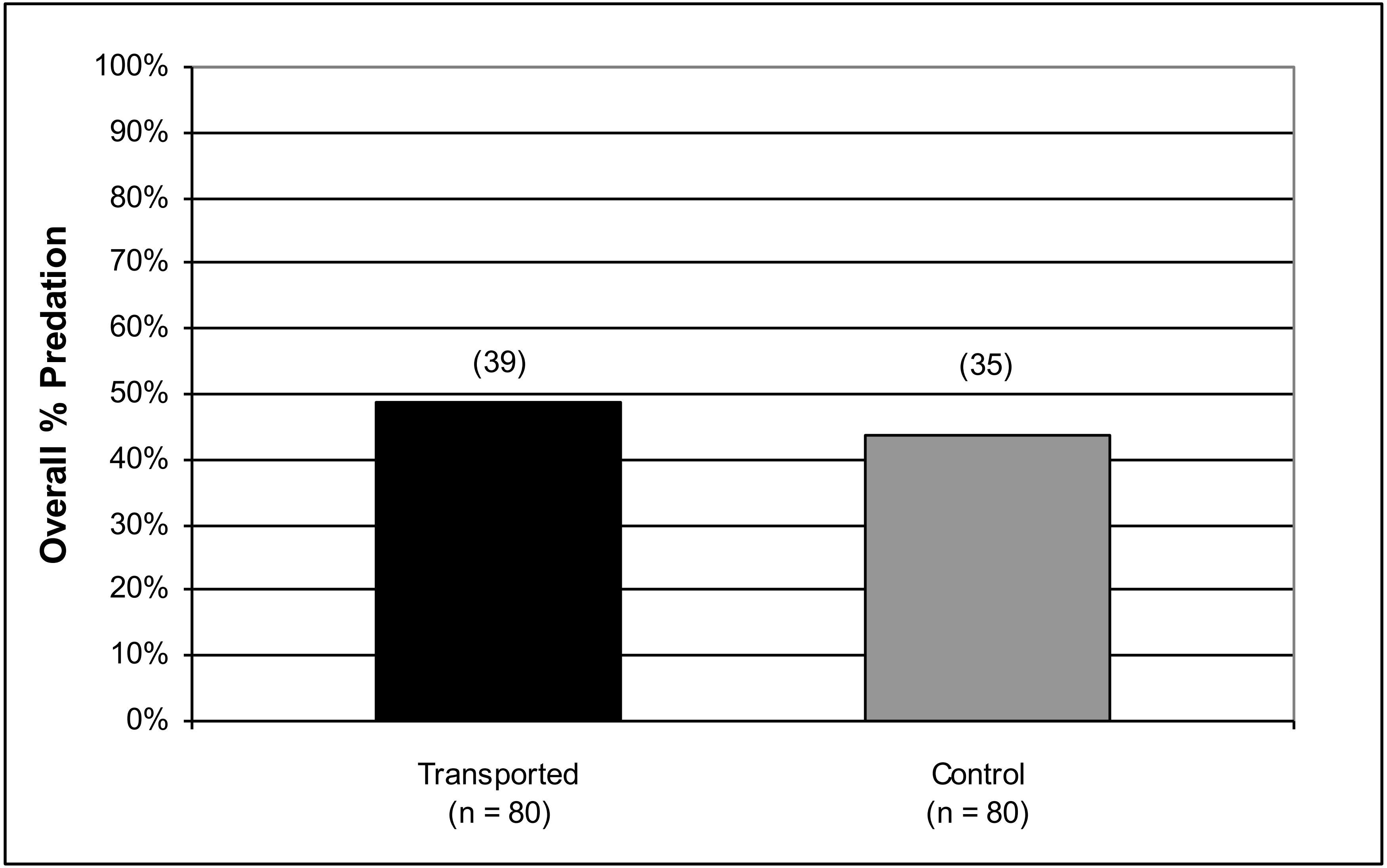


Anaesthesia Trials

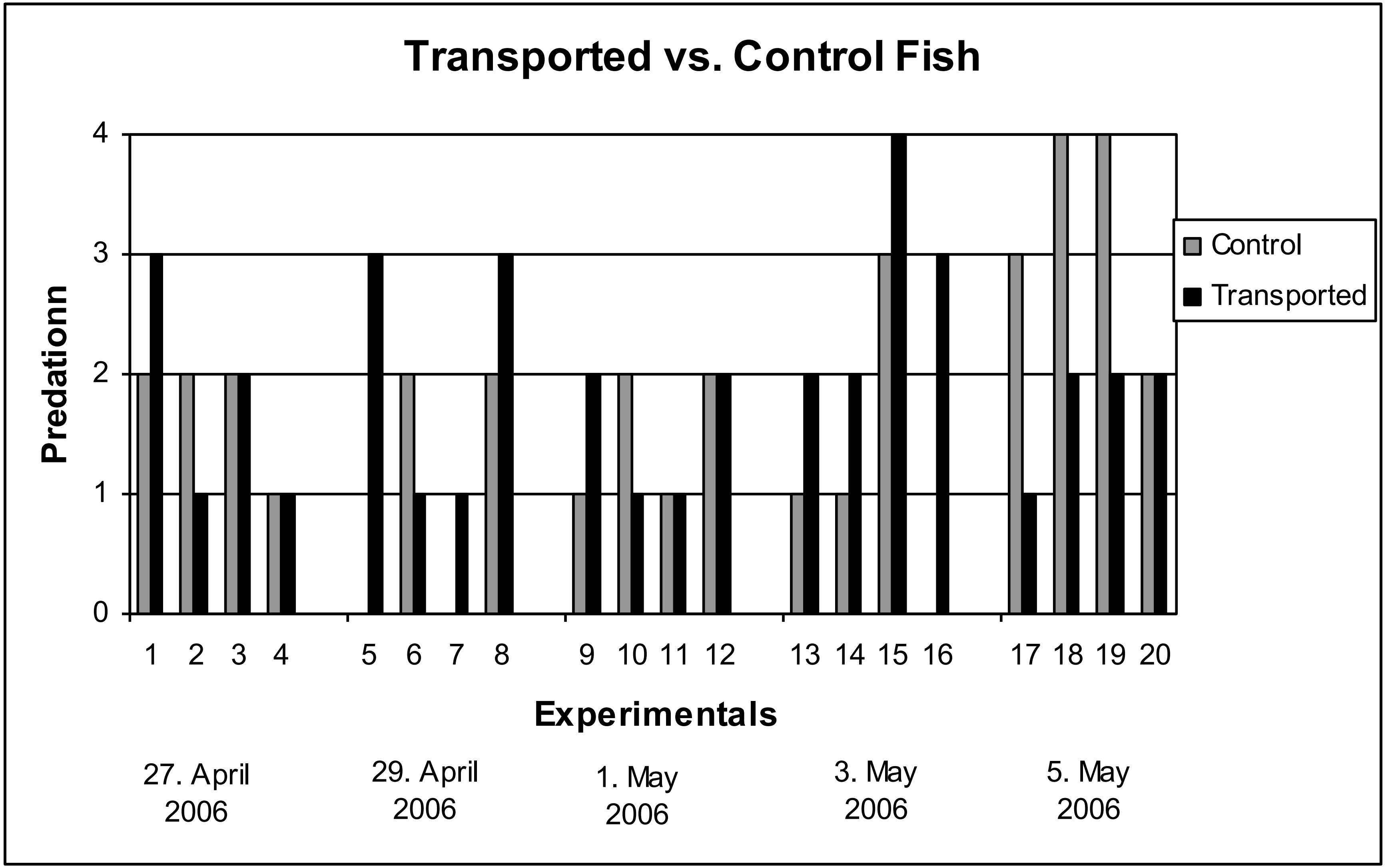
- Mimicking transport of fish for stocking

- 30 minutes of stress

- Wild brown trout v. pikeperch

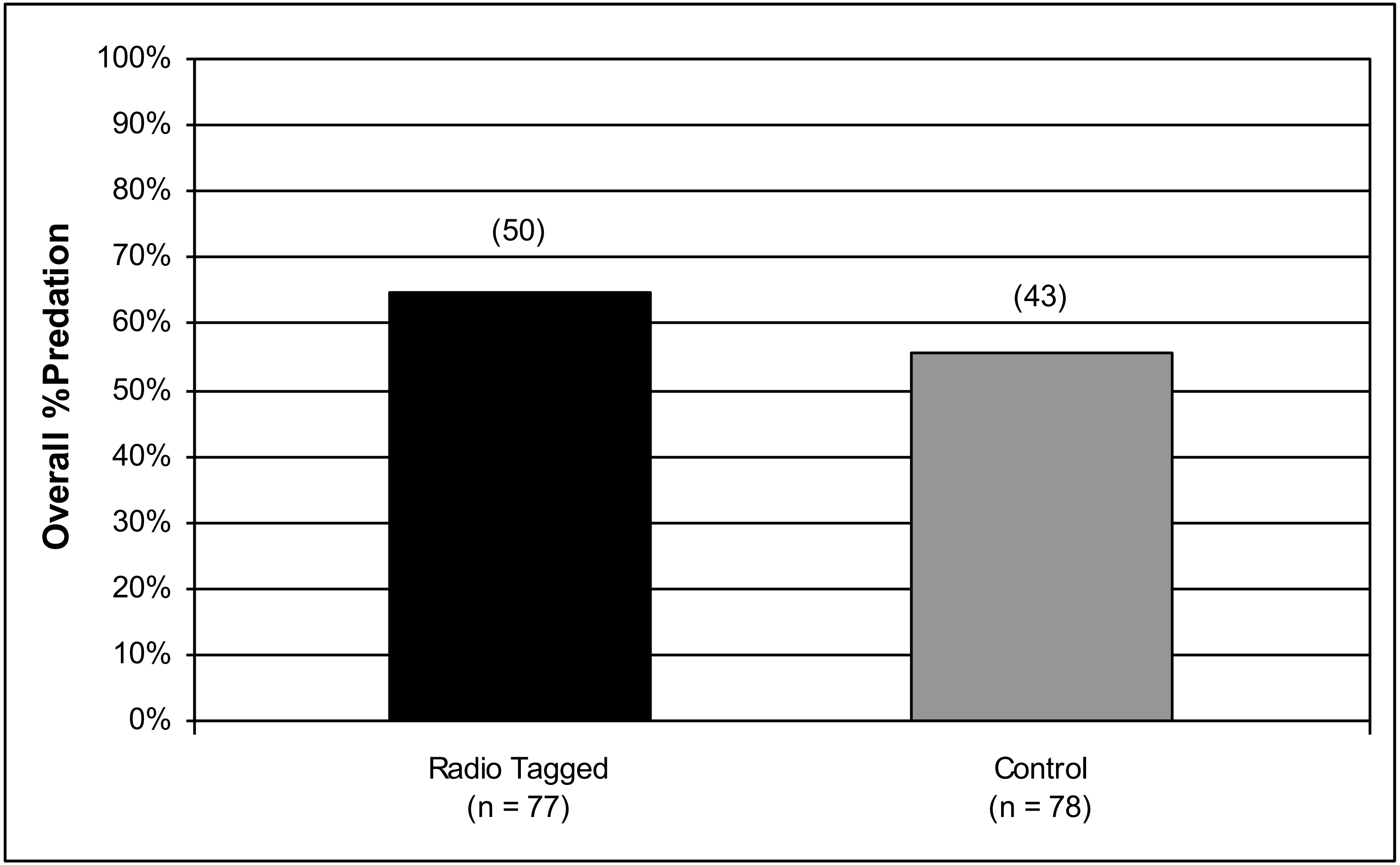


Transport - stress

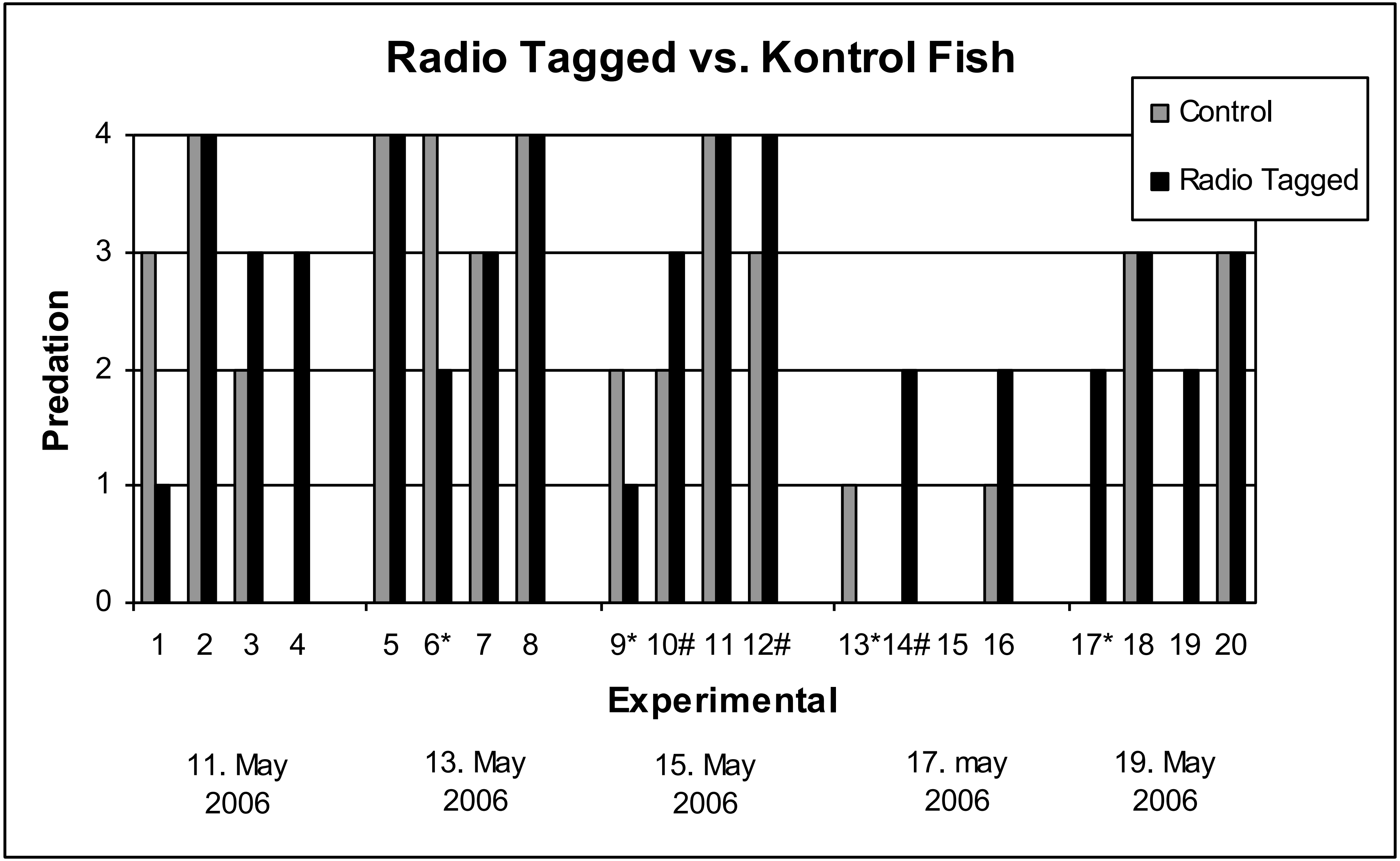


Transported vs. Control Fish
Predationn
0
1
2
3
4
Control
Transported
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Experimentals
27. April 2006
29. April 2006
1. May 2006
3. May 2006
5. May 2006

- Normal surgical implant procedure
- Fish released 24 hours post surgery
- Wild brown trout v. pikeperch



Tagging – surgical implant


Radio Tagged vs. Kontrol Fish
Control
Radio Tagged
Predation
0
1
2
3
4
1 2 3 4 5 6* 7 8 9* 10# 11 12# 13* 14# 15 16 17* 18 19 20
Experimental
11. May 2006
13. May 2006
15. May 2006
17. may 2006
19. May 2006

Conclusion

Treatment seems not to influence predation, but….

Counterintuitive, but match field results

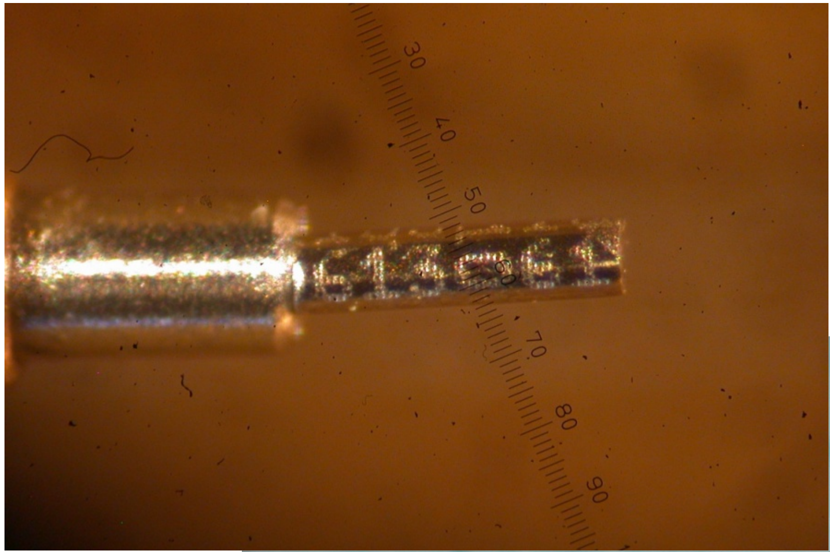
The pursuing of this issue is important

Evaluation of tagging effects in the field

Lille-Å: 188 wild brown trout were captured by E-fishing

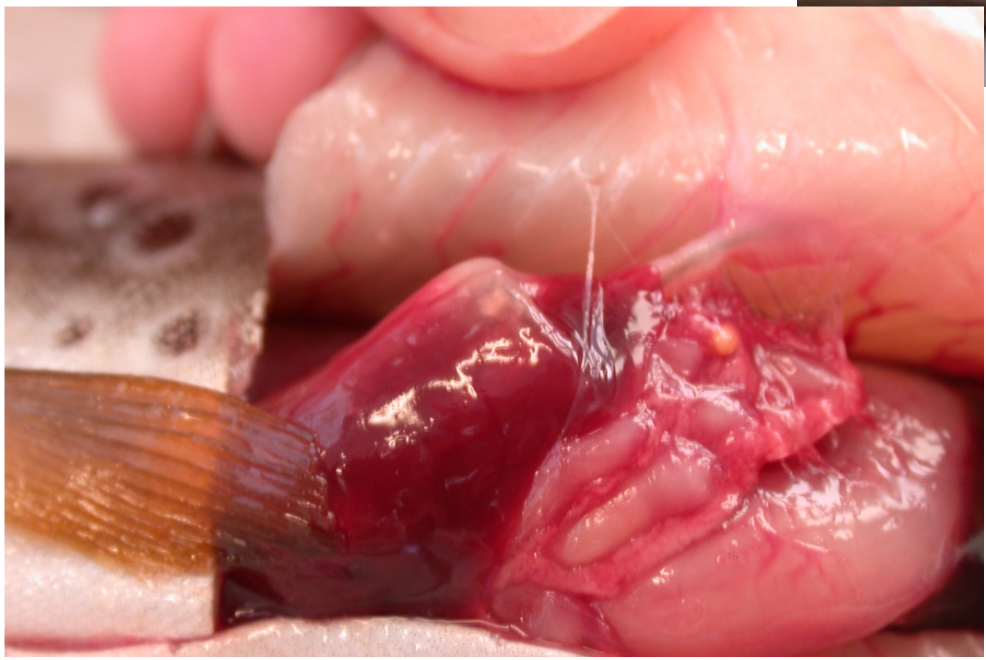
3 different treatments, and released back in the river

Recaptured (blind E-fishing) 6 months later and evaluated



CW-tagging for individual ID






Suture material	No antenna (recaptured)	Antenna (recaptured)	Expulsed	Wound healing (Score 1-4)	Specific growth (weight)
Vicryl	30 (16)	32 (19)	5 (14%)	1.40	0.29
Ethilon II	32 (17)	31 (15)	10 (31%)	1.72	0.36

Treatment	No. tagged	No. recaptured (relative survival)	Specific growth (weight)	Specific growth (length)	Tag expulsions
Control	63	36 (57 %)	**0.412**	0.1158	-
Antenna	62	33 (53 %)	0.342	0.0926	7
No antenna	63	34 (54 %)	0.311	0.0778	8

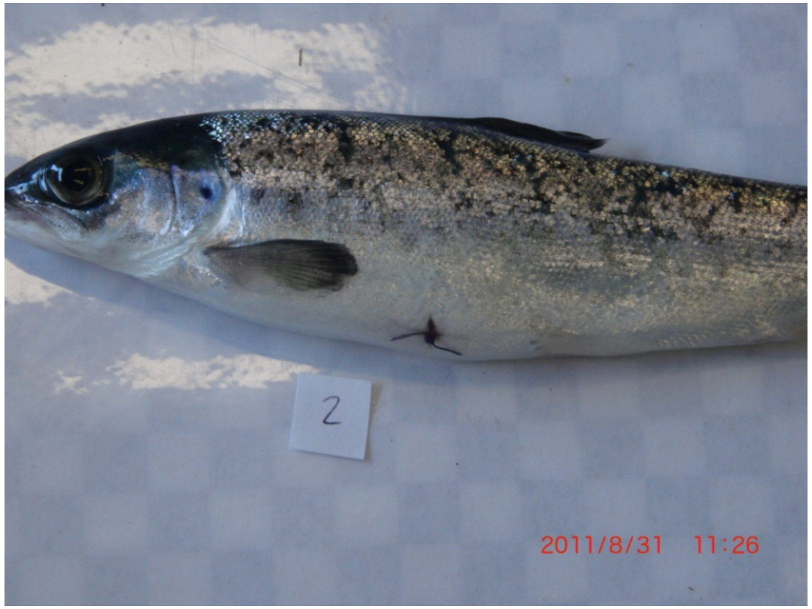
No significant effect of treatment

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”


2011/8/31 17


2
2011/8/31 11:26

8
2011/8/31 11:59

36
2011/8/31 14:52

22
2011/8/31 13:53

Treatment	N-tagged	Mortalities	Specific length growth (Var)	Healing (Median)
Clean-A	25	3	0.04 (0.0155)	**1.88**
Dirty-A	25	4	0.06 (0.0054)	**1.76**
Clean-NA	**25**	**1**	**0.09 (0.0065)**	**1.79**
Dirty-NA	**25**	**2**	**0.07 (0.0139)**	**1.71**

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

Jepsen, N., Boutrup, T.S., Midwood, J. & Koed, A. (2013). Does the level of asepsis impact the success of surgically implanting tags in Atlantic salmon? *Fisheries Research* **147**, 344-348.

Mulcahy & Harms (2014). Experimental methods fail to address the questions posed in studies of surgical techniques. *Fisheries Research 156, 1-5.*

Jepsen, N., Boutrup, T.S., Midwood, J. & Koed, A. (2014). Fish surgery – a dirty business? Comments to a letter submitted by D. Mulcahy and C.A. Harms. *Fisheries Research*

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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

Factor	External	Stomach	Implantation
Installation time	moderate	quick	slow
Difficulty	moderate	low	highest
Recovery time	moderate	quick	longest
Balance Problems	greatest	least	least
Transmitter Size	Large	Dependent on morphology	Dependent on morphology
Infection potential	moderate	least	highest
Snagging potential	yes	no	no
Drag/resistance	yes	no	no

Comparison of transmitter attachment methods

Factor	External	Stomach	Implantation
Abrasions	yes	no	no
Regurgitation	no	sometimes	no
Feeding interference	lowest	highest	lowest
Spawning interference	lowest	lowest	highest
Transmitter sensors external internal	 easy harder	 harder harder	 harder easier
Transmitter expulsion	no	no	sometimes

Most important:

Use common sense and -

TEST your methods!!!

Animal welfare

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)