Establishing best practices for implanting Passive Integrated Transponder (PIT) tags in American Eel (*Anguilla rostrata*)

Workshop Report

Ontario Species at Risk Stewardship Program Project #18-19-CWF

Sarah Rabideau

Dirk Algera

Jacqueline Pallard

Nicolas Lapointe

Canadian Wildlife Federation

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Project Summary

Researchers and practitioners implant Passive Integrated Transponders (PIT) tags into American Eel for a variety of reasons, though the practices used to tag these fish can vary widely, leading to inconsistencies in animal response, data collection, and reporting. Such inconsistencies may lead to lost opportunities to gain knowledge, or unnecessarily harm for this at-risk species. Standard practices ensure that harm is minimized, and maximum information is gained whenever an American eel is handled and tagged. Best management practices can guide practitioners on practices for handling, anaesthetizing, tagging, and releasing American Eel, with guidance on equipment choice.

Prior to the workshop, we conducted a science review to synthesize empirical, peer-reviewed literature on tagging processes for anguillid species. We reviewed scientific literature to determine common methods and synthesize comparisons of methodologies for PIT tagging eels. Over 550 articles were screened (title/abstract/keywords) from a targeted search of online scientific literature databases (Web of Science Core Collection) using pre-determined screening criteria. Additional articles were identified through multiple searches of Google Scholar using related combinations of key words (first 10 pages of each search), and through reviewing the reference sections of relevant articles. Relevant data were extracted from 50 articles published from 1998 to 2019. Screening criteria (at title, abstract, keywords) included the species used in the study, tagging type (satellite, data-storage, VIE, coded wire, PIT, acoustic, or radio), and whether they were mark/recapture studies.

Structured interviews were held with 18 practitioners to discuss their procedures for implanting American Eel with PIT tags and rationale for methods used. We then shared evidence (literature review and interview results) with practitioners and obtained recommendations for best practices via an online questionnaire. The questionnaire is included in Appendix 1, along with supplementary materials that were circulated along with the questionnaire (Appendix 2). The survey was sent to 32 practitioners, and 12 responses were received. Survey results were summarized and presented at the workshop (Appendix 2) Evidence and survey results were discussed in a workshop on 19 February 2020 to jointly develop recommendations for best management practices. Minutes from the workshop are included in Appendix 4. Tables 1 and 2 provide brief summaries of recommendations made at the workshop. The information below will be used to develop a document on best management practices for implanting PIT tags in American Eel.

Table 1: Summary of results of votes held during the workshop on best management practices for implanting PIT tags in American Eel. The % that voted "Yes" was not calculated if >50% of participants abstained. The total number of voters differed among questions given that all participants were not always present in the room or via teleconference and webinar.

Question asked at Workshop	Yes	No	Abstain	Total	% Abstained	% Yes

Do you agree with the use of FDX-A tags?	1	2	10	13	77%	
Do you agree with the use of FDX-B tags?	8	1	4	13	31%	89%
Do you agree with the use of HDX tags?	8	0	5	13	38%	100%
Do you agree with the use of encrypted tags?	0	8	5	13	38%	0%
Should the use of a universal handheld reader be the recommended best practice?	11	0	2	13	15%	100%
Do you recommend the use of Clove Oil : Ethanol to anesthetize eels?	9	0	3	12	25%	100%
Do you recommend the use of Eugenol to anesthetize eels?	5	0	7	12	58%	
Do you recommend the use of MS-222 to anesthetize eels?	9	0	3	12	25%	100%
Do you recommend the use of no anesthetic (i.e. cotton cloves) to anesthetize eels?	2	2	8	12	67%	
Do you recommend the use of an ice bath to anesthetize eels?	0	6	6	12	50%	0%
Do you recommend the use of metomidate to anesthetize eels?	0	0	12	12	100%	
Do you recommend the use of benzocaine to anesthetize eels?	0	0	12	12	100%	
Do you recommend the use of 2-phenoxyethanol to anesthetize eels?	0	0	12	12	100%	
As a best management practice, do you think 200mm should be the minimum fish length when using 8mm tags?	7	1	5	13	38%	88%
As a best management practice, do you think 200mm should be the minimum fish length when using 11mm tags?	4	1	8	13	62%	
As a best management practice, do you think 250mm should be the minimum fish length when using 12mm tags?	6	0	7	13	54%	
Do you support the use of multi-use syringes to insert PIT tags?	10	0	3	13	23%	100%
Do you support the use of single-use syringes to insert PIT tags?	10	0	3	13	23%	100%
Do you support the use of an empty needle to puncture the skin follow by manual insertion of the PIT tag?	5	2	6	13	46%	71%
Do you support the use of a scalpel to make an incision for PIT tag insertion?	8	0	5	13	38%	100%

Do you support the use of an empty needle to puncture the skin follow by a loaded needle to insert the PIT tag?	1	5	7	13	54%	
Do you agree with the use of adhesives for wound closure?	0	7	4	11	36%	0%
Do you agree with the use of sutures for wound closure?	1	6	4	11	36%	14%
Do you agree with leaving the wound open?	9	0	2	11	18%	100%
Do you agree with releasing eels after they have displayed normal swimming behaviour post-surgery?	10	0	1	11	9%	100%

Table 2: Summary of results of the workshop on best management practices for implanting PIT tags in American Eel

Topic	Summary
Tag Type	• Practitioners support use of FDX-B and HDX tags for American Eel work.
Handheld Reader	• If using encrypted tags, they should be used in a closed system. • BIOMARK has universal readers now read FDX-B, HDX, and encrypted tags.
Anesthetic	• Practitioners support use of multiple anesthetics (MS-222, clove oil, Eugenol), but very few "no" responses to other types, suggesting uncertainty and need for more research to find which anesthetics are best. • More research is needed on the benefits or drawback of no anesthetic for American Eels. • More comparative research needed for all anesthetics, particularly MS-222 on olfactory system. • No clear recommendation for 2 phenoxytethanol, benzocaine, or metomidate (e.g., AquaCalm) due to a lack of experience.
Anesthetic & Eel Size	• Electro anesthesia requires future research for American Eels, and this method is not up for recommendation currently. • No response for recommendations of anesthesia based on life stage.
Ice + Water with MS-222	• Support for cooling waters to match ambient temperatures, but less support to cool water down to enhance effectiveness of MS-222.
Fish and Tag Size	• Mueller (YEAR) is the most useful methodological study (at present) to suggest recommendations with respect to tag size.

	• Tagging American Eels above 200-mm could use multiple insertion methods. • Girth is an important factor, as is volume of the tag, relative to its mass. • Playing it conservative is the most appropriate action, despite the trade off in fish size and ramifications for tag loss. • Larger tags are used for American Eel, but for specific objectives. No recommendation was proposed for these larger tag sizes.
Tag Insertion Location	• Preference for behind the head exists along Quebec border due to consumption concerns. • Practitioners have confidence with intracoelomic (IC) implantation for American Eels of any size. • Practitioners recommend study on tag effects on different locations if not body cavity; Zimmerman and Welsh (2008) methodological study best reference at present (e.g., similar retention for IC, intramuscular, and behind head). • Practitioners caution colleagues that use larger-scale antennas in studies that include implanting PIT tags and radio tags together, as radio tags may influence PIT tags and restrict detection range.
Tag Methods & Tools	• Strong support for and opposition for scalpel incision with manual insertion. • Strong support for use of multi-use syringes. • Recommend gleaning information from literature on most appropriate surgical tools (e.g., sutures, needles, etc.)
Operative Care	• Recommendations include: o using ambient water temperature throughout operative process; o using yoga mats as padding, cleaning with Virkon between sites; o keeping “field clean” with Betadine as a disinfectant for surgical tools o avoiding the use of antibiotics or analgesics; o releasing American Eels as soon as they regain ‘normal’ equilibrium, use behavioural cues; o continuing to incorporate rigorous testing studies into empirical field studies.
Data Sharing	• Ocean Tracking Network and BIOMARK have options to manage PIT tag database, and both organizations have vetting process regarding public vs. private information from tags.

	• BIOMARK INSTREAM program shares burden of cost with partners. • Biggest potential for database management in Great Lakes and other specific basins along the east coast (e.g., Nova Scotia).
Measurements	• Other key traits to measure, in addition to those captured by researchers, include life stage, age, anesthetic dosage used, maturity phase (e.g., silver), sex, lateral line development, colour contrast, swim bladder parasites, and injuries.
Needle: Under Skin or In Muscle	• Smaller Eels (250-mm or less) should be tagged under the skin. • More research is needed to determine which option is more appropriate to retain tags in larger American Eels, if tags are not placed intracoelomically.
Wound Closure & Antiseptic Practices	• Vetbond is considered ineffective, with strong support for leaving wound open. • Betadine as an effective antiseptic for tool sterility ONLY (internal contact). • No antiseptic on wound was recommended. • Check with Cooke for citations to back recommendation if endorsing Betadine, otherwise rely on expertise, or make no specific antiseptic recommendation.
Surgery Considerations	• Ensure trap mesh size does not permit escape, which may cause potential injury or mortality to American Eels. • Reduce light conditions (e.g., PVC pipe for Eels to hide). • Consider anesthetic endpoints (e.g., opercular movements, respiration)., • Create a fresh bath of anesthetic after a low density of American Eels have been knocked out. It is good practice to pay attention to how many batches per bath.

Appendix 1. American Eel (*Anguilla rostrata*) PIT tagging questionnaire, shared with 32 experts in January 2020. Each section of the questionnaire began with a summary of the literature and previous practitioner interview results. Respondents were then asked for their recommendations for best management practices, based on the information provided and their own experience.

Methods for implanting American Eel with passive integrated transponder (PIT) tags are known to vary widely amongst practitioners and projects. Evidence-based research and expert knowledge can inform the development of best practices for PIT tagging American Eel.
The goal of the following questionnaire is to identify a suite of standards and practices that will be summarized in a best management practices document and shared with practitioners.
This questionnaire summarizes information on methods derived from the scientific literature and technical reports on all anguillid species (Appendix F), and interviews with 19 practitioners from across Canada and the United States that work with American Eel specifically.

This questionnaire focuses on the following topics:

- PIT tag type (HDX vs FDX)
- PIT tag size and minimum tagging size
- Anesthetic
- Tag insertion location
- Tagging process
- Tools (scalpel vs. needle)
- Wound care

We kindly ask that you review each section summary prior to answering the associated questions. Though insight is drawn from the published literature on all anguillid species, the questions are mean to focus on tagging practices for American Eel specifically. Responses will be summarized and presented at a subsequent multi-stakeholder workshop for further discussion.

Tag Type and Reader

PIT tags are either full-duplex (FDX) or half-duplex (HDX), where duplex refers to the capacity to send and receive data. An FDX system can receive and transmit information simultaneously (i.e., a phone call), whereas an HDX system transmits then receives (i.e., a walkie talkie). All HDX tags meet ISO standards (ISO 11784 and ISO 11785) of having an unencrypted microchip with a 15-digit code that can be read by all universal PIT tag readers. FDX tags are available in two sub-technologies: FDX-B tags meet the ISO PIT tag standards, but FDX-A tags do not. FDX-A tags can have various code lengths (i.e., AVID FriendChip, 9 digits; FECAVA FriendChip, 10 digits) and can also be encrypted (i.e., AVID FriendChip), meaning they can only be read by that company's readers.

PIT tags can be read via handheld readers or passive antenna systems. With handheld readers, a fish is manually scanned for a PIT tag and the number recorded, whereas passive antenna

systems read and record tag numbers automatically as fish pass through the antenna. As such, the PIT tag type selected depends on the objectives of the research, the morphology of the specimens, and the study environment.

Handheld readers can be brand-specific for encrypted tags (i.e., AVID Friendchip, Destron FECAVA), tag-type specific (i.e., FDX-A, FDX-B, or HDX), or universal (FDX-A, FDX-B, HDX, and encrypted). However, some universal readers may not be able to read all encrypted tag types. Most antenna arrays will be designed to read a single tag type (i.e. FDX or HDX), however they can be designed to read both types with the correct equipment and if specific criteria are met (i.e. tag size).

Table A1.1: Examples of some of the primary differences between FDX and HDX PIT tags and readers.

Criteria	FDX	HDX	Reference
Tag cost (USD)	1.70	1.70	Biomark, 2020; Oregon RFID, 2020
Antenna reader cost (USD)	3647.00	2150.00	Biomark, 2020; Oregon RFID, 2020
Read rate (#/s)	30	14	Avid, 2019; Biomark, 2020; Oregon RFID, 2020
Detection range (mm)	20-360	<1000	Johnston et al. 2009
Minimum tag size (mm)	8	12	Avid, 2019; Biomark, 2020; Oregon RFID, 2020

Literature Review

Authors used FDX or HDX tags for a variety of project types (Table 2). HDX tags were most used for field studies (N = 19) compared to FDX tags (N = 4). No studies compared the performance of the two PIT tag types in anguillid species; however, these differences are well understood and do not vary among target species.

Table A1.2: Number of studies that reported PIT tag type used and the associated research objectives. N >18 as some studies reported multiple research objectives.

Research Objective	Tag Type	
	HDX	FDX
Tagging methodology	3	2
Hydroelectric facilities	6	0
Local movement & dispersal	4	1
Migration	6	1

Practitioner Interview Results

Practitioners used different tag types: three used HDX and five used FDX, exclusively; three used both types, another four were unsure of the tag type they used, and two did not answer. One practitioner identified using an HDX tag under 12-mm.

Though practitioners were not asked about their reader equipment, most stated they used handheld tag readers or that they expected their PIT tags would be read by fisherman using handheld readers. To this end, read range, read rate, and tag type were not considered a concern, because these practitioners assumed that handheld readers could read both HDX and FDX tag types.

One practitioner stated they select FDX tags because of their smaller size, to implant in smaller American Eel, while another selected HDX tags because of their higher detection range by fixed arrays, and because HDX antennas are less expensive. However, if FDX tags and antennas were cheaper, one practitioner would prefer FDX over HDX thanks to their increased read rate.
One practitioner cautioned against the use of encrypted PIT tags (i.e., FDX-A) because readers and antennas from other manufacturers cannot read these tags. This could lead to fish being double tagged, cause tag collisions, or result in lost data if other practitioners fail to read the tags.

Tag Type Questions

Indicate if best practices should permit the use of each tag type?

	Yes	No	Unsure
FDX-A	○	○	○
FDX-B	○	○	○
HDX	○	○	○
Encrypted	○	○	○

Should best practices include the use of universal handheld readers that are able to read all unencrypted tag types?

- ○ Yes
- ○ No
- ○ Unsure
- ○ Other/Comments:_____

Fish and Tag Size

Tag size and body size are important factors to consider when tagging a fish. A tag that is too large relative to the fish's body size can have physiological and behavioural effects. Currently, HDX tags are a minimum of 12-mm and FDX tags a minimum of 8-mm in length.

Literature Review

Twelve different PIT tag sizes were identified by authors (N = 38) in our literature review, with 12-mm tags used most often and for the greatest size range of eels (Table 3). With exception of 32-mm tags, which were only used for mature eels >500 mm, the sizes of eels tagged with each PIT tag size varied greatly (Appendix B).

Table A1.3: PIT tag sizes reported by authors in the literature review. Eel size range indicates the smallest and largest (when reported) eels tagged by each tag size. N indicates the number of times each tag size was reported by an author. N >38 as some authors used multiple tag sizes in a study.

Tag Size (mm)	Eel Size Range (mm)	N
8.5	*>250*	1
9	*320-630*	2
11	*90-110*	1
11.4	*115-208*	1
11.5	*>200*	4
12	*87-1650*	11
12.5	*>161*	4
15	*>400*	1
23	*>230*	6
32	*>500*	9
50	*N/R*	1
120	*N/R*	1

Most studies designed to assess post-implantation effects in anguillid species reported 100% survival. Mueller et al. (2017) surgically implanted 11.4-mm Eel/Lamprey Acoustic Tags (ELAT) tags (with dimensions designed to mimic PIT tag dimensions) in 115- to 208-mm long American Eel and observed 100% survival, though tag retention was limited. Only two studies of PIT tagging effects on smaller eels reported less than 100% survival. Hirt-Chabbert & Young (2012) surgically implanted 11-mm PIT tags in small (90-110 mm) Short-finned Eel (*A. australis*) and observed 5% mortality.

Mazel et al. (2013) observed a 1.8% mortality rate when injecting 12-mm PIT tags in the intracoelomic cavity of European Eel (*A. anguilla*), due to the death of one of the smallest fish they tagged (276-mm).

In contrast with survival, body size did appear to influence tag retention. Smaller eels appeared to have lower tag retention relative to larger eels, clearly illustrated by Mueller et al. (2017) who studied tag retention for a range of size classes, holding other tagging methods constant. Tag retention was significantly lower in eels <150 mm, with none retained in the smallest eels tagged (115- to 130-mm). Tag retention ranged from 50 to 75% in 134- to 208-mm eels but did not significantly increase with body size in fish > 150-mm. Overall, eels <209 mm had lower tag retention compared to larger eels, even when smaller PIT tags were used. Lower tag retention in fish >208 mm was attributed to wound care and insertion location (see corresponding topics later in the survey).

Table A1.4: Comparison of survival and tag retention for various PIT tags and eel body sizes. Studies presented here explicitly measured survival and retention.

Tag Size (mm)	Body Size (mm)	Survival (%)	Retention (%)	Species	Reference
11	90-110	95	96	*A. australis*	Hirt-Chabbert & Young, 2012
11.4	115-117	100	0	*A. rostrata*	Mueller et al., 2017
	121-130	100	0		
	134-139	100	50		
	145-150	100	75		
	151-159	100	67		
	161-208	100	57		
12	350-805	100	100	A. anguilla	Charrier et al., 2012
12	247-732	98	100	*A. anguilla*	Mazel et al., 2013
12	590-790	100	100	*A. anguilla*	Thorstad et al., 2013
12	270-725	95	94	*A. rostrata*	Turner et al., 2018
	270-725	100	79		
12.5	161-458	100	100	A. rostrata	Rudershausen et al., 2019
12.5	205-370	100	88	*A. rostrata*	Zimmerman & Welsh, 2008
	205-370	100	100		
	205-370	100	100		
15	428-570	100	100	*A. australis*	Jellyman & Crow ,2016
23	>250	100	100	*A. rostrata*	Sweezey, 2014
23	>313-376	100	100	*A. anguilla*	Wright et al., 2015

Practitioner Interview Summaries

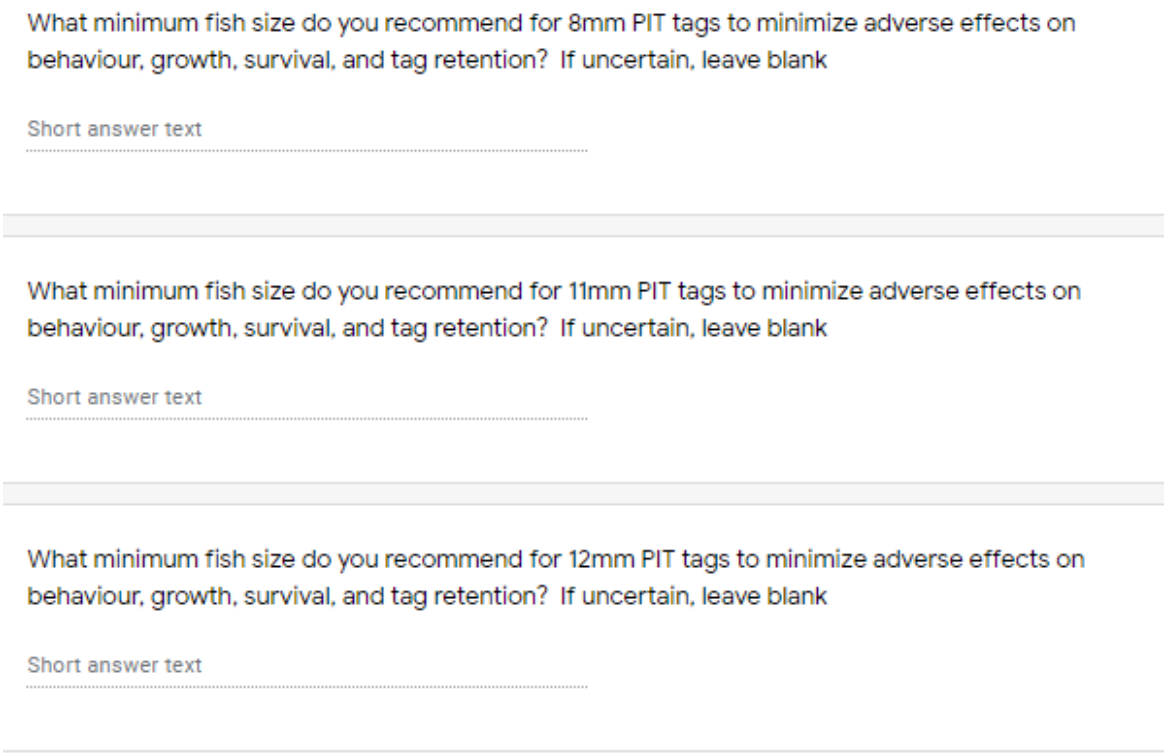
Practitioners selected tag sizes based on the size of eels they intended to tag, but also user preference and what was on hand from previous projects. Practitioners often used different tag sizes with the same study, depending on the size of the eel, to minimize tagging effects.

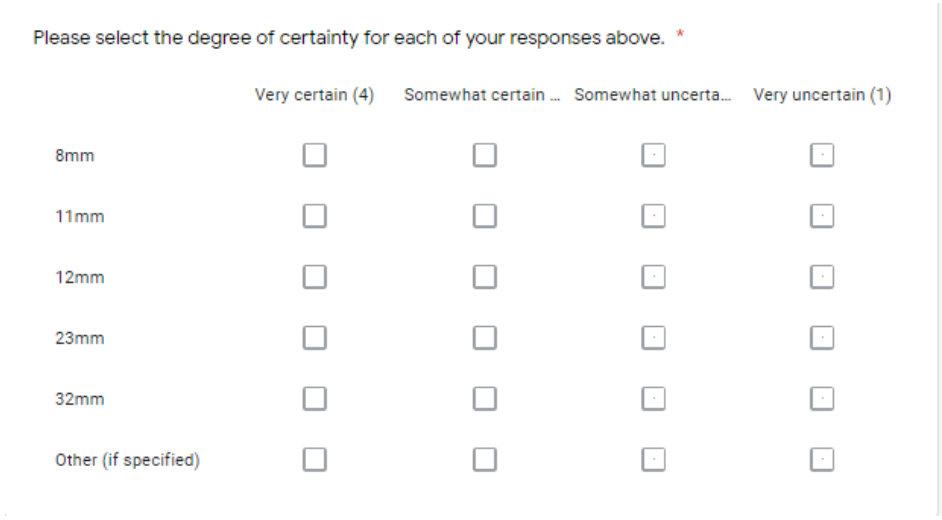
The most common tag size used was 1-mm for American Eel >200-mm in length, though smaller tags (i.e., 8- to 11-mm) tags were used for 90- to 300-mm American Eel (Table 5).

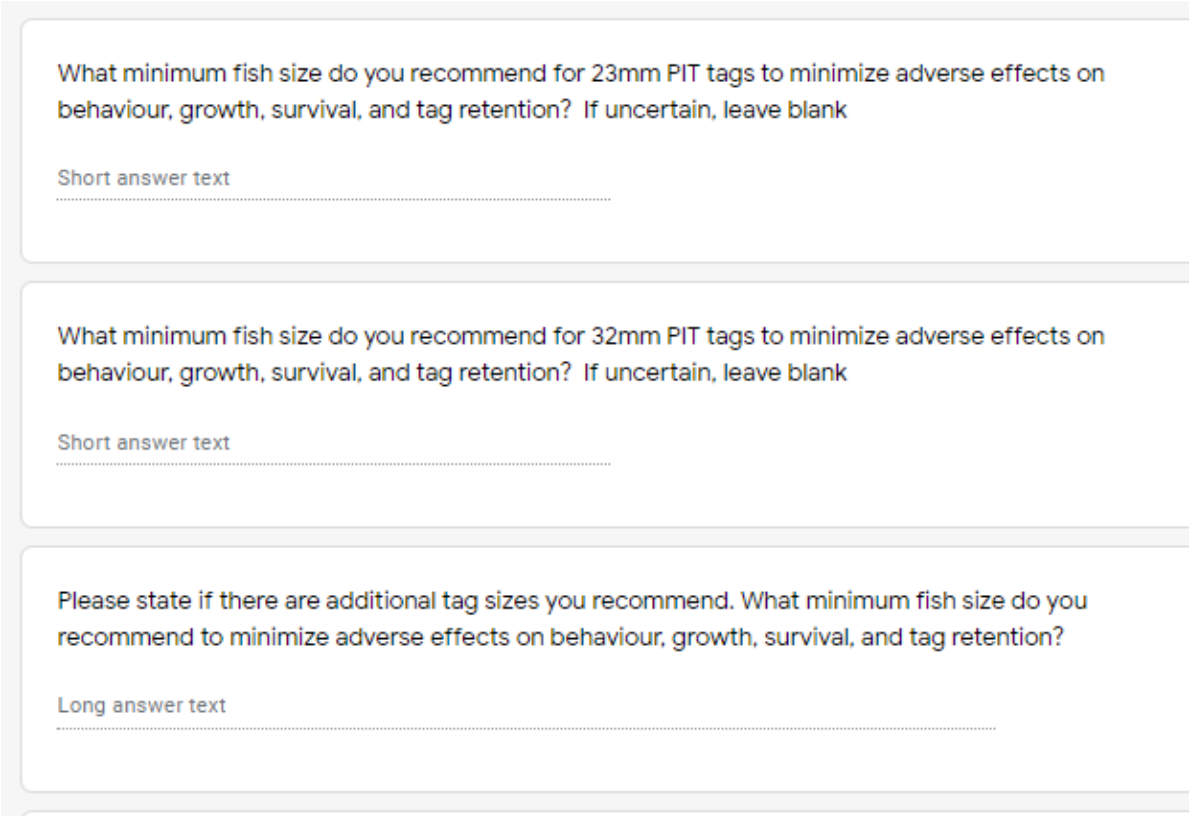
Table A1.5: Summary of tag sizes used, and associated eel lengths based on a range of reported minimum sizes. More than one method (tag and eel size combination) was reported by some practitioners. The number of times a method was reported may sum to more than 19 as some practitioners reported using more than one tag size. If a practitioner did not identify the size(s) of eel they tagged, their practice was not reported in this table.

Tag length (mm)	Minimum American Eel size range (mm)	# of times tag length was reported
8	90 - 300	7
11	200	1
12	130 - 500	6
12.5	200	4
23	250	1
32	250	1

Fish and Tag Size Questions







Anesthetic

When performing surgical procedures on fish, anesthesia is typically considered achieved when the fish exhibits a loss of equilibrium. Water temperature, water chemistry, and animal stress levels influence dosage response.

In the 43 studies reviewed, seven methods were reported by 40 authors to anesthetize anguillid species for PIT tagging (Figure 1). Electronarcosis was the only non-chemical method used to anesthetize eels. Of the six chemical anesthetics reported, five had a range of dosages used for a wide range of eel sizes (Table 6 & Appendix C).

Figure 1: Percent of studies that used each anesthesia method (N = 41). Data includes studies (N = 9) that anesthetized eels to implant both a PIT tag and an acoustic or radio tag. N >40 because one study included two anesthetic types.

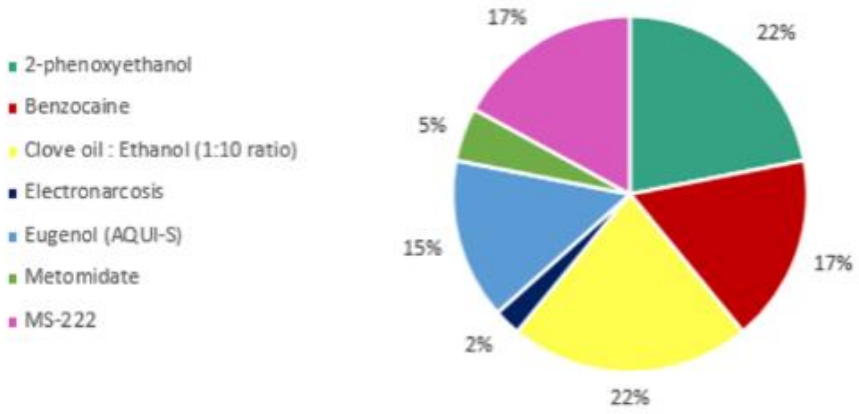


Table A1.6: Chemical anesthetic dosages used to anesthetize eels in the literature review. Eel size range indicates the smallest and largest (when reported) eels anesthetized using each chemical anesthetic. N indicates the number of times a dosage was reported. Data includes studies (N=7) that anesthetized eels to implant both a PIT tag and an acoustic or radio tag.

Anesthetic	Dosage	Eel Size Range (mm)	N
2-phenoxyethanol	0.2-1.5 mL/L	175-1100	6
Benzocaine	100-200 mg/L	>90	8
Clove oil : Ethanol (1:10 ratio)	90-250 mg/L	>205	4
Eugenol (AQUI-S)	0.2-0.5 mL/L	87-1005	2
Metomidate	40 mg/L	>580	2
MS-222	240-300 mg/L	>115	3

There were two methodological studies comparing anesthetic use. Iversen et al (2013) compared the efficacy of metomidate (40 mg/L) and eugenol (Aqui-S vet; 300 mg/L) for anesthetizing European Eel. Induction time did not differ significantly between the two anesthetics, though sample size was small (N = 10 eels each). For eugenol, induction time was 3.8 +/- 1.6 min and recovery time was 6.8 +/- 3.1 min. For metomidate, induction time was 2.6 +/- 0.6 min and recovery time was 4.8 +/- 2.0 min. There was also no difference in long-term

European Eel survival between the two anesthetic treatments after a four-month holding and assessment period.

Walsh and Pease (2002) conducted a comprehensive study comparing the effects of anesthetic type (clove oil, benzocaine) on Longfinned Eel (*A. reinhardtii*). They initially found that eels had a variable response to benzocaine, even at relatively high concentrations. Because of this, human health risks if eels are later consumed, and cost, Walsh and Pease recommended clove oil, and conducted further experiments. They explored the effects of combinations of concentration (60 or 80 mg/L), water temperature (17 or 25C), salinity (6 or 18 g/L), eel source (hatchery, wild), and life stage on the induction time (i.e., loss of equilibrium) and recovery time of Longfinned Eel. They found that induction time converged between 17 and 25^{o}C at a concentration of 100 mg/L, and recommended this as a general optimum concentration.

Practitioner Interview Summary

Anesthetics used on American Eel were dependent on jurisdictional regulations, given that clove oil is restricted in some regions. To this end, one practitioner used clove oil under an experimental analysis permit because it anesthetized eels more quickly than MS-222. Practitioners used three chemical anesthetics: MS-222, clove oil, and eugenol (Figure 2, Table 7). Two practitioners preferred MS-222 for smaller eels and clove oil for larger, mature eels. Two did not use anesthetics and instead used an ice bath or cotton gloves to slow down or immobilize the eel. No practitioners reported immediate mortalities.

Two practitioners that used MS-222 noted that higher doses are required for eels to lose equilibrium in cold water. One placed blocked ice in their MS-222 anesthetic bath when anesthetizing larger, mature eels to extend the unconscious period. A different practitioner noted that ice cannot be added to an anesthetic bath containing clove oil, because it will come out of suspension.

One practitioner who used eugenol worked with a veterinarian to determine the best anesthetic. The veterinarian switched their recommendation from MS-222 to eugenol after observing long (>15 minute) induction times. This increased stress and reduced oxygen uptake. Though nearly half of practitioners interviewed provided a specific concentration used, there was no consensus on the dosage for either clove oil or MS-222 because these were frequently adjusted based on behaviour and eel size.

Figure 2. Percent use of each anesthesia method reported by practitioners (N = 19).

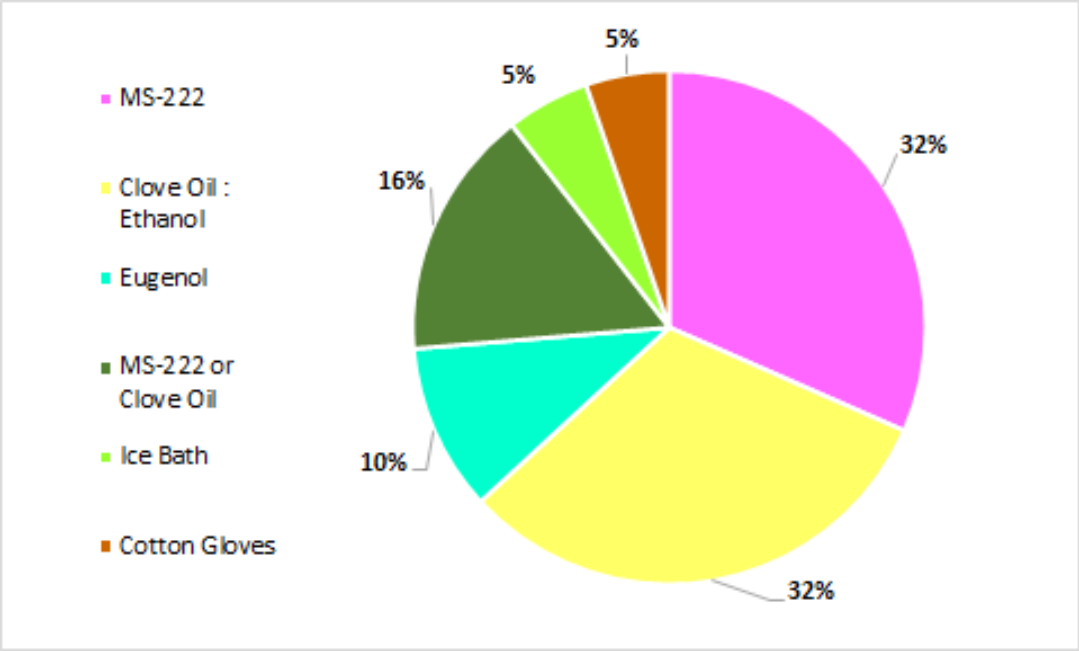


Table A1.7: Dosage range of anesthetics used by practitioners for American Eel. 'N/A' is noted when eel size range was not indicated.

Anesthetic	Dosage (mg/L)	Eel Size Range (mm)
Clove oil	40-80 100 200	>200 >300 N/A
Eugenol	90 120	<750 >750
MS-222	75 100 300 400	<130 >250 >130 >90

Anesthetic Questions

Indicate if you recommend the use of each anesthetic to sedate and PIT tag American Eel to ensure negligible adverse effects.

	Yes	No	No recommendation
2-phenoxyethanol	☐	☐	☐
Benzocaine	☐	☐	☐
Clove Oil mixed with Ethanol	☐	☐	☐
Eugenol (AQUI-S)	☐	☐	☐
Metomidate (AquaCalm)	☐	☐	☐
MS-222	☐	☐	☐
Ice bath	☐	☐	☐
No anesthetic (i.e., only cotton gloves)	☐	☐	☐

Select the degree of your certainty with each choice above.

	Very certain (4)	Somewhat certain (3)	Somewhat uncertain (2)	Very uncertain (1)
2-phenoxyethanol	☐	☐	☐	☐
Benzocaine	☐	☐	☐	☐
Clove Oil mixed with Ethanol	☐	☐	☐	☐
Eugenol (AQUI-S)	☐	☐	☐	☐
Metomidate (AquaCalm)	☐	☐	☐	☐
MS-222	☐	☐	☐	☐
Ice bath	☐	☐	☐	☐
No anesthetic (i.e., only cotton gloves)	☐	☐	☐	☐

Please recommend a dosage or dosage range for the use of 2-phenoxyethanol.

Short answer:_____

Please recommend a dosage or dosage range for the use of benzocaine.

Short answer:_____

Please recommend a dosage or dosage range for the use of clove oil mixed with ethanol.

Short answer:_____

Please recommend a dosage or dosage range for the use of metomidate (AquaCalm).

Short answer:_____

Please recommend a dosage or dosage range for the use of MS-222.

Short answer:_____

Where jurisdictional regulations permit, should different anesthetic types be used based on American Eel size (i.e. adult vs. juvenile)?

- Yes
- No
- Unsure
- Other/Comment:_____

Should ice be added to water (if using MS-222) to prolong the unconscious period?

- Yes
- No
- Unsure
- Other/Comment:_____

Tag Insertion Location and Retention

The placement of an electronic tag within an eel may influence its physiology and survival, and may also influence detection by antenna arrays (based on where a fish sits in the water column). It may also influence the likelihood of tag retention. The placement of the tag may also affect detection by handheld readers if the operator does not scan the correct area of the fish.

Literature Review

Of the 41 PIT tag studies that reported insertion location, PIT tags were inserted into the intracoelomic cavity along with a radio or acoustic tag in 9 studies. In the remaining 32 studies, only PIT tags were inserted. Three of these studies used multiple locations. Turner et al. (2017) changed from implanting PIT tags in the dorsal musculature to the intracoelomic cavity in a

subsequent year to reduce handling stress and increase tag retention based on the results of two tagging location studies and one long-term tagging effects study. Morrison and Secor (2003) did not indicate why tagging location varied between years, and Zimmerman and Welsh (2008) conducted a methodological study on the effects of insertion location on survival and retention.

Three tagging locations were indicated: the dorsal musculature behind the head (1), the dorsal musculature near the origin of the dorsal fin (2), and the intracoelomic cavity (3) (Figure 3). Of the reported PIT tag insertion locations that were not associated with radio or acoustic tag implantation (N = 35), 6% inserted the tag behind the head, 11% inserted near the origin of the dorsal fin, and 83% inserted in the intracoelomic cavity. All studies that inserted both a PIT and an acoustic or radio tag inserted the PIT tag in the intracoelomic cavity.

Zimmerman & Welsh (2008) compared survival and tag retention among three tagging locations. They found no difference in survival for American Eel according to tagging location. Eighty eight percent of tags implanted behind the head were retained, compared to 100% of those implanted in the intracoelomic cavity and 100% of those implanted near the dorsal fin origin. Turner et al. (2017) cited three studies of tag retention by location including Baras and Jeandrain (1998), Zimmerman & Welsh (2008), and Mazel et al. 2013. Baras and Jeandrain (1998) used larger epoxy dummy acoustic transmitters rather than PIT tags. Mazel et al. (2013) implanted 12-mm PIT tags in the intracoelomic cavity of 56 European Eel ranging from 247-732 mm in length. One fish (276 mm) died. All remaining fish retained tags for the 28-day observation period, by the end of which 54 of 56 incisions had fully healed. No other pattern on the effects of insertion location on survival or tag retention was evident in comparisons across survival and tag-retention studies.

Practitioner Interview Summary

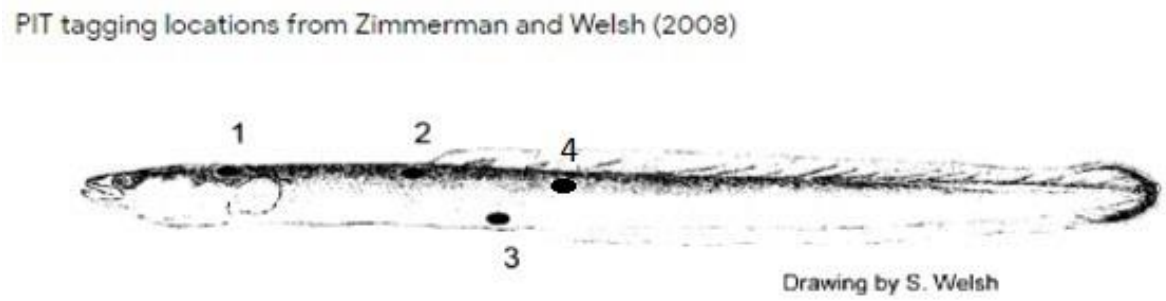
Practitioners inserted PIT tags in four different locations: 45% implanted PIT tags behind the head, 25% in the dorsal musculature anterior to the dorsal fin, 5% in the dorsal musculature above the cloaca, and 25% in the intracoelomic cavity.

A variety of rationale were provided for implanting PIT tags behind the head. One practitioner did so to promote tag retention, because they assumed that tags in the dorsal musculature could be dislodged or relocated because of the species' serpentine movement. Two practitioners implanted PIT tags behind the head because it was the way their predecessors tagged eels. Two practitioners did so to coordinate with Quebec commercial fishers, who are accustomed to scanning this location. One practitioner did so to avoid damage to internal organs and to avoid a tag ending up in people's food if harvested.

In contrast to practitioners who thought that tags in the dorsal musculature might be dislodged, one practitioner noted that PIT tags were eventually pushed out when implanted behind the head and expected better retention in front of the dorsal fin. The only practitioner to implant tags in the dorsal musculature above the cloaca did so to prevent infection and keep infections

away from the head and fins. The two practitioners that used a combination of PIT tags and acoustic/radio telemetry implanted the PIT tag in the body cavity for convenience, because a surgical incision was already made.

Figure 3 – Common tagging locations for eels (adapted from Zimmerman and Welsh 2008). Tagging locations include: 1) dorsal musculature behind the head; 2) dorsal musculature near the dorsal fin origin; 3) intracoelomic cavity; and 4) dorsal musculature above the cloaca.



Tag Insertion Location Questions

Do you recommend a standard location for implanting PIT tags in American Eel, and if so, where?

- Behind the head
- Dorsal, anterior of the dorsal fin
- Dorsal, above the cloaca
- Intracoelomic cavity
- Do not standardize location
- Unsure
- Other/Comments:_____

If a surgical incision is being made for another purpose (i.e., implanting an acoustic or radio transmitter), should the PIT tag be placed with the other tag in the intracoelomic cavity, or in a different, consistent location?

- Intracoelomic cavity
- Other consistent location (i.e., your answer from Q.13)
- Unsure
- Other/Comments:_____

Tagging Methods and Tools

There are two common methods for PIT tagging eels: surgery and injection. Surgery involves making an incision with a scalpel to implant the PIT tag into the chosen location. With injection, a needle or similar device punctures the skin and places the tag into the chosen location.

Literature Review

Of the 39 PIT tag studies that reported insertion method, 30 studies involved only PIT tags. In one of these studies (Hirt-Chabbert & Young, 2012), PIT tags were inserted using both methods. In the remaining nine studies, PIT tags were inserted along with an acoustic or radio transmitter.

There was a nearly equal split in studies that inserted PIT tags (N = 32): 53% inserted tags via injection compared to 47% through surgical incision. All studies that reported making a surgical incision noted that tags were then inserted manually, rather than using an injector. Two studies reported using a needle to puncture the skin, then manually inserting the PIT tag. In all studies that involved inserting a PIT tag plus an acoustic or radio transmitter (N = 10) the PIT tag was inserted through the surgical incision made for the larger transmitter.

In most studies, tag depth was not reported; however, two studies reported inserting PIT tags subcutaneously (i.e., between the skin and musculature), and four studies reported inserting PIT tags in the dorsal musculature.

No methodological study was found in the literature that compared surgical to needle injection methods. No pattern of effects of insertion method on survival or tag retention was evident in comparisons across all survival and tag-retention studies.

Practitioner Interview Summary

Most practitioners – 85% - injected American Eel with tags by needle (e.g., preloaded implanting guns, multi-use syringes), whereas 15% manually inserted PIT tags through an incision made with a scalpel. Practitioners that used needles considered them effective tools that created minimal wounds. They also noted that needles were cheap enough to replace once dull.

Practitioners that used an injection method did so using one of four approaches:

- 5 practitioners pierced the skin and inserted a PIT tag using a multi-use needle (discarding them as they became dull);
- 4 practitioners used a single-use, preloaded needle that came with an implanting gun; or
- 3 practitioners created a small incision with a scalpel to break the skin then injected a PIT tag with a multi-use syringe.
- 1 practitioner pierced the skin with a needle then inserted the PIT tag manually

One practitioner directly implanted PIT tags in the intracoelomic cavity using a multi-use syringe after a colleague first punctured a hole through the skin using an empty syringe. Interestingly,

four biologists that used multi-use syringes to implant PIT tags into the intracoelomic cavity recognized an increased risk of damaging internal organs and that care should be taken to prevent this when tagging American Eel. The one practitioner that used a scalpel to make an incision before injecting a PIT tag with a multi-use syringe did so to avoid dulling syringe tips. To improve retention, several practitioners inserted PIT tags underneath the skin at a shallow, 30-35 degree angle, rather than in the musculature. To improve tag retention, these practitioners recommended that the needle be inserted 2- to 3-cm along the musculature below the skin, that a finger be pressed over the tag after the plunger is deployed, and that the tag be firmly massaged into place.

Tag Insertion Questions

Indicate if you recommend the use of each anesthetic to sedate and PIT tag American Eel to minimize adverse effects.

	Yes	No	No recommendation
Puncture and injection via Implanting gun (i.e., single-use syringe)	☐	☐	☐
Puncture and injection via multi-use syringe	☐	☐	☐
Empty needle puncture followed by multi-use syringe injection	☐	☐	☐
Empty needle puncture followed by manual insertion	☐	☐	☐
Scalpel incision followed by multi-use syringe injection	☐	☐	☐
Scalpel incision followed by manual insertion	☐	☐	☐

Please select the degree of certainty for each of your responses above.

	Very certain (4)	Somewhat certain (3)	Somewhat uncertain (2)	Very uncertain (1)
Puncture and injection via Implanting gun (i.e., single-use syringe)	☐	☐	☐	☐
Puncture and injection via multi-use syringe	☐	☐	☐	☐
Empty needle puncture followed by multi-use syringe injection	☐	☐	☐	☐
Empty needle puncture followed by manual insertion	☐	☐	☐	☐
Scalpel incision followed by multi-use syringe injection	☐	☐	☐	☐
Scalpel incision followed by manual insertion	☐	☐	☐	☐

If implanting tags behind the head or along the dorsal fin, should PIT tags be planted under the skin (i.e., between the skin and musculature) or within the musculature, as a best practice?

Long answer:_____

Wound Closure

Wounds occur regardless of tagging procedure, which can potentially lead to tag loss, or infection or other adverse effects on an animal. There are a variety of wound closure options including letting the wound heal on its own, sutures, and various medical-grade adhesives.

Literature Review

Of the studies that solely inserted PIT tags (N = 37), 16% left the wound open, 5.5% used sutures to close the wound, 5.5% used an adhesive to close the wound, and 73% did not report the method used; 11% of these studies used an antibacterial solution on the wound in addition to one of the two wound closure methods or to leaving the wound open.

Of the studies that inserted both a PIT and radio or acoustic tag (N = 9), 67% used sutures to close the wound and the remaining 33% did not report the method used. One study reported using an antibacterial solution on the wound when sutures were used.

One methodological study evaluated survival and tag retention for American Eel implanted with PIT tags but subjected to different wound-closure techniques (Turner et al. 2018). They treated one group with a wound closure aid (surgical glue) and left the wound open to heal in a second group. They found that American Eel survival was 100% in both the open wound and surgical glue treatment groups; however, treatment groups differed in PIT tag retention. The retention rate was 88% when using the surgical glue compared to 100% when leaving the wound open to heal.

Practitioner Interview Summary

Practitioners avoided using sutures or adhesives to close wounds. Some indicated that they were concerned that sutures may introduce an additional infection site. They also indicated that the incision site healed quickly and that tag retention rates were high without the use of sutures or adhesives. One practitioner noted that eels would bite off sutures if applied.
One practitioner suggested the use of Vetbond to seal the incision point for the PIT tag. Another practitioner noted from experience that Vetbond should not be used in combination with sutures as it can lead to infection and poor wound healing.

Wound Closure Questions

Please indicate if you recommend the use of each wound closure method when PIT tagging American Eel to minimize adverse effects.

	Yes	No	No recommendation
Adhesive (i.e., Vetbond)	☐	☐	☐
Sutures	☐	☐	☐
No wound closure	☐	☐	☐

Select the degree of certainty for each of your responses above.

	Very certain (4)	Somewhat certain (3)	Somewhat uncertain (2)	Very uncertain (1)
Adhesive (i.e., Vetbond)	☐	☐	☐	☐
Sutures	☐	☐	☐	☐
No wound closure	☐	☐	☐	☐

Should antibacterial solution be used to prevent wound infection following PIT tag implantation, as a best practice?

- Yes
- No
- Unsure
- Other/Comments:

Concluding Questions

How many years' experience do you have working with American Eel?

Short Answer

Approximately how many American Eel have you implanted PIT tags in?

Short Answer

Which reasons/objectives do you have for PIT tagging American Eel (select more than one if needed)?

- Mark-recapture population estimation
- Tracking movement
- Measuring growth
- Estimating survival
- Marking fish as having been handled (e.g., fish implanted with additional radio or acoustic transmitters)
- Other (list multiple if applicable):_____

To ensure our best practices document encompasses all relevant information, should any other factors or requirements be covered that were not discussed in this survey? Please describe below.

Long answer

Thank you for taking the time to complete this questionnaire.

Appendix 2: Supplementary materials included with the questionnaire on best management practices for implanting American Eel with PIT tags, circulated January 2020.

Appendix B: Tag sizes used to PIT tag eels in the literature review. Fish size represents the size range of eels tagged by each tag size in the study. Retention is the % of eels that retained their tags and survival is the % of eels that survived after PIT tag insertion. Injection represents PIT tags inserted via a needle or similar device that punctures the skin and surgical represents tags inserted via a surgical incision. PIT tags inserted at the head are in the dorsal musculature near the head and dorsal insertion represents tags inserted in dorsal musculature near the origin of the dorsal fin. N/R is noted for any value that was not reported in a study. References noted with an * inserted both a PIT tag and a radio or acoustic tag in the study.

Tag Size (mm)	Fish Size Range (mm)	Retention (%)	Survival (%)	Insertion Method	Insertion Location	Wound Care	Species	Reference
8.5	>250	N/R	N/R	Injection	Head	N/R	*A. rostrata*	Stevens, 2015
9	320-450	N/R	N/R	Surgical	Intracoelomic	N/R	*A. anguilla*	Weltersbach et al., 2016
	360-630	N/R	N/R	Surgical	Intracoelomic	N/R	A. anguilla	Weltersbach et al., 2018
11	90-110	N/R	N/R	Surgical	Intracoelomic	N/R	*A. australis*	Hirt-Chabbert & Young, 2012
	90-110	N/R	N/R	Injection	Intracoelomic	N/R	*A. australis*	Hirt-Chabbert & Young, 2012
	90-110	95.6	95	Surgical	Intracoelomic	Antibacterial	*A. australis*	Hirt-Chabbert & Young, 2012
11.4	115-117	0	100	Surgical	Intracoelomic	N/R	*A. rostrata*	Mueller et al., 2017
	121-130	0	100	Surgical	Intracoelomic	N/R	*A. rostrata*	Mueller et al., 2017
	134-139	50	100	Surgical	Intracoelomic	N/R	*A. rostrata*	Mueller et al., 2017
	145-150	75	100	Surgical	Intracoelomic	N/R	*A. rostrata*	Mueller et al., 2017
	151-159	67	100	Surgical	Intracoelomic	N/R	*A. rostrata*	Mueller et al., 2017
	161-208	57	100	Surgical	Intracoelomic	N/R	*A. rostrata*	Mueller et al., 2017
11.5	>200	N/R	N/R	Injection	Intracoelomic	N/R	*A. anguilla*	Acou et al., 2005
	240-633	N/R	N/R	Injection	Intracoelomic	N/R	A. anguilla	Imbert et al., 2010
	389-405	N/R	N/R	Surgical	Intracoelomic	N/R	*A. anguilla*	Cucherousset et al., 2010
	N/R	N/R	N/R	Surgical	Dorsal	N/R	A. anguilla	McCarthy et al., 2014
12	87-461	100	100	Surgical	Intracoelomic	N/R	A. anguilla	Nzau Matondo et al., 2019
	175-542	N/R	N/R	Injection	Intracoelomic	Antibacterial	*A. anguilla*	Riley et al., 2011
	202-604	N/R	N/R	Injection	Intracoelomic	N/R	*A. anguilla*	Mazel et al., 2013
	247-732	100	98.2	Injection	Intracoelomic	N/R	*A. anguilla*	Mazel et al., 2013
	270-725	N/R	N/R	Injection	Intracoelomic	N/R	*A. rostrata*	Turner et al., 2018
	270-725	N/R	N/R	Injection	Dorsal	N/R	*A. rostrata*	Turner et al., 2018
	325-475	N/R	N/R	Surgical	Intracoelomic	N/R	*A. anguilla*	Cucherousset et al., 2011
	336-1650	100	N/R	Injection	Intracoelomic	N/R	*A. australis*	Baker et al., 2017
	336-1650	100	N/R	Injection	Intracoelomic	N/R	*A. dieffenbachii*	Baker et al., 2017
	350-805	100	100	Injection	Intracoelomic	N/R	A. anguilla	Charrier et al., 2012
	590-790	100	100	Surgical	Intracoelomic	Sutured & Antibacterial	*A. anguilla*	Thorstad et al., 2013*
	590-790	100	100	Injection	Intracoelomic	Open	*A. anguilla*	Thorstad et al., 2013
	591-908	N/R	N/R	Surgical	Intracoelomic	Sutured & Antibacterial	*A. anguilla*	Trancart et al., 2018*
	N/R	100	N/R	Injection	Intracoelomic	N/R	A. anguilla	Dainys et al., 2018
	N/R	N/R	N/R	N/R	N/R	N/R	*A. anguilla*	De Oliveira & Tetard, 2016
	N/R	94	94.74	Injection	N/R	Open	*A. rostrata*	Turner et al., 2018
	N/R	79	100	Injection	N/R	Adhesive	*A. rostrata*	Turner et al., 2018

Tag Size (mm)	Fish Size Range (mm)	Retention (%)	Survival (%)	Insertion Method	Insertion Location	Wound Care	Species	Reference
12.5	161-458	100	100	Surgical	Intracoelomic	Open	A. rostrata	Rudershausen et al., 2019
	205-370	88	100	Injection	Head	N/R	*A. rostrata*	Zimmerman & Welsh, 2008
	205-370	100	100	Injection	Intracoelomic	N/R	*A. rostrata*	Zimmerman & Welsh, 2008
	205-370	100	100	Injection	Dorsal	N/R	*A. rostrata*	Zimmerman & Welsh, 2008
	>250	N/R	N/R	Injection	Head	N/R	*A. rostrata*	Stevens, 2015
	254-1100	N/R	N/R	Injection	Dorsal	N/R	*A. dieffenbachii*	McEwan & Joy, 2011
15	>400	95	N/R	Injection	Intracoelomic	N/R	*A. australis*	Jellyman & Crow, 2016
	428-570	100	100	Injection	Intracoelomic	N/R	*A. australis*	Jellyman & Crow ,2016
23	230-715	N/R	N/R	N/R	N/R	N/R	*A. anguilla*	De Oliveira & Tetard, 2016
	>250	100	100	Surgical	Intracoelomic	Adhesive	*A. rostrata*	Sweezey, 2014
	287-836	N/R	N/R	Surgical	Intracoelomic	N/R	A. anguilla	Nzau Matondo et al., 2017
	500-884	N/R	100	Surgical	Intracoelomic	Sutured	A. anguilla	Piper et al., 2018*
	>300	100	100	Surgical	Intracoelomic	N/R	*A. anguilla*	Wright et al., 2015
	569-921	95	100	Surgical	Intracoelomic	Sutured	*A. anguilla*	Piper et al., 2013*
32	>500	N/R	N/R	Surgical	Intracoelomic	Sutured & Antibacterial	*A. rostrata*	Brown et al., 2009
	500-1100	N/R	N/R	Surgical	Intracoelomic	N/R	A. anguilla	Travade et al., 2010*
	526-1005	N/R	100	N/R	Intracoelomic	N/R	*A. rostrata*	Baker et al., 2019
	597-940	N/R	100	N/R	Intracoelomic	N/R	*A. rostrata*	Haro et al., 2016
	635-827	N/R	100	Surgical	Intracoelomic	N/R	*A. anguilla*	Piper et al., 2017*
	639-921	N/R	N/R	Surgical	Intracoelomic	N/R	*A. anguilla*	Piper et al., 2015*
	705-1000	N/R	N/R	Surgical	Intracoelomic	N/R	*A. anguilla*	Dębowski et al., 2016
	N/R	N/R	N/R	Surgical	Intracoelomic	Open	*A. australis*	Boubée & Williams, 2006
	N/R	N/R	N/R	Surgical	Intracoelomic	Open	*A. dieffenbachii*	Boubée & Williams, 2006
	N/R	100	100	Surgical	Intracoelomic	Sutured	A. dieffenbachii	Boubée et al., 2008*
	N/R	100	100	Surgical	Intracoelomic	Sutured	A. australis	Boubée et al., 2008*
50	N/R	N/R	N/R	Surgical	Intracoelomic	Sutured	*A. australis*	Boubée & Williams, 2006
	N/R	N/R	N/R	Surgical	Intracoelomic	Sutured	*A. dieffenbachii*	Boubée & Williams, 2006
120	N/R	N/R	N/R	Surgical	Intracoelomic	Sutured	*A. dieffenbachii*	Boubée & Williams, 2006
N/R	>200	14	N/R	Injection	Intracoelomic	N/R	*A. anguilla*	Feunteun et al., 2000
	>200	N/R	N/R	Injection	Intracoelomic	N/R	*A. rostrata*	Strickland, 2002
	>300	100	100	Injection	N/R	N/R	*A. rostrata*	Morrison & Secor, 2003
	>300	89	N/R	Injection	Intracoelomic	N/R	*A. rostrata*	Morrison & Secor, 2003
	>300	N/R	N/R	Injection	Dorsal	N/R	*A. rostrata*	Morrison & Secor, 2003
	>580	N/R	N/R	Surgical	Intracoelomic	Sutured	*A. rostrata*	Béguer-Pon et al., 2015*

Anesthesia methods and dosages of chemical anesthetics used in the literature review

Appendix C: Anesthesia methods and dosages of chemical anesthetics used by authors in the literature review. Fish size represents the size range of eels anesthetized by each dosage. Dosages for MS-222, clove oil : ethanol (1:10 ratio), benzocaine, and metomidate are in mg/L. Dosages for 2-phenoxyethanol and eugenol (AQUI-S) are in mL/L. N/R is noted for any value that was not reported in a study. References noted with an * inserted both a PIT tag and a radio or acoustic tag in the study.

Anesthetic	Dosage	Fish Size (mm)	Species	Reference
2-phenoxyethanol	0.2	254-1100	*A. dieffenbachii*	McEwan & Joy, 2011
	0.5	287-836	*A. anguilla*	Nzau Matondo et al., 2017
	0.8	N/R	*A. anguilla*	Dainys et al., 2018
	1	175-542	*A. anguilla*	Riley et al., 2011
	1.5	320-450	*A. anguilla*	Weltersbach et al., 2016
	1.5	360-630	*A. anguilla*	Weltersbach et al., 2018
	N/R	325-475	*A. anguilla*	Cucherousset et al., 2011
	N/R	336-1650	*A. dieffenbachii*	Baker et al., 2017
	N/R	336-1650	*A. australis*	Baker et al., 2017
	N/R	N/R	*A. anguilla*	Cucherousset et al., 2010
Benzocaine	100	90-110	*A. australis*	Hirt-Chabbert & Young, 2012
	150	>200	*A. anguilla*	Acou et al., 2005
	150	591-908	*A. anguilla*	Trancart et al., 2018*
	200	500-844	*A. anguilla*	Piper et al., 2018*
	200	569-921	*A. anguilla*	Piper et al., 2013*
	200	635-827	*A. anguilla*	Piper et al., 2017*
	200	639-921	*A. anguilla*	Piper et al., 2015*
Clove oil : Ethanol (1:10 ratio)	90	N/R	*A. dieffenbachii*	Boubée & Williams, 2006
	90	N/R	*A. australis*	Boubée & Williams, 2006
	120	205-370	*A. rostrata*	Zimmerman & Welsh, 2008
	250	>250	*A. rostrata*	Stevens, 2015
	N/R	>150	*A. anguilla*	Boulenger et al., 2016
	N/R	>300	*A. rostrata*	Morrison & Secor, 2003
	N/R	>400	*A. australis*	Jellyman & Crow, 2016
	N/R	>500	*A. rostrata*	Brown et al., 2009
	N/R	N/R	*A. dieffenbachii*	Boubée et al., 2008*
	N/R	N/R	*A. australis*	Boubée et al., 2008*
	N/R	N/R	*A. anguilla*	McCarthy et al., 2014
Electronarcosis	N/A	500-1100	*A. anguilla*	Travade et al., 2010*
Eugenol (AQUI-S)	0.2	240-633	*A. anguilla*	Imbert et al., 2010
	0.5	87-461	*A. anguilla*	Nzau Matondo et al., 2019
	N/R	350-805	*A. anguilla*	Charrier et al., 2012
	N/R	202-732	*A. anguilla*	Mazel et al., 2013
	N/R	526-1005	*A. rostrata*	Baker et al., 2019
	N/R	597-940	*A. rostrata*	Haro et al., 2016
Metomidate	40	>580	*A. rostrata*	Béguer-Pon et al., 2015*
	40	590-790	*A. anguilla*	Thorstad et al., 2013*
MS-222	240	115-208	*A. rostrata*	Mueller et al., 2017
	250	>250	*A. rostrata*	Sweezey, 2014
	300	>300	*A. anguilla*	Wright et al., 2015
	N/R	161-458	*A. rostrata*	Rudershausen et al., 2019
	N/R	>200	*A. rostrata*	Strickland, 2002
	N/R	259-725	*A. rostrata*	Turner et al., 2018
	N/R	>300	*A. rostrata*	Morrison & Secor, 2003

List of questions asked in practitioner interviews

1. Where do you insert PIT tags in American Eel?
2. What method(s) do you use to insert PIT tags in American Eel?
3. What PIT tag size(s) do you use for American Eel?
4. What PIT tag type (HDX versus FDX) do you use for American Eel?
5. What are your anaesthesia practices when implanting PIT tags in American Eel?
6. What are your handling processes when implanting PIT tags in American Eel? Including surgical equipment care, capture, and release.
7. What is your PIT tag reading format (decimal versus hexadecimal)?
8. What measurements do you take when implanting PIT tags in American Eel?
9. How do you manage your PIT tagging data for American Eel?

List of interviewees and their affiliation

Name	**Affiliation (category)**
Caumartin, Jean	Hydro Quebec (industry) – retired
Casselman, John	OMNRF (government)
Dussureault, Johanne	MFFP (government)
Galbraith, Heather	USGS (government)
Haro, Alex	USGS (government)
Lavictoire, Michelle	Bowfin Consulting (consultant)
Leach, Steve	Normandeau Associates (consultant)
Mathers, Alastair	OMNRF (government)
Newhard, Josh	USFWS (government)
O'Connor, Lisa	DFO (government)
Oliveria, Ken	UMass researcher (academia)
Pallard, Jacquie	Canadian Wildlife Federation (NGO)
Pernette, Danielle	Bluenose (NGO)
Pratt, Thomas	DFO (government)
Punt, Kirby	OMNRF (government) – retired
Runderhausen, Paul	North Carolina State University Researcher (academia)
Stanely, Dave	OPG (industry)
Turner, Sara	Researcher (government)
Windle, Matt	River Institute (NGO)

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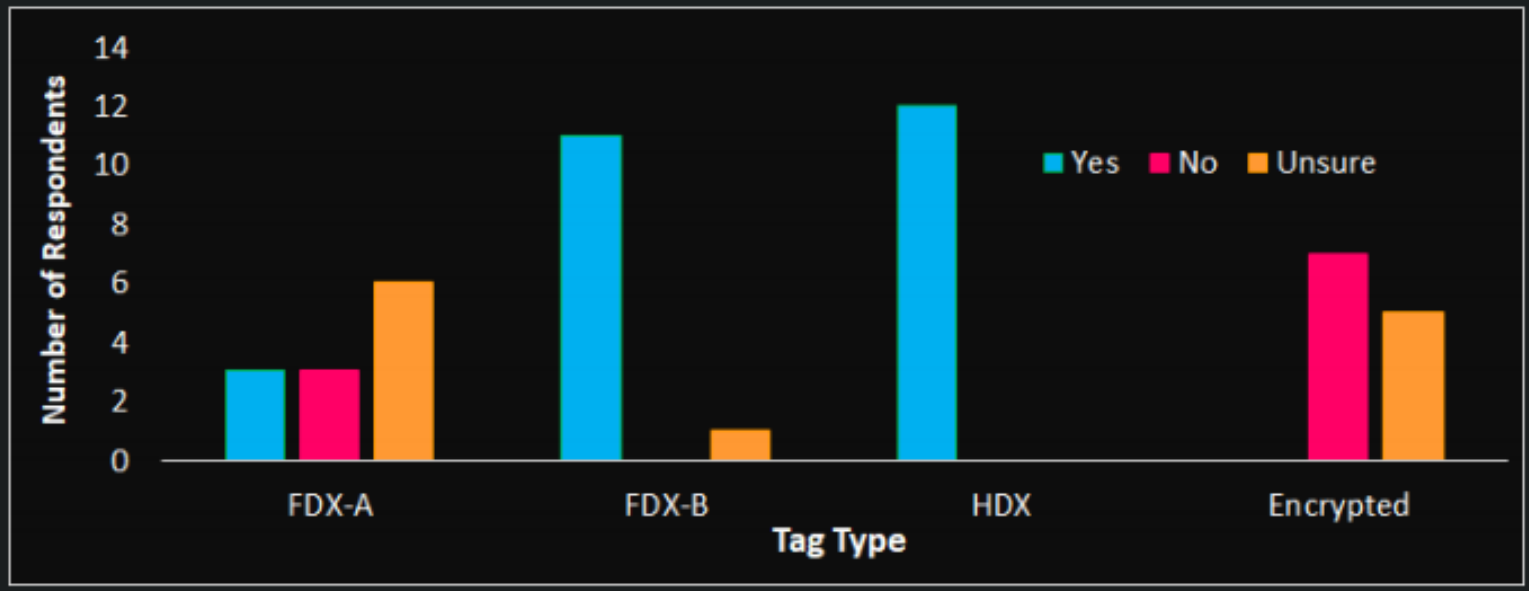
Appendix 3. Summary of results from the questionnaire on best management practices for implanting American Eel with PIT tags, circulated January 2020 (N=12).

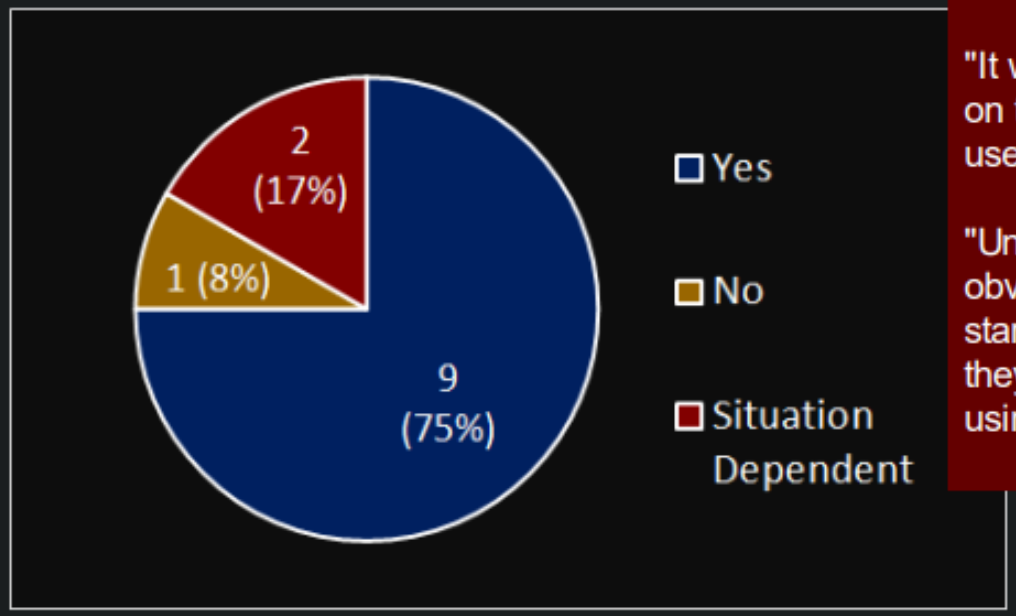
QUESTIONNAIRE RESULTS
Tag Type (HDX vs FDX)

- Respondents agree the BMP should include use of HDX and FDX-B tag types
- Uncertainty exists about FDX-A



QUESTIONNAIRE RESULTS
Handheld Readers

- Most respondents agree that Universal handheld readers are a must



Comments:

"It would obviously depend on the type of tag already in use".

"Universal would be the obvious choice, but if one started with encrypted tags, they would need to keep using an encrypted reader".

QUESTIONNAIRE RESULTS

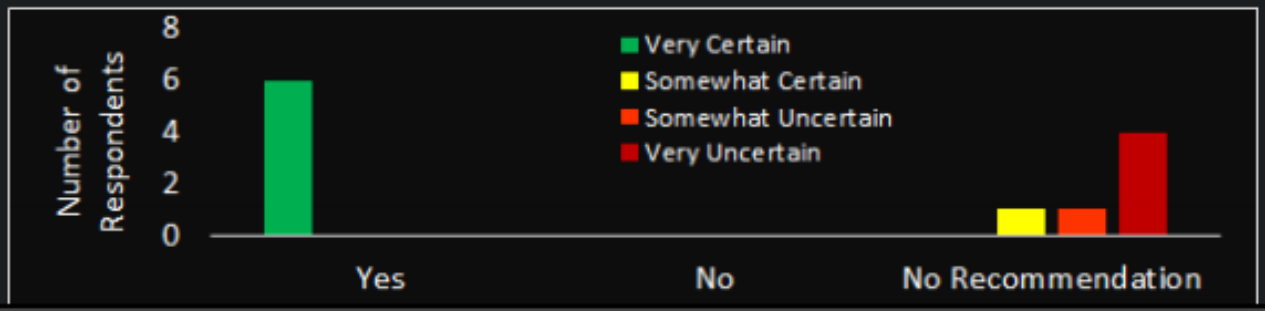
Anesthetic Type

Anesthetic	Yes	No	Unsure	Very Certain	Somewhat Certain	Somewhat Uncertain	Very Uncertain
2-phenoxyethanol	0	2	10	0	1	2	9
Benzocaine	0	3	9	0	2	2	8
Clove Oil / Ethanol	7	1	4	7	1	0	4
Eugenol (AQUI-S)	6	0	6	6	1	1	4
Metomidate (AquaCalm)	2	1	9	1	1	2	8
MS-222	10	1	1	7	3	1	1
Ice Bath	4	4	4	3	5	0	4
Cloth Gloves (No anesthetic)	0	9	3	7	1	1	3

"Shorter exposure to clove oil is enough to knock them out (so they don't have an extreme reaction to the gloves) but the fish recover very quickly after release from the gloves"

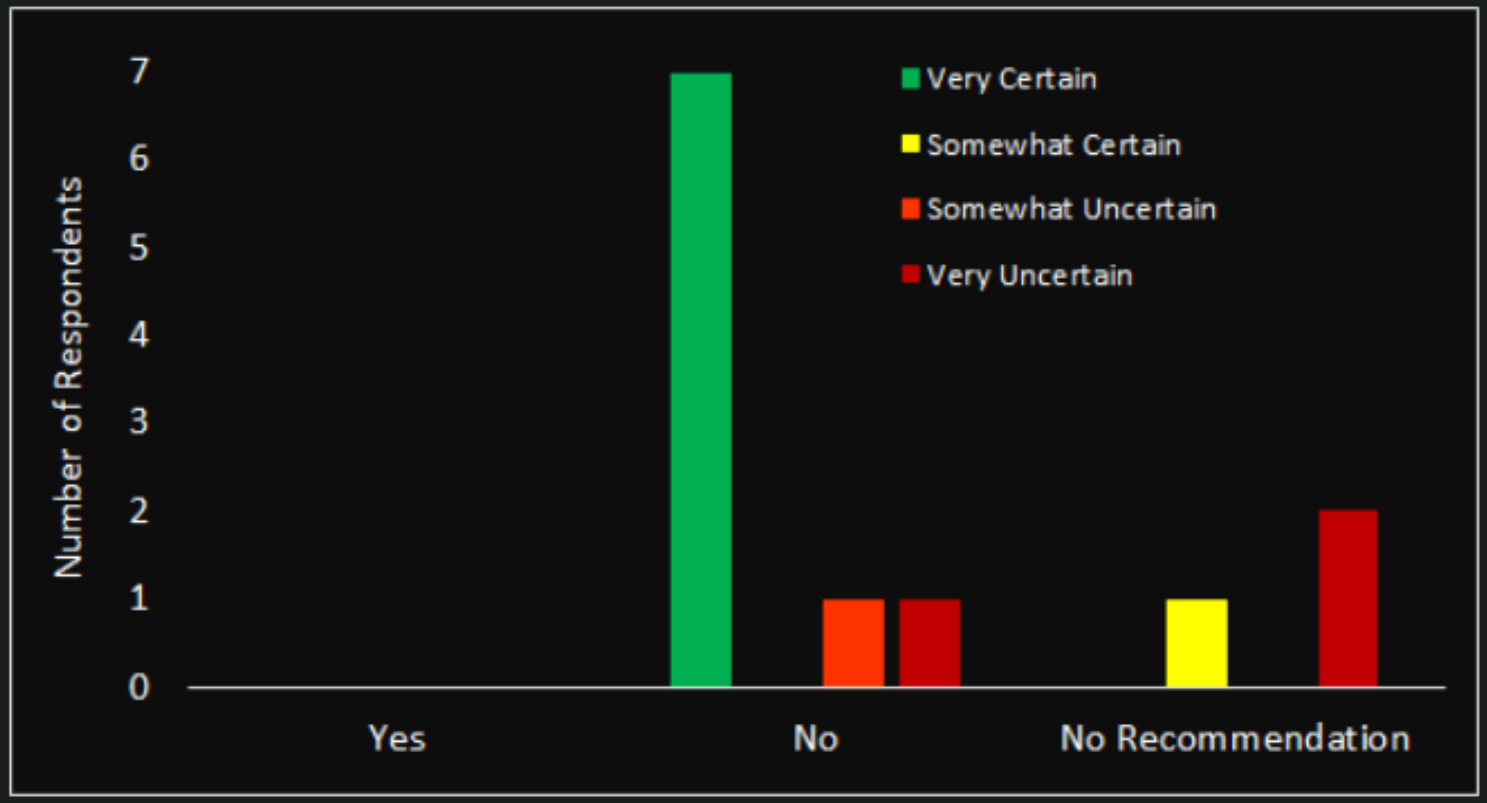
QUESTIONNAIRE RESULTS – Clove Oil : Ethanol

QUESTIONNAIRE RESULTS – Eugenol (AQUI-S)

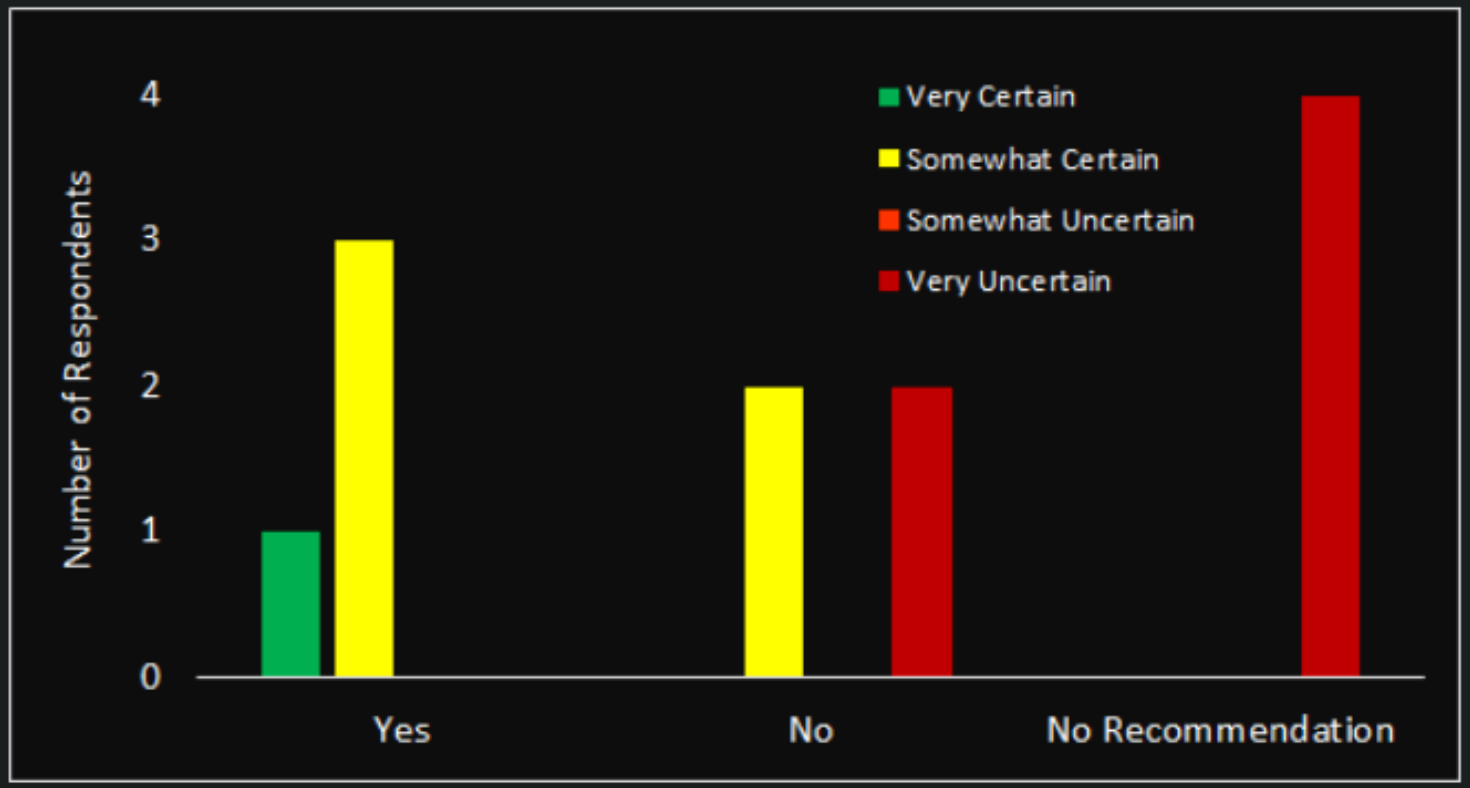


QUESTIONNAIRE RESULTS – MS-222

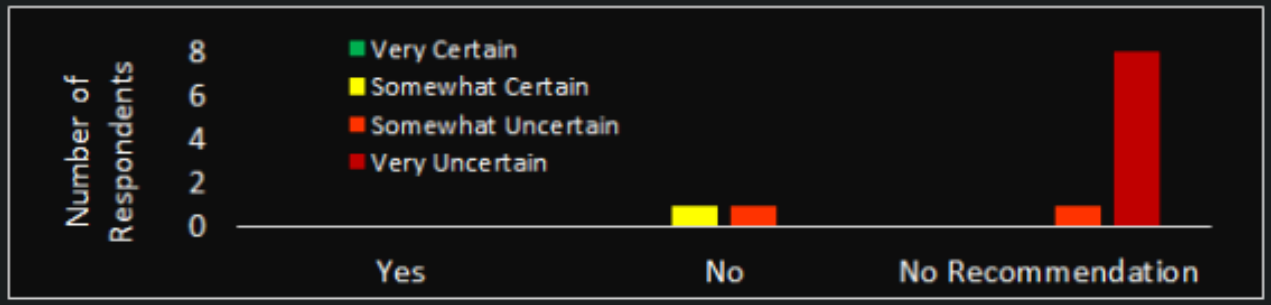
Q. RESULTS – No Anesthetic



QUESTIONNAIRE RESULTS – Ice Bath

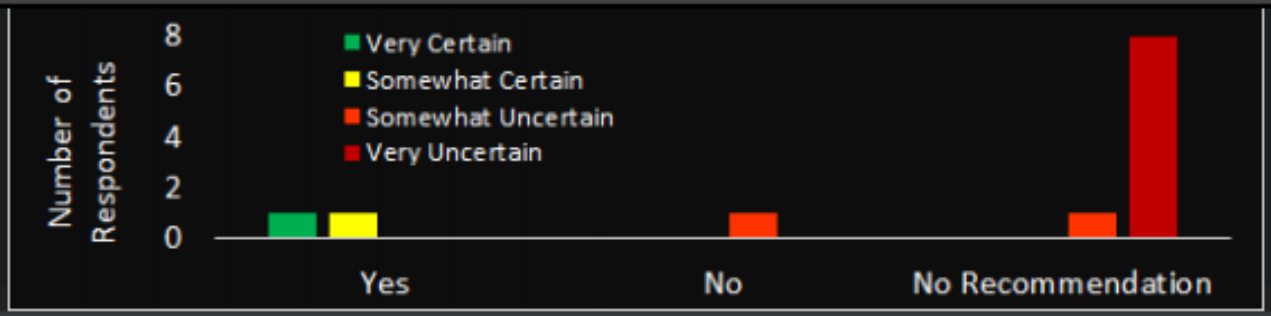


QUESTIONNAIRE RESULTS – 2-phenoxyethanol



QUESTIONNAIRE RESULTS – Benzocaine

QUESTIONNAIRE RESULTS – Metomidate (AquaCalm)



Q. RESULTS - Anesthetic Dosage

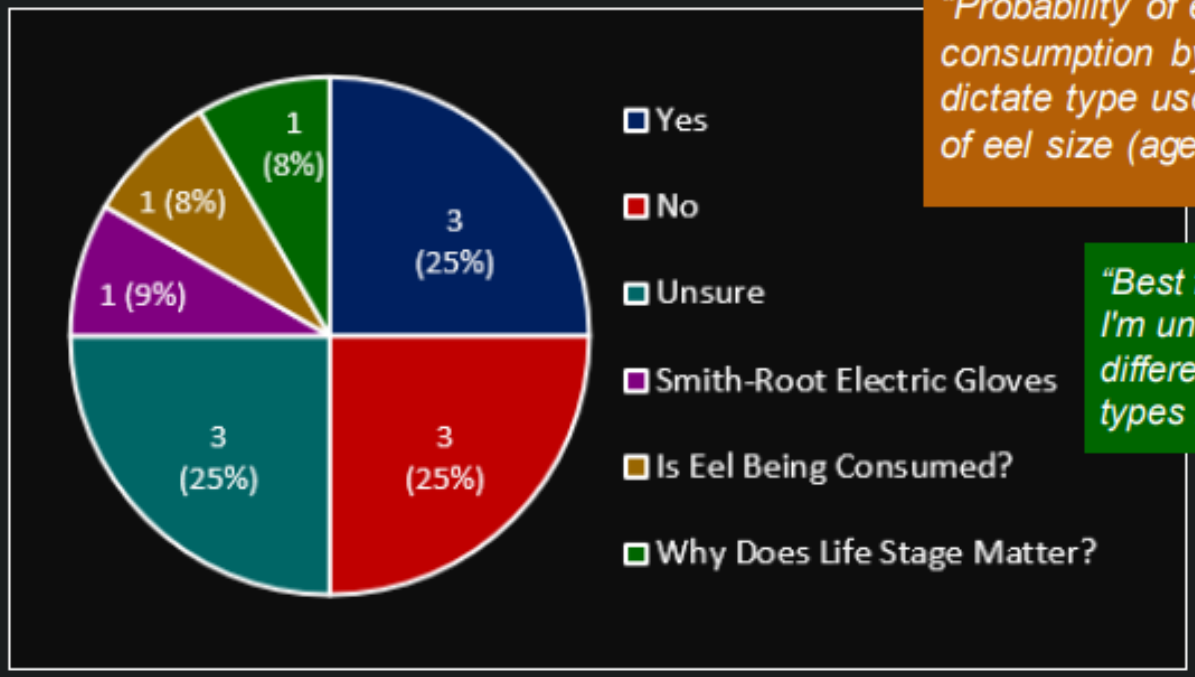
Clove Oil
100 mg/L
1/10
Not approved to be used in Ontario
Low temps (below 15 C) will require higher doses
We use max. recommended dosage at 20-25C (100 mg/l for 15 min exposure); sometimes 1.5 to 2 x at lower temps
30 mg/L
0,1 ml clove oil / Liter of water, eels: 200mm to 500mm

MS-222
150 mg per liter
240 mg/L
50-100 mg/L is usually sufficient to sedate Eels enough for intracoelomic PIT tagging.
Temperature may affect sedation time/level.
MS-222 may have effects of inhibiting olfaction.
Water temperature is important when comes to dosage and dilution so this need to be monitored over time during procedures and sometime more be added.

* No comments were received on any other anesthetic type

Q. RESULTS - Anesthetic Type by Eel Size

- Respondents are split on whether anesthetic should depend on life stage

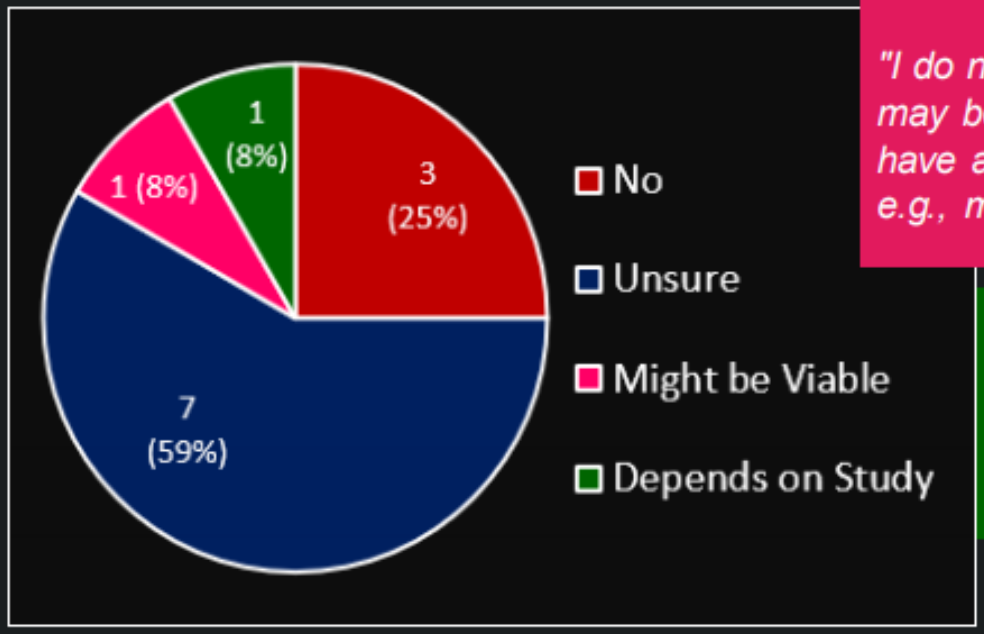


"Probability of eel consumption by humans may dictate type used, regardless of eel size (agency decision)"

"Best for the situation, but I'm unsure why there are differences in anaesthetic types between life stages"

Q. RESULTS – Ice & Water with MS-222

- Most respondents are unsure about adding ice to MS-222
- Benefits? Negative consequences?



"I do not use this technique, but it may be viable. Thermal shock may have additional unknown effects; e.g., metabolic acidosis."

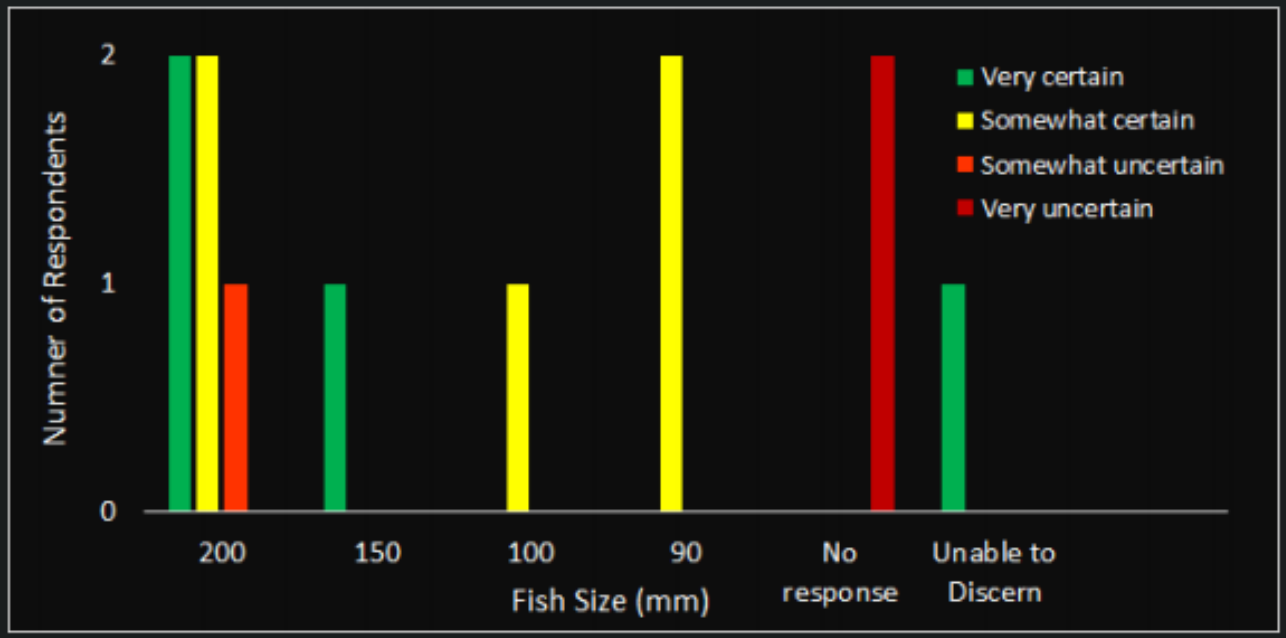
"Depends on the study and what needs to be done."

QUESTIONNAIRE RESULTS
Fish & Tag Size

"Any tagging procedure should strive to minimize effects of behaviour, growth, survival, and tag retention. A comprehensive tagging study (varying eel size and tag size) should be conducted to better answer these questions...Alternatively, the "3% rule" (i.e., maximum permissible weight of any telemetry tag should be <3% of total body weight) could be applied...However, PIT tags are rigid and elongate and may incur unanticipated negative effects even below the 3% threshold."

"If you are only using PIT then size of fish should increase with PIT size. Some internal transmitters you don't like to go above 2% body mass."

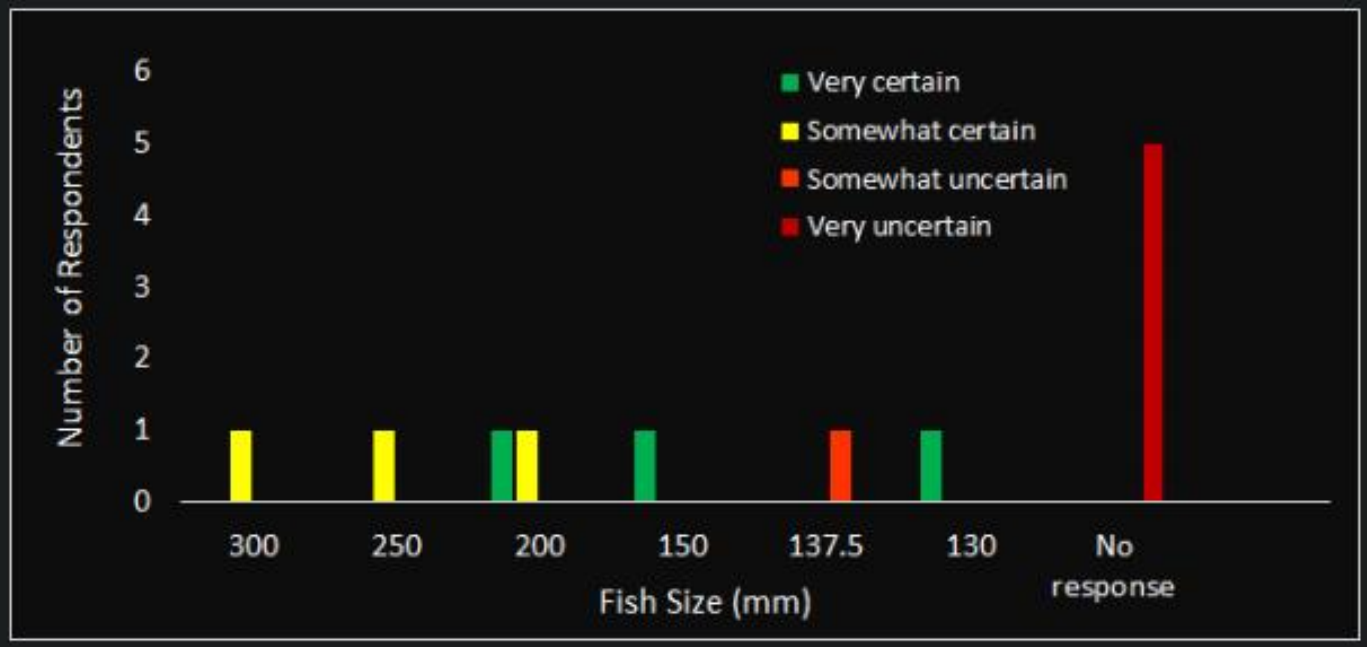
Q. RESULTS - Fish Sizes for 8-mm Tag



Unable to Discern. There are obvious differences in species for tag retention/mortality.

I also am unable to determine if all the tagging methods and tag locations were the same - which may also make a difference for survival and retention - as will tagger experience and handling conditions.

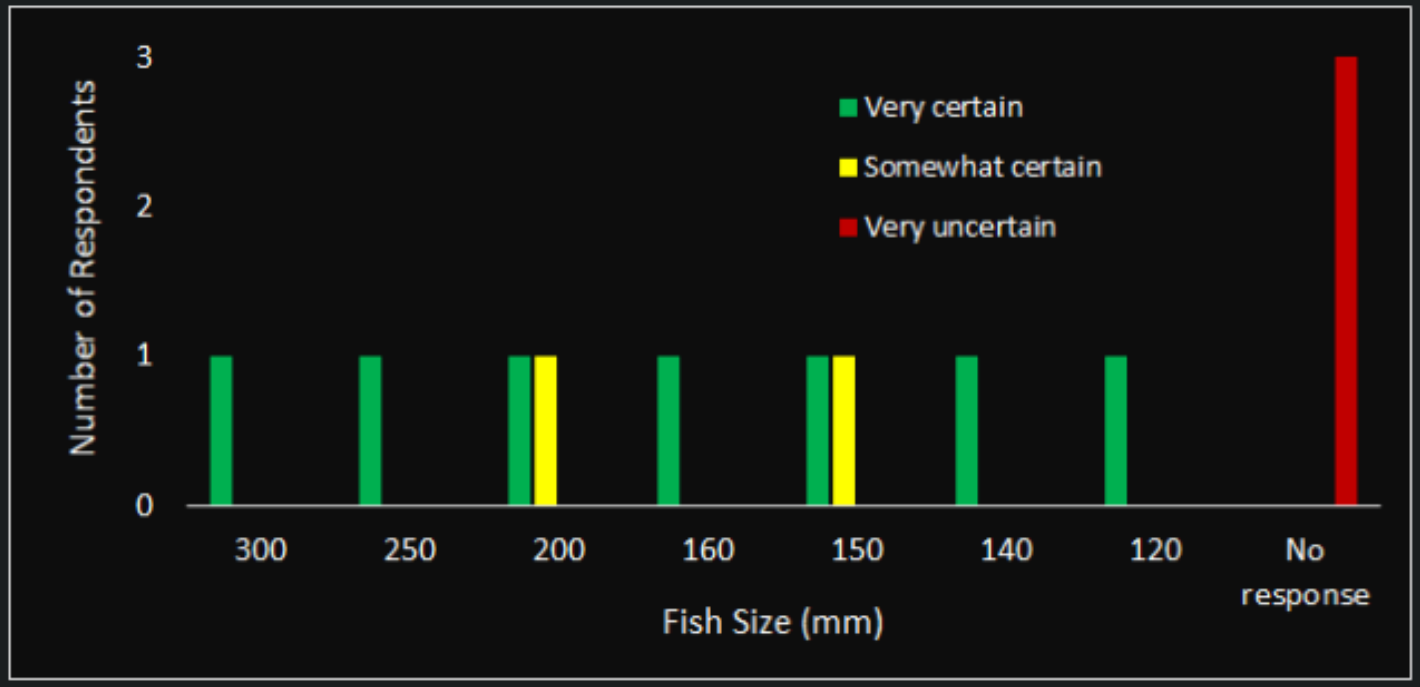
Q. RESULTS - Fish Sizes for 11-mm Tag



*Given above answer, proportional minimum size for 11 mm tags should be (11/8)*100 = **137.5 mm**.*

However, this should be tested with a tag retention/mortality study.

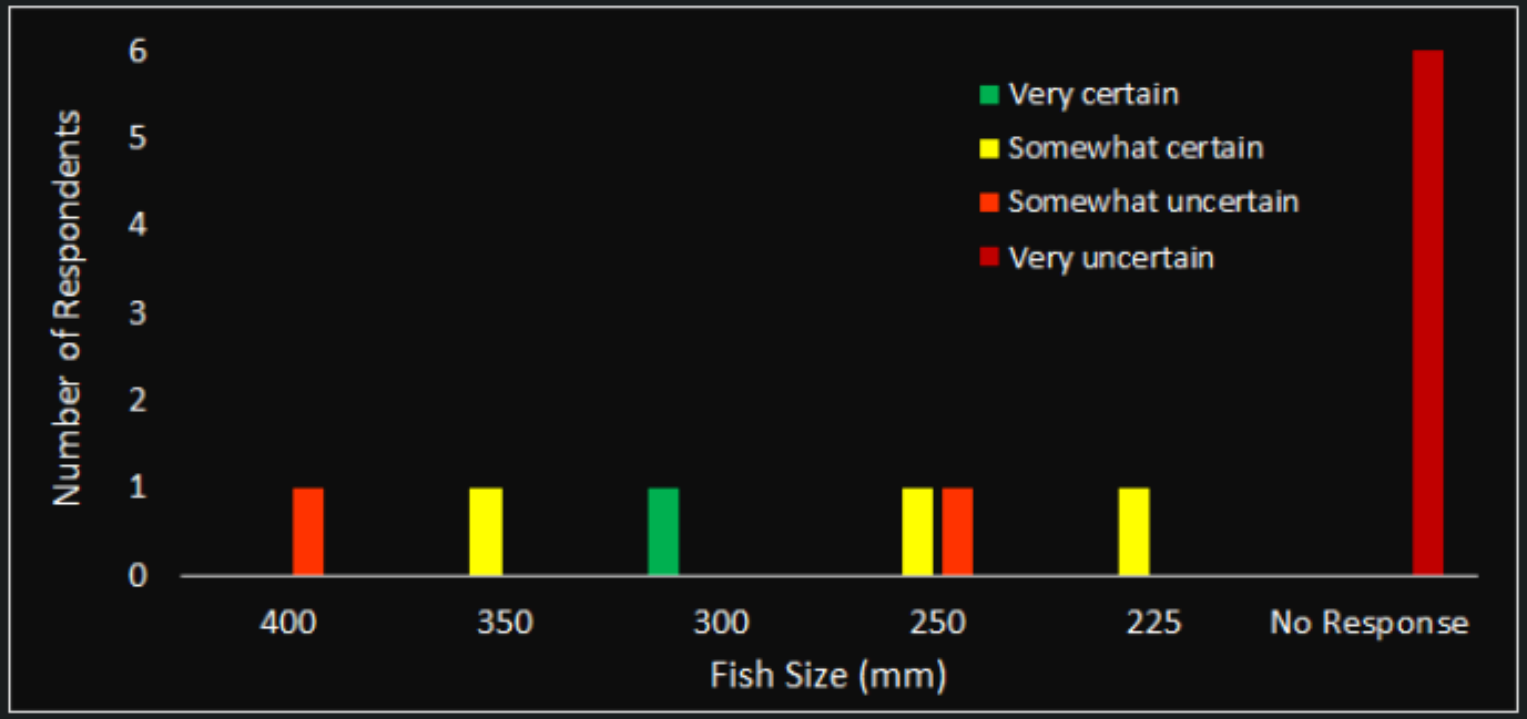
Q. RESULTS - Fish Sizes for 12-mm Tag



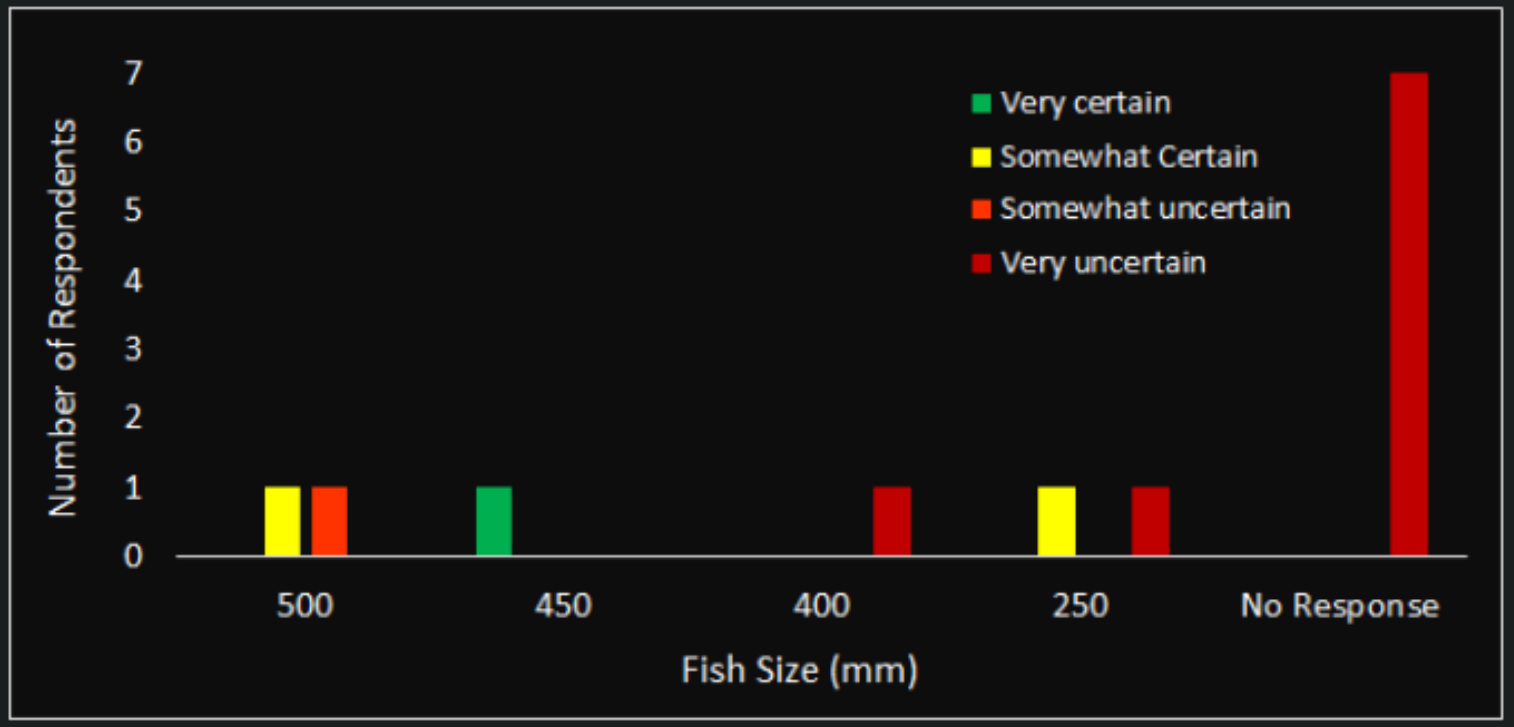
*Given above answer, proportional minimum size for 12 mm tags should be (12/8)*100 = **150** mm (included in graph)*

However, again this should be tested with a tag retention / mortality study.

Q. RESULTS - Fish Sizes for 23-mm Tag



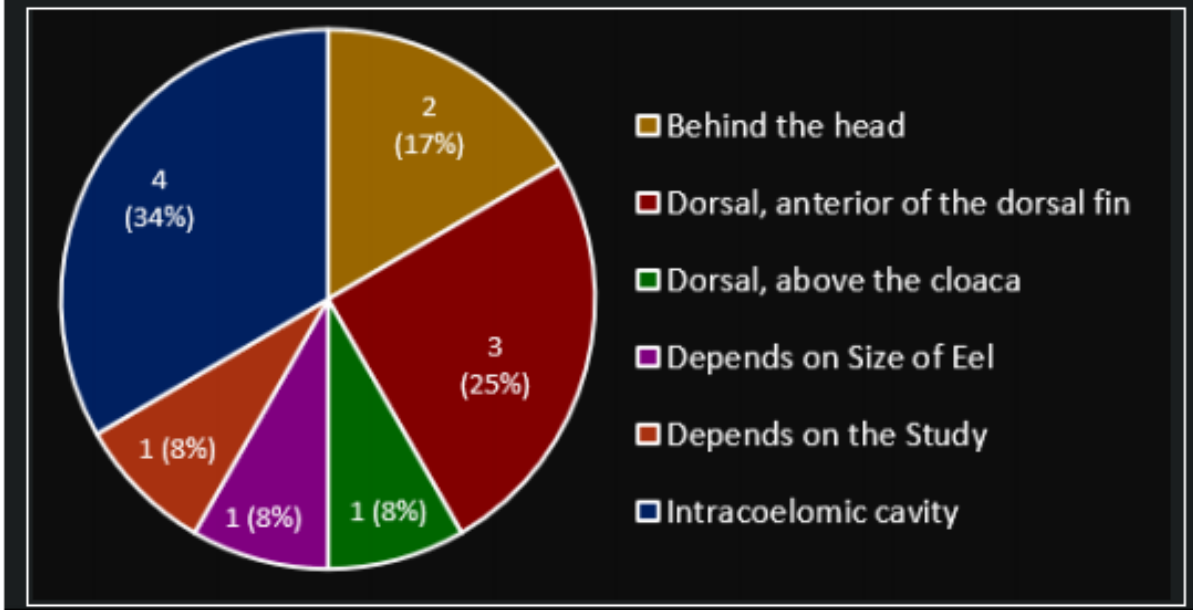
Q. RESULTS - Fish Sizes for 32-mm Tag



Q. RESULTS - Tag Insertion Location

"The rationale for PIT tag insertion behind the head was done in consultation w/ MFFP and PQ fishers, because there is a risk that PIT tagged eels could be harvested and consumed, this location was chosen as this area is not typically cleaned and potentially consumed."

- Intracoelomic cavity & dorsal musculature preferred



"Intracoelomic is the most common and easiest to implement."

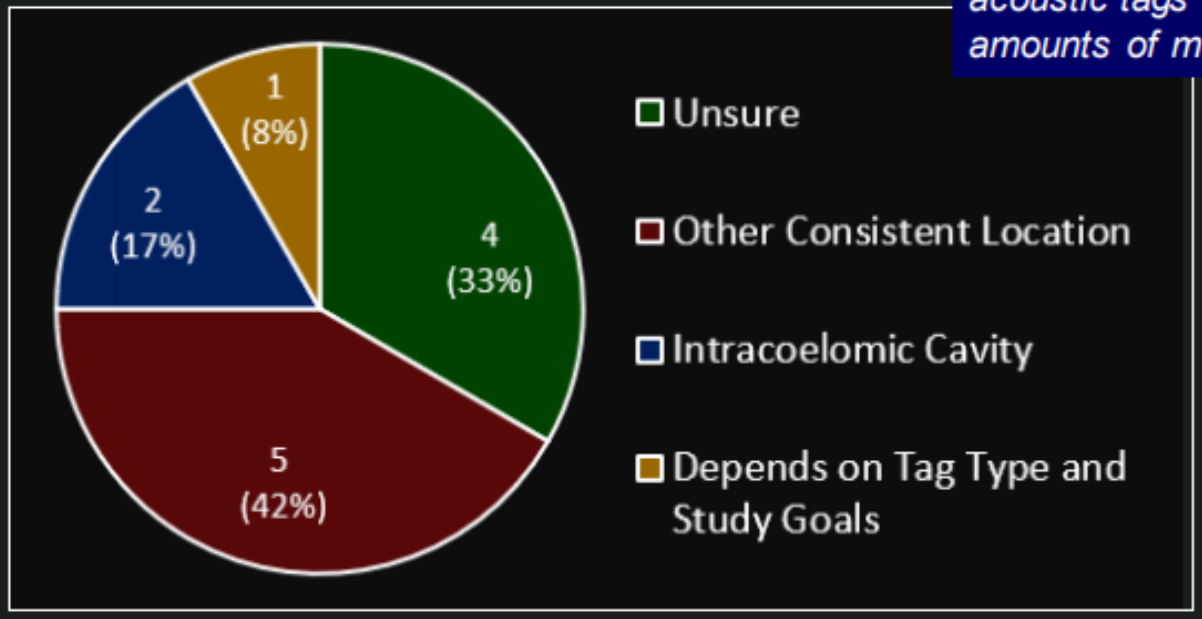
"IC has good tag retention (but few long-term studies exist) and has less risk re. human consumption."

"My experience with subdermal and IM injection is that tag retention is not as good as IC."

Q. RESULTS – Implant PIT Tags in IC with transmitters?

- Prefer a consistent location (intracoelomic or dorsal)
- Unsure about where this should be

"Prefer IC implantation in this case although PIT tag detectability may be impaired if PIT tags come in close contact with large radio or acoustic tags with significant amounts of metal".

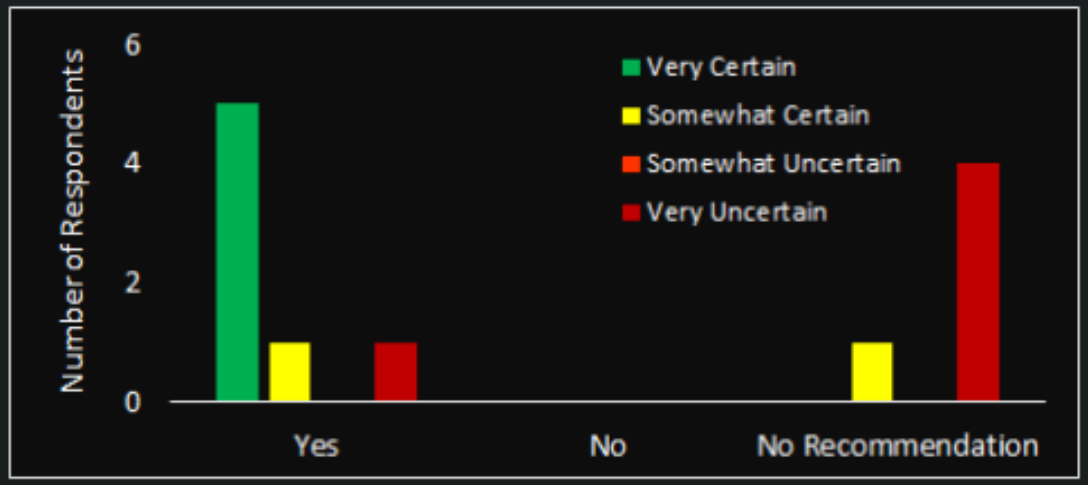


Q. RESULTS - Methods and Tools

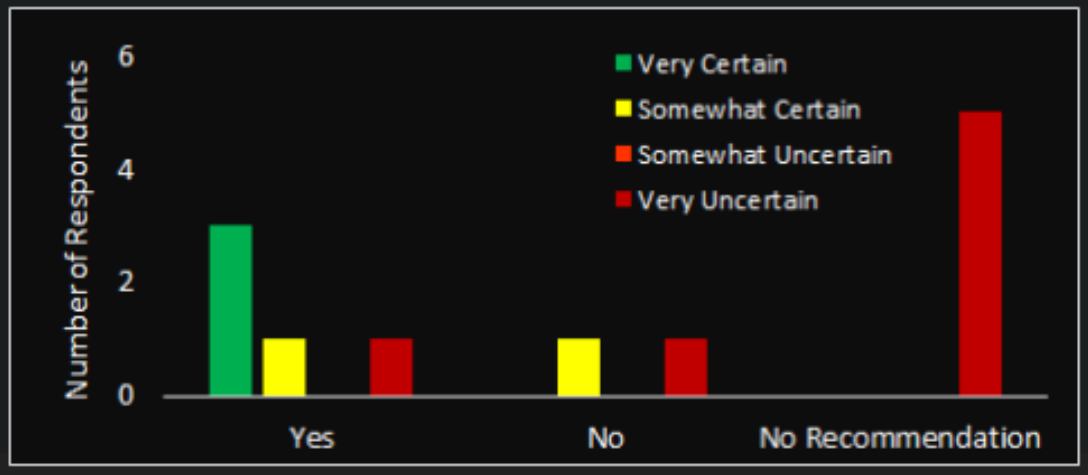
Tagging Tool and/or Method	Yes	No	NR*	Very Certain	Somewhat Certain	Somewhat Uncertain	Very Uncertain
Puncture & Injection via Implanting Gun	7	0	5	5	2	0	5
Puncture & Injection via Multi-Use Syringe	5	2	5	4	2	0	6
Empty Needle Puncture / Multi-Use Syringe Injection	0	3	9	0	3	2	7
Empty Needle Puncture / Manual Insertion	1	5	6	1	3	3	5
Scalpel Incision / Multi-Use Syringe Injection	3	3	6	2	4	2	4
Scalpel Incision / Manual Insertion	4	4	4	4	4	2	2

R – No Recommendation

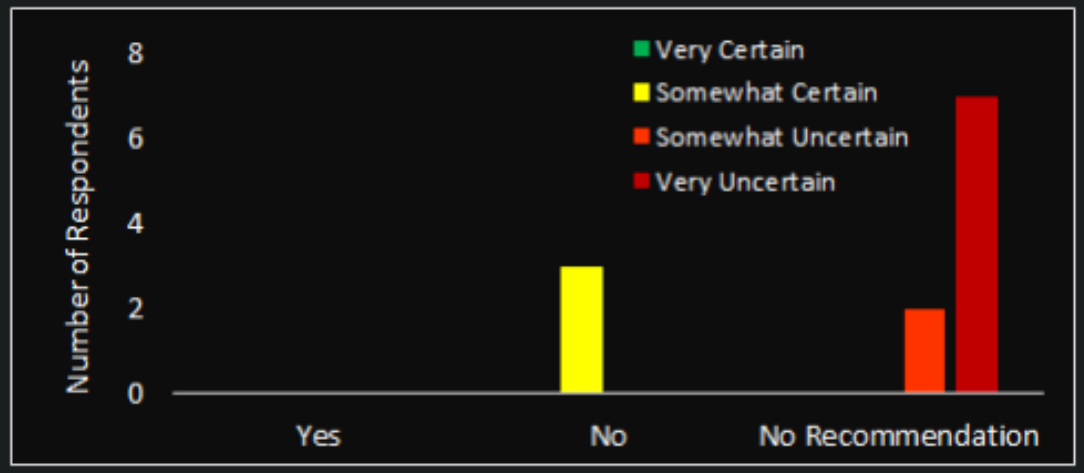
Q. RESULTS – Puncture & Injection via Implanting Gun



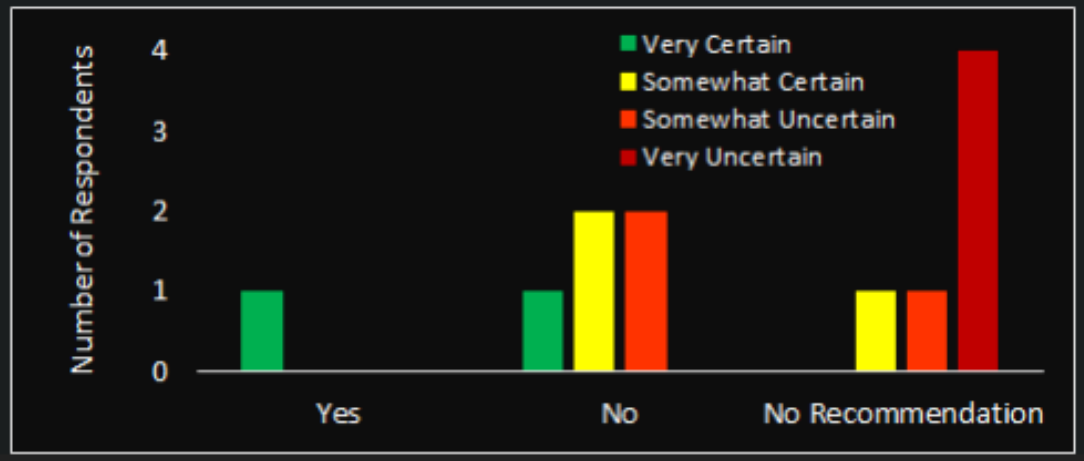
Q. RESULTS – Puncture & Injection via Multi-Use Syringe



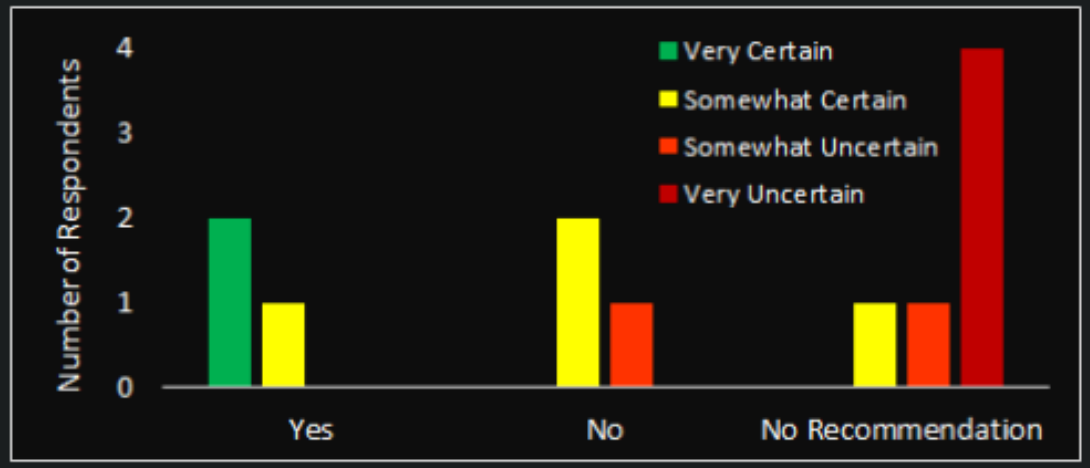
Q. RESULTS – Empty Needle Puncture / Multi-Use Syringe Injection



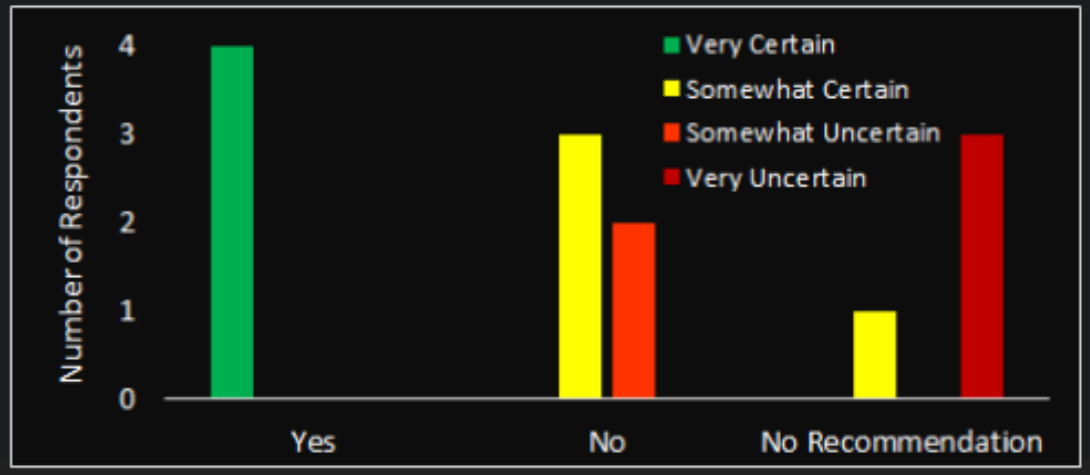
Q. RESULTS – Empty Needle Puncture / Manual Insertion



Q. RESULTS – Scalpel Incision / Multi-Use Syringe Injection



Q. RESULTS – Scalpel Incision / Manual Insertion



Q. RESULTS - Under Skin or Muscle?

Under Skin	4	Within Muscle	2	Unsure	3

"Behind the head within the musculature; unknown for dorsal fin"

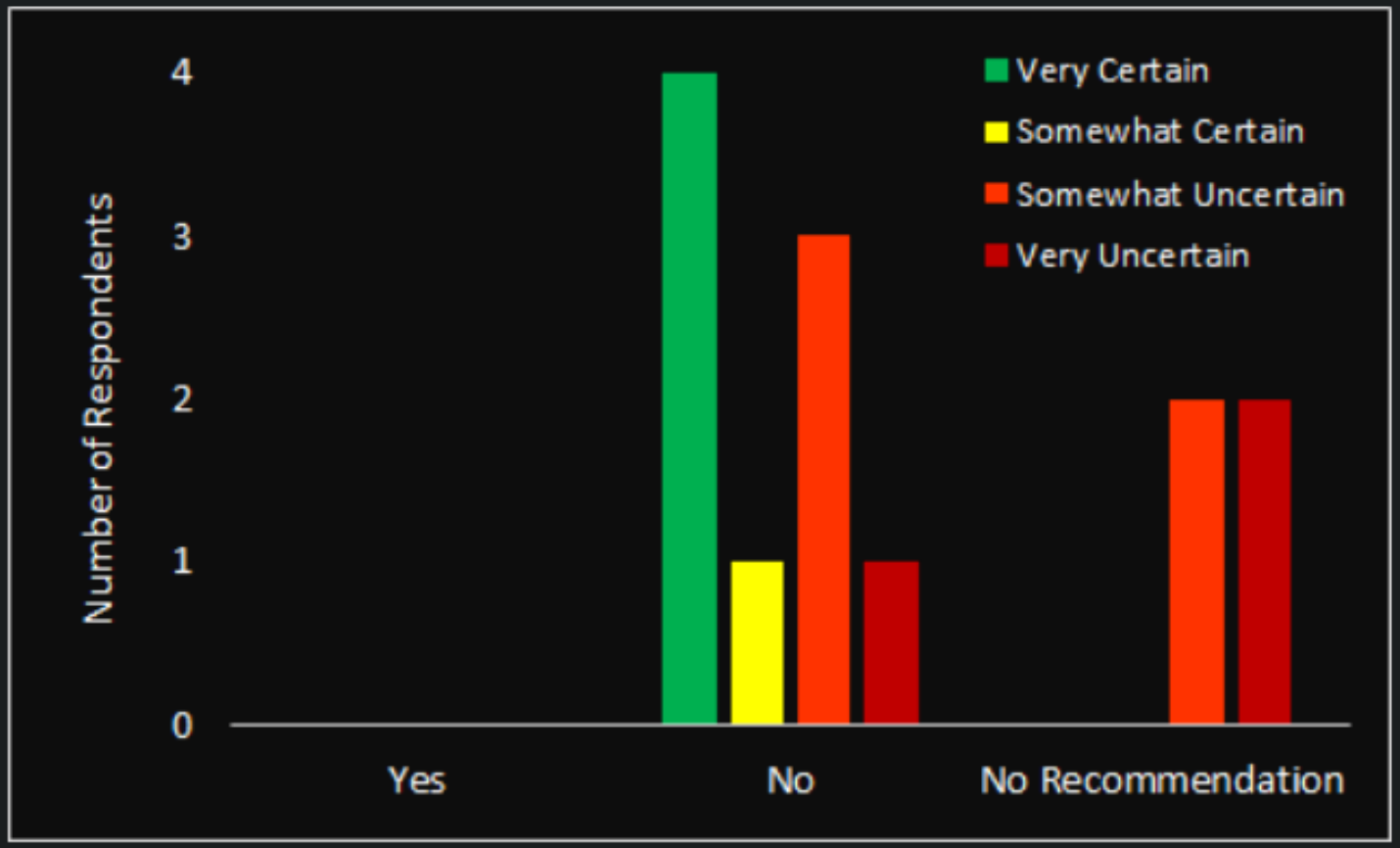
"I do not use these techniques, as a rule, given their uncertainty in tag retention. A definitive study comparing subdermal / intramuscular with IC implantation should be conducted."

Q. RESULTS - Wound Closure

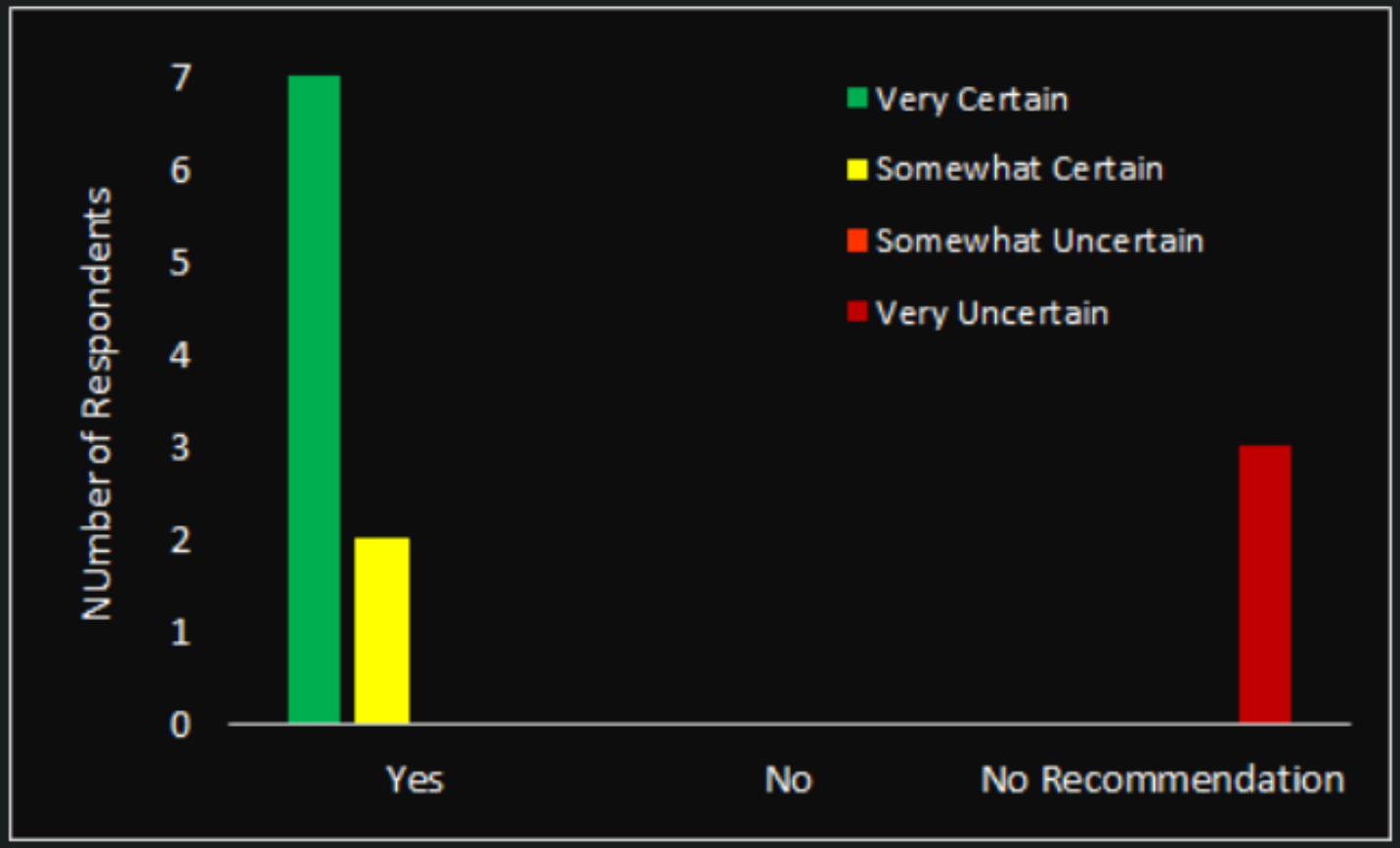
Technique	Yes	No	NR*	Very Certain	Somewhat Certain	Somewhat Uncertain	Very Uncertain
Adhesive	2	5	5	5	2	2	3
Sutures	0	8	4	4	1	5	3
Open	9	0	3	7	2	0	3

NR – No Recommendation

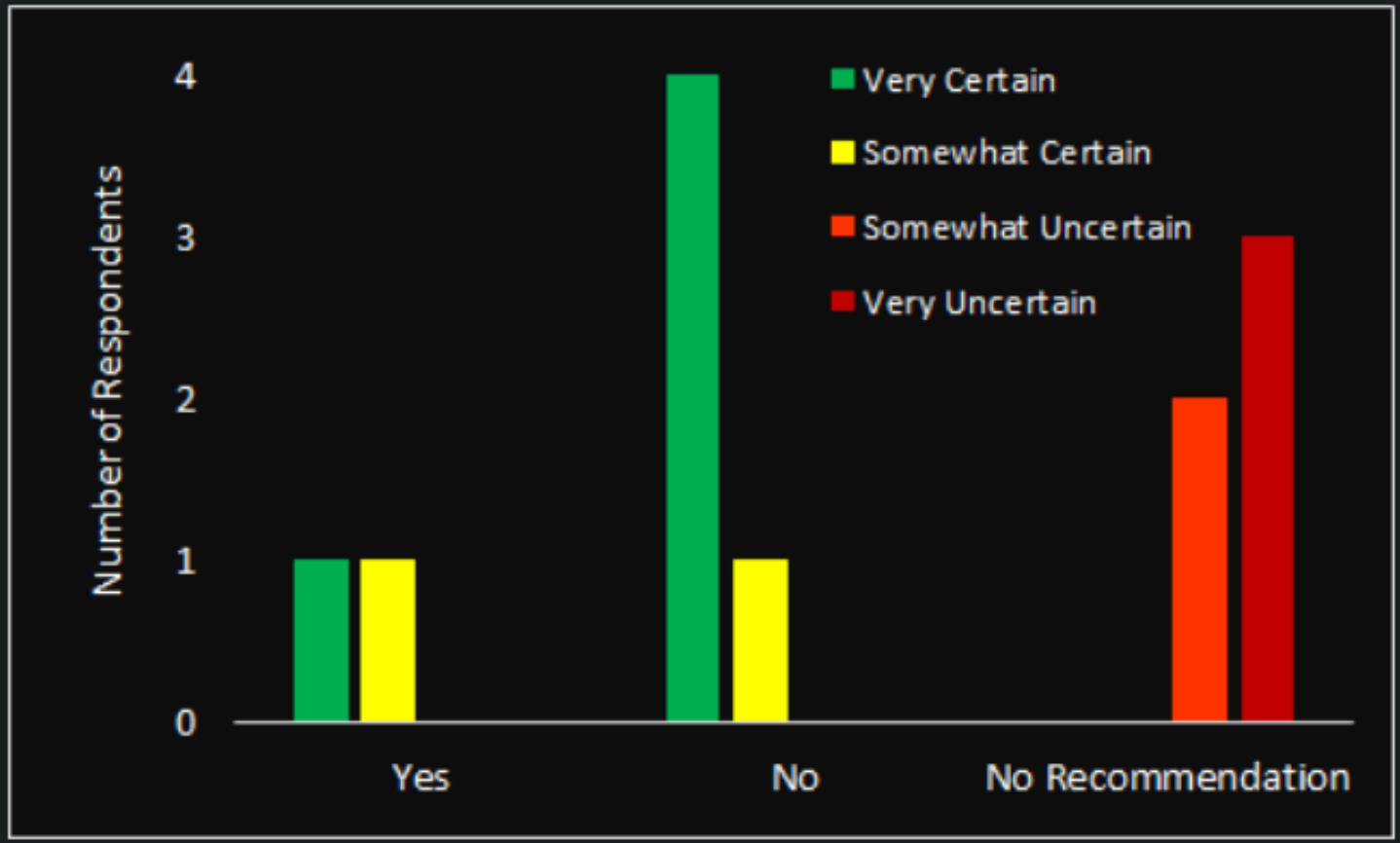
Q. RESULTS – Closure Sutures



Q. RESULTS – Closure Open



Q. RESULTS – Closure Adhesive



Data Sharing and Management Options

What data to record when tagging?

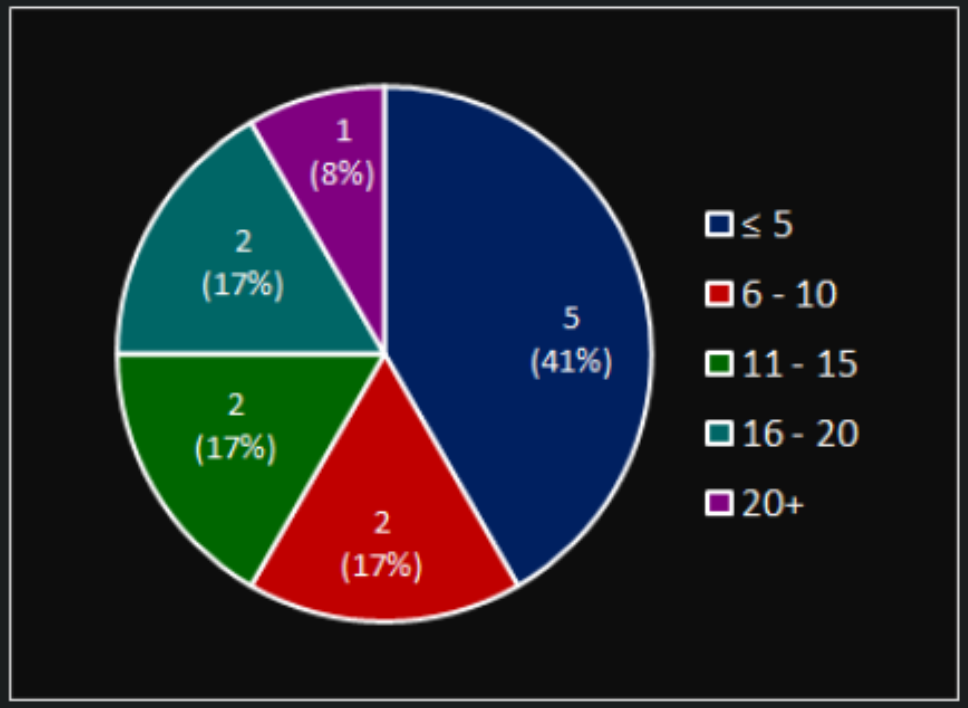
- Location
- Date
- Time
- Tag number
- Length
- Animal ID/Name (if null, use tag serial #)

Additional options?

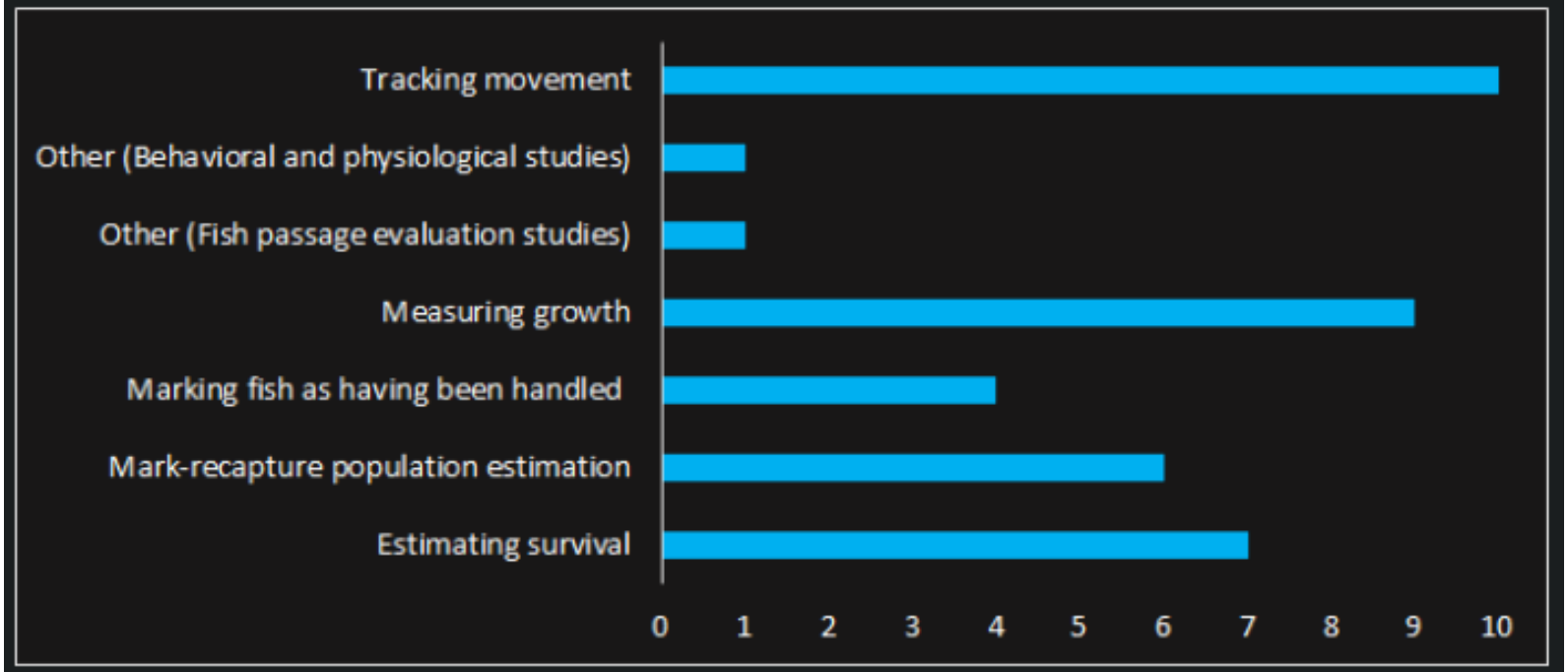
- Weight
- Eye diameter
- Girth
- Head width
- Eel Phase/Life stage
- Sex if known
- Notes (injuries, anesthetic dosage, swim bladder parasite, age)
- Images
- Fate following recapture/date of removal from the system

QUESTIONNAIRE RESULTS
Years Tagging Eels

- Many respondents had over ≥10 years' experience



RESULTS – Tagging Purpose



- Several practitioners tagged eels for more than one purpose
- Tracking was the most common reason

Appendix 4. Minutes from the workshop on best management practices for implanting American Eel with PIT tags, February 2020.

Introductory Remarks

- Purpose and goal of workshop to obtain expert advice on best practices for implanting American Eels with PIT tags.
- No concerns raised with decision-making framework (e.g., an amended Ad-Hoc Delphi process; see Appendix) to develop a standard or general recommendation.

Workshop Participant List

Role at Workshop	Last Name	First Name	Organization
In-Person Attendee	Algera	Dirk	Carleton University
In-Person Attendee	Pallard	Jacquie	Canadian Wildlife Federation
In-Person Attendee	Rabideau	Sarah	Canadian Wildlife Federation
In-Person Attendee	Lapointe	Nick	Canadian Wildlife Federation
In-Person Attendee	Cooke	Steven	Carleton University
In-Person Attendee	Deny	Angela	Ocean Tracking Network
In-Person Attendee	Leblanc	Molly	Blue Nose Coastal Action Foundation
In-Person Attendee	Lavictorie	Michelle	Bowfin Environmental
In-Person Attendee	Narezny	Andy	Ottawa River Hydro Electric
Remote Attendee	Creaser	Taylor	Blue Nose Coastal Action Foundation
Remote Attendee	Haro	Alex	United States Geological Service
Remote Attendee	Henning	Aaron	Susquehanna River Basin Commission
Remote Attendee	Newhard	Josh	US Fish and Wildlife Service
Remote Attendee	Mueller	Bob	Pacific Northwest National Laboratory
Remote Attendee	Sevigny	Kathleen	Ministère des Forêts de la Faune et des Parcs
Remote Attendee	Turner	Sara	University of Maine

Discussion Points - Selecting Best Practices

Tag Type

- Most participants were in favor of FDX-B and HDX tags
- Debate over using FDX-A tags
- Haro: no reason to switch to FDX-A unless cheaper. Remote operators write their own software, if a new coding scheme a standard program cannot read that tag; they can retrofit, but it can cause problems RE: setting up an entire study, may lead to compatibility issues; diversity of tags is continually growing.
- Haro: FDX-A tag coded tags read differently which has implications for their studies, scrambled to get system reading those tags.

Handheld Reader

- Lapointe: under impression that not many encrypted tags used in the system now
- Narezny: not using any encrypted tags.
- Lapointe: if using encrypted tags, they should be used in a closed system, on other fish species

Anesthetics

MS-222, Clove oil, Eugenol

- Lapointe: concern about Eels being consumed and chemical anesthetic, jurisdictional approvals
- Newhard: cost and local jurisdiction regs key issues, success with low dose of Aqui-S, success with MS222 on elvers (no consumption issues), do not use MS-222 anymore.
- Lapointe: advantage of recommending several types, so flexibility
- Cooke: institutional culture – animal care. MNRF has no jurisdiction over what is used by staff, Health Canada issue, so there can be confusion as to what is legal. Can be a fall back without any evidence to back choices, gets passed on over the years. Practices may be forced onto others even in the face of evidence or other experience
- Cooke: likes that there is support for multiple (3) anesthetics among respondents. Calls for research on comparative research on anesthetic type. Support but very few "no" responses, suggesting uncertainty and need for more research to find which anesthetics are best
- Lavictoire: how effective one anesthetic vs other for PIT tagging vs. full surgery?
- Phone: Aqui-S and MS222 for acoustic tagging (full surgery)
- Mueller: used Eugenol for field studies (yellow eels), lab studies MS-222.
- Leblanc: clove oil used as well
- Lavictoire: MS-222 has high inter-individual variability, Pallard agreed.
- Haro: American Eels have to be put down further for full surgery, cold temps (< 15C) takes much longer for induction (higher or longer). MS222 use: unbuffered water increase acidity and may impact olfactory epithelium (not confirmed in eels but noted in other species, including salmon smolts). Agree with Cooke on more comparative research on effects of exposure, recovery and MS-222 dosage on silver eel. Subsequent behaviours have implications for eel health and studies.
- Paul: 240 mg/L MS-222 buffered with sodium bicarb in lab, procedure lasts 60 s per eel. Published in JoVE video of eel tagging procedure. No sutures used.
- Narezny: provincial regulators didn't approve of Clove oil, used MS-222

No Anesthetic

- Leblanc: uses wet cotton glove method. Clove oil paralyzes but doesn't provide analgesic (?) Is use of clove oil for animal care? Animal care vs. handling point of view (?)
- Cooke: anesthesia is an assault on the system, so this is a consideration. Uses Anesthesia to immobilize difficult species or large specimens. Think to oneself "if I fail to anesthetize, it will hurt the animal or researcher; do not know enough about analgesics to know if they play a role in 'pain'
- Cooke: RE: wet cotton gloves. Recap specimens you can see glove marks from removing slime, also transfer other substances/particles to surgery specimen
- Leblanc: recap many eels from 10-yr mark recap and not seen any ill effects from cotton glove method (e.g., finger imprints). Seems to work well if you the cover eyes, PIT tag is fast process so only held for 20 s.
- Lavictoire: May cause more harm to animals from increased handling/holding to admin anesthetic than if not using anesthetic
- Leblanc: working in remote locations often, so transport or disposal of chemicals an issue. High success in tagging without anesthetic; take length measurements and PIT tag using a specialized trough to force Eel in a straight line.
- Phone: Disease transport may be an issue with cotton gloves.
- Question Posed: Ice Bath or Cotton Gloves? (None in favour of ice baths, two for cotton gloves)
- Phone: Difficulty in tagging an un-sedated eel difficult; concerns with removing slime coat

Ice Bath

- Phone: used as emergency procedure when lacking anesthetic. Takes lots of time. Doesn't work when water temp is already cold, immersion into ice bath was ineffective.
- Haro: variable outcome. Wouldn't use for full surgery. PIT tag procedure could be ok in combination with cotton gloves. Could cause metabolic acidosis in fish (other fish) with the severe temp drop, but not sure if an issue for eels. Try to use a conventional anesthetic.
- Lapointe: wasn't approved to use ice bath to kill invasive species (animal care) so likely wouldn't be approved for anesthetic.
- Lapointe: temp shock may be a problem, more research on this needed.
- Phone: what is the physiology of the fish? What happens when the fish is immersed? Ice bath vs cotton glove, what would be better?

- Cooke: physiological assault, cold shock is a known stressor. That said, if comparative work indicates that its ok then would consider.
- Cooke: very concerned about clove oil messing with fish ability to 'smell' their way home; MS-222 used as clove oil is a concern RE: olfactory sense, so should also be flagged for American Eels.

2 phenoxyethanol, Benzcaine, Metomidate

- Phone: More comparative research needed for all anesthetics, particularly MS-222 on eel olfactory sense.
- Lapointe: Metomidate as effective as Eugenol, but lack of support may be from unfamiliarity. More research warranted.

Dosage

- Phone: 30 mg/L AQUI-S (Eugenol) considered a good option.
- Phone: PIT tagging relatively quick, fish doesn't need to be completely out so standard (e.g., 100 mg/L clove oil, 150 mg/L MS222) might be adequate. Maybe not for full surgery.
- Lapointe: MORE research needed on anesthetic specifically for American Eel and for PIT tagging vs full surgery.
- No discussion for dosage on MS-222
- Phone: Clove Oil – some use higher concentrations than 'normal', that is when I need higher level for surgery, PIT tagging (quick, rapid procedure), does not need to be out, maybe standard dosage (100 mg/L CO, 150 mg/L MS-222) are adequate? Not enough?

Anesthetic and Eel Size

- Lapointe: results included use of electric gloves. Any experience?
- Haro: not used glove in eel, but on other fish. Shad and lamprey failed miserably. Worked well for sturgeon because could lay out strait. Anguillid cannot do this with gloves. If you can straighten out Eel to be parallel to electric field; put inside plastic screen tube; might work, have not tried it myself
- Haro: Electro anesthesia could work, but jury is still out. Has little faith in gloves in the way they are supposed to be used.
- Mueller: Conducted a small study on lamprey, found gloves would leave scarring; 24% had injuries. Opinion that gloves not a good way forward for adult eels.
- Cooke: Can it be optimized? Works well with some species and not others. Creativity and R+D could facilitate effective use, but in current format no appropriate

- Cooke: AFS Fisheries Mag article reviewed electro immobilization techniques (lots of unknowns); TENS units a good option; Chris Vandergoot has fish lay against socks; STILL POTENTIAL; just effective for American Eel if using snout to tail angle
- Lavictorie: Based on electofishing behaviour would not recommend using gloves for anesthetics. Do not use until studies have been done. It's not a reasonable approach.

Ice + Water with MS-222

- Cooke: use ice that is not chlorinated. Instead use frozen lake water or a frozen plastic bottle.
- Pallard: use block ice so not diluting the anesthetic concentration.
- Cooke: ambient is best (bass work), but mimic ambient water where fish was pulled from (e.g., depth). Avoid large temp swings. Modest cooling not a big issue. Consideration of where animals will be released is key.
- Cooke: Use cooling temps that emulate where they will go upon release (e.g. depth).
- Phone: why bother using both MS-222 and ice water? Not a big benefit in using in combination. Cooler temp will require more anesthetic to compensate. Use one or the other, not the combo.

Fish and Tag Size

- Mueller: speaking on study: 113- to 120-mm tags were 11-mm; retention test; held for 45 days; small fish had higher tag burden of 3.5-5%; tag loss started occurring 25-30 days of holding (didn't know mechanism). No differences in swimming abilities.
- Phone: what was mass of tag used in Mueller study? Answer: 0.88 g in air
- Phone: Tag insertion method? Answer: incision, 25-mm posterior of pectoral fin, body cavity. Small incision was made with scalpel, inserted by hand; body cavity was found to be best for placement, did X-rays; overall procedures lab and field worked well
- Mueller: overall procedure and study worked well.
- Lapointe: 30-day window in study for tag retention disconcerting for PIT tags; long term nature of study
- Mueller: recommend putting one suture to help prevent tag loss for long term study. One suture on another study using lamprey found that tag retention was very high.
- Cooke: not all about mass, also about shape and volume of tag and body.
- Cooke: 2% tag size: body size generally accepted for publishing (need some benchmark to measure against) such that anything above 2% (in water) is good (generally speaking), and anything above that need justification.

8-mm Tag Size

- Lavictoire: girth very important consideration regardless of size, they are so pencil thin at times.
- Newhard: 200-mm min size for 8mm tag size. 12-mm tags at 200-mm fish is questionable, avoided doing it. Surgeon experience in handling 200mm or below is very important. Could do smaller but thinks 200-mm and above is good.
- Mueller: fish 150-mm + suitable for tagging using 11.4-mm tags. Tag loss of 48% at 170- to 175-mm range, mechanism might not be related to fish size, could be other. Tag burden on 170- to 175-mm burden was lower than 2%; 8-mm tag would be even less burden.
- Haro: Mueller data is best in terms of controlled study, a good starting point. Tag retention in excess of 90% for long term study appropriate. Long term study can encompass lengthy durations for some of these fish (15+ years) and have no idea for tag retention for these durations. Research is needed but would be difficult.
- Lapointe: interpreted Mueller data different such that tag retention is low among all groups. Mueller response: agreed but results confounded by small sample size, data is a starting point. Sample size 3 – 7 fish, what might be applicable is the larger group value of 30 – 43% tag loss started to occur at day 30.
- Lapointe: BMP should be established based on well known and high confidence outcomes. Based on this 200-mm seems like a good start point. Thoughts?
- Phone: above 200-mm could use multiple insertion methods. Below 200-mm insertion method of high importance (Mueller agrees)
- Bob: playing it conservative is best. Trade off in fish size exist and ramifications for tag loss.
- Lapointe: objectives of study / research important to consider.
- Leblanc: Coastal Action does not tag fish below 200-mm.

11-mm Tag Size

- Lapointe: propose 200-mm, Pallard agrees

12-mm size

- Lavictoire: switched to 8-mm tag after using a 12-mm tag on 200- to 250-mm eels. Girth related issues. Use needles to tag. Proposes 250-mm minimum for 12-mm tags.
- Newhard: use 300-mm but comfortable with 250-mm. Some biologists they work with uncomfortable with using 250-mm for 12-mm tags. Conservative is a good thing, so favors the higher side of 250-mm.

23-mm and 32-mm tag size

- Lapointe: 23-mm size tag, fish size? No size proposed

Tag Insertion Location

- Lavictoire: commercial fishers scan the whole eel, prefer behind the head because this area is not an area of concern for consumption
- Lapointe: there is a strong preference to standardize but not strong preference for any one location; requires a strong rationale
- Haro: quickest and easiest and most confidence in intracoelomic (IC); tagged close to 100 in IC – tagging shifting around in body cavity over time, whether at risk of human consumption – less experience with intramuscular (IM) – makes a pitch to research different size tags – used scalpel for IC – size of fish might have bearing on where to put tag – small fish might have few options to place tag (IC) – recommend study on tag effects on different locations if not body cavity.
- Haro: in talks with others, intramuscular less reliable. More research is needed using different size tags, long term retention times, etc.
- Mueller: size of fish bearing on tag placement. IC is best option for all fish. Tag effects study on different sizes/locations, swimming capability.
- Turner: 2015-2018 tags detection using IM, so decent tag detection over longer term. Suggests using IC because easy to tag in this location and have had a high degree of recaps (4 years) indicating good retention rates for IC. Easy to standardize for teaching techs. Confident with high retention rates.
- Lavictoire: tagged behind the head, recaptured 2 fish from 2012, find it easy to tag behind the head (but does not necessarily indicate a high retention rate).
- Lapointe: will have to rely on month long study by Mueller.
- Lavictoire: a little trickier but once you have it down, easy to standardize dorsal musculature (Pallard agrees)
- Newhard: good success dorsal musculature (DM, not difficult, smaller ones under the skin (sub dermal) – good returns 7 years later in DM – heal up well
- Leblanc: anyone experienced tagging in the IC with a needle?
- Cooke: IC with needle is default for other species.
- Lapointe: desire to research tagging locations/methods, but no grounds to set a standard. Best practice currently. When PIT tagging with other tags, any issues with that?
- Phone: with big tag relative to eel size, IC better location
- Mueller: no interference in detections or surgery with salmon smolts, 12-mm PIT and small acoustic tag placed together, never any issues, common practice.
- Haro: opposite experience with 32-mm HDX PIT and radio tag. If PIT tag snugs up to radio tag (large battery) it severely restricts detection range. May not make a difference

in hand scanning but could impact antenna detection. Used large home-build antennas, but handheld reader will have better strength. Caution using larger scale antennas.

Tagging Methods and Tools

- Lapointe: more opposition around multi-use needles relative to implanting gun. How do we feel about multi-use syringes?
- Newhard: can tell when needle gets dull. Can sanitize needle between uses. Unsure of how to change out implanting gun between uses, especially on Silver Eels.
- Pallard: prefer single use because of sharpness and disinfecting.
- Turner: use empty PIT tag needle to puncture. Use alcohol to wipe needle and tags as cleaner. Once tissue builds up inside, throw needle away. Time restrictive when tagging many eels so easier and more efficient to puncture and manually insert for 50-60 Eels.
- Leblanc: use needle multiple times and use needle to insert it. Problems with tag retention when inserting tag by hand. Faster to use needle for insertion to avoid dropping tag, especially with smaller tags.
- Mueller: started using 22 g hypodermic to start the hole, then PIT tag needle to insert the tag. Since then revised to using scalpel blade and insert tag by hand, tag retention was high (3/100 lost tag).
- Lapointe: cost is higher for preloaded but always have a sharp needle. Is extra cost of single use prohibitive. Sharp needle with disease transfer (single use) vs. cost.
- Leblanc: cost would be prohibitive and needle method works. But can understand proposing for BMP.
- Newhard: single use would be cost prohibitive. Could file them down and re use in a pinch though.

Scalpel Incision / Insertion

- Haro: small scalpel incision, yellow eels with 23- or 32-mm tags. Insert by tag by hand. Tag retention is high, so method seems to work well. Old school method.
- Narezny: volume of fish big influence on insertion method (e.g., efficiency of implant gun vs scalpel time wise)
- Lavictoire: difficult to reach consensus when there are different sized Eels. Could be based on number of fish – which method to use? When on a boat, maybe use needle – when out on boats, better to use scalpel or needles?
- Mueller: scalpel 45 s to 1 min duration.
- Leblanc: cannot think of any benefit using scalpel vs needle on dorsal side. (Cooke: DM, needle is only puncture vs a cut for a scalpel).
- Cooke: there are numerous studies examining suture types and needle types, maybe glean info from these studies.

- Lapointe: Strong support for and opposition for scalpel incision with manual insertion – maybe due to tagging location.
- Leblanc: better control with scalpel for IC? Yes

Cooke Presentation

- Think about surgery as the whole process, it's irrelevant to be good at one thing
- Pre and post surgery very important
- Knotless nets
- To capture a fish, you cause injury and stress – everything we do stresses them out – check catch-and-release science.
- Bycatch and catch and release literature – front end handles precluding care
- Generalizing across aquaculture and live wells – lots of ambient water - more important than using chemicals (no science to back up chemical water conditioners, etc.)
- Aquaculture – minimize handling and use wet surfaces
- Surgical set up will depend on the type of project, number of fish, field conditions (e.g., lighting, ample support staff, comfortable for surgeon)
- Yoga mats best surfaces – cheap and no antimicrobial properties – use Virkon between sites.
- Using pumps and push water over gills – static water OK if held for short period of time – if water temperatures are cool – remarkable how quickly fish can suck out DO
- Pump water over same direction water naturally flows over the gills
- Several recovery totes, ergonomics – trailers can be purpose built
- A lot about perception, surgical professionalism
- Anesthetic Is used primarily to immobilize fish; it's a physiological challenge for fish.
- Electro anesthetic (electronarcosis) would be great to develop for AE given its effectiveness in other species
- "Field clean? Addresses animal care, professional, cost effective.
    - Betadine is good for tool cleaning, not toxic.
    - Avoid cidex and alcohol – kills cells.
    - Advise switching gloves between fish and/or betadine dip.
- Keep water out of the wound generally.
- Antibiotics not necessary, kills good bacteria

- Analgesics – no evidence for efficacy of analgesics in fish, so why use them? Implications for studying wild fish in the wild. Not necessary based on recommendation from USGS vet.
- Recovery and release – release as soon as "normal" (equilibrium, swimming, vigour); use reflex tests, does the fish try to escape, vigorous enough so it does not get displaced downstream
- Practice matters – especially in suturing. Retain animals to see if methods work well.
- Make vets your ally.

Comments on Presentation

- Mueller: Cordon – artificial slime layer. Effective? Steve: found to be neutral (did not benefit nor hurt) and in healthcare do not do things that are neutral because not beneficial but are wasteful resources if not beneficial. Comparative research in depth (e.g., microbiome level) needed. Salmonids more sensitive, might get fungal infections, work with microbiologists. Maybe differences in abrasions and mucus loss (sub lethal).
- Cooke: many are unregulated products, not tested. Be confident in human/vet product, but not market based unregulated products.
- Haro: Have vet in USGS, very helpful to get feedback from vet. Tech always changing. What is the next thing? Be adaptive in approach. May take a little longer for certain steps but if beneficial should adapt and push for that.
- Lapointe: discussing appropriate community practice is what we are doing here today, need the rigour, manipulate as you go
- Cooke: not everything needs to be standalone study, can incorporate into empirical studies.

Data Sharing

Ocean Tracking Network (OTN)

- Track acoustic telemetry – when an animal is detected on someone else's array, will get information, but also contact so encourage connection
- Publish data, protect by having an Embargo, default is 2 years after tags end date
- Use three different elements / nodes to pick up data across networks

BIOMARK

- Columbia River basin – great example of collaboration among millions of tags
- How are our detections working? Antennas, handheld readers
- Sea turtle community in similar boat to us
- Build a database end-user group – American Eel group
- Data privacy – all users (governing body) – vetting process of who has access

- All data broken down by project – register of tag decides whether data is public or private
- If marked as public, user can see all tagging metadata and biological data of fish
- If tag is recapped and private, you get contact info from entity that originally tagged
- No data publishing option
- All data is marked however practitioner wishes to mark it
- Customizable in terms of tag recaps and privacy - Two-way privacy system – up to researchers to communicate data they want to share
- BIOMARK readers are inclusive of all PIT tags (HDX, FDX-B, encrypted); non BIOMARK pit tags as well as non-pit tags can be included in database.
- Tools that clean PIT tag data (occupancy, etc.); built in visualization tools

Responses to BIOMARK

- Leblanc: may be concerns with data; great option to reach out to other groups; what is the fee associated with housing this data (both parties need to talk to supervisors RE: costs - host and server fees to store data would come into play eventually
- BIOMARK: unsure if cost is associated, but could be host/server fees
- OTN: unsure if cost is associated with hosting/database, defer to boss.
- Lapointe: challenge to NGO (costs, etc.) and gov (international species) - will end up being an international database (CAN & US).
- BIOMARK: push for databases for handheld scanners (can read all tag types)
- BIOMARK: Payment scheme for INSTREAM working to share hosting burden working with partners
- Lapointe: CWF will continue to engage with BIOMARK and OTN to determine database feasibility

Measurements to Save to Database

- OTN: ID number for animal in addition to the core fields, can customize beyond that (e.g., life stage), image storage available on repository
- BIOMARK: add in as many IDs from the tag as you want, customize fields
- OTN: allows for additional fields, not restricted, includes life stage and age, anesthesia dosage
- Creaser: phase of Eel (yellow, silver, silvering), development of lateral line and colour contrast
- Newhard: Sex of gender if a mortality, swim-bladder parasites other important fields
- BIOMARK: Can track injuries over time – add as many photos as you want

- Newhard: Criteria for life stage – how is that identified? Darkening of fins (dealing with tides)
- OTN: capture date field – handle recapture dates – date upon recapture
- BIOMARK: electronic data collection system; we pipeline data into database

Why PIT tag American Eels?

- Lapointe: are there scenarios that researchers/workers encounter eels that should not be PIT tagging eels?
- Lavictoire: if you don't have the right equipment to tag the eel (e.g., size is too small for tag size). If this isn't the intention of the study and happen to capture one, shouldn't be part of the permitting. Should not be required or at least not a "hard condition". Another reason is if you have a licence to collect fish in ON and you catch an SAR they are "done their work", so cannot ask them to tag the eel because they won't be able to, they cannot perform the work.
- Newhard: we tag a subset of Eels, not all animals captured in one tributary, but some from each trib. Not many come across Eels opportunistically while conducting other research because they are rare in our area. Maintains database of recaps, which often comes from anglers. Most biologists do not have equipment or experience for collecting PIT data or tagging Eel as they opportunistically encounter them, so likely will not tag. If all biologists had PIT tag readers it would be great but not practical.
- Leblanc: resources, data management and time concerns, so don't tag eels outside of actual eel research projects. Not in the current state, but perhaps in the future. Until a database management is in place, it is challenging to PIT tag every Eel.
- Lapointe: if nobody is monitoring eel recaps, then low benefit in placing a PIT tag in eels in that system and scanning eels in that system.
- Leblanc: impact to the eel from lack of experience in tagging, etc.
- Lavictoire: where deliberate monitoring program then useful, Great Lakes fishers recap lots (about 10%) and do scan them. Government application should outline who is tagging Eels.
- Lapointe: potential for database management in GLB and other specific basins; government knows who PIT tags as a condition of their permit license

Under the Skin or In the Muscle

- Newhard: under the skin for smaller, less intrusive. Syringes preferred, plunger on injector pushes tag further under the skin, tucks tag under the skin nicely, fits better (Lavictoire agrees)
- Leblanc: under skin regardless of size of eel using a needle. But larger tags use IC all the time.

- Lavictoire: based on size of tag and fish, so an area of research for larger Eels RE: tag retention outcomes.
- *Lapointe: general rec, smaller eel (250 mm or less) under the skin. More research needed for this in larger eel.*

Wound Closure

- *Turner*
  - Tagging in DM, framing retention as such (used one tagger for retention study, as tagger can influence retention.
  - Ambient water from nearby pond pumped into hatchery – mixed treatment tanks
  - Used Vetbond adhesive. Should have done IC and DM.
  - No issues but adhesive irritating their skin, using bricks (that were placed in tank to hold components down) to scratch backs to rid themselves of adhesive.
  - Used needle puncture and manually inserted tags.
  - Used 10 fish untagged, 18 and 19 with each treatment.
  - No irritation on open wound fish but had to release fish to migrate (silvering).
  - Tags were starting to protrude so would have had higher tag loss.
  - More protruding tags with adhesive but a few started protruding with open wound at time of release. 4 weeks study, but not conclusive to extrapolate onto long term tag retention studies. Tagged in the musculature, rather than under skin. Size range used was just under 30cm ranging to 67 cm.
  - Good retention with tagging in IC now. Go through anal pore and go up 2 cm, into cavity at a steep angle – not stabbing vital organs – get into cavity and manually insert tag.
  - Seen Eels with marks that indicate previous tagging events, no tagging
- Mueller: uses Vetbond for juvenile lamprey, did not work well, mucus layer caused issues, Vetbond came off. Not very effective!
- Leblanc: dependent on how tagging fish (training) having a person to physically show you rather than reading a protocol, need repeat staff, even with needle injection
- Lavictoire: dependent on skill and comfort level

Release Time

- Lapointe: barring release of fish for experimental purposes, any max. holding times?
- Newhard: leery of holding out-migrating Eels to disrupt migration
- Lapointe: reason for holding Eels more than an hour? (Newhard could not think of a good reason)

- Lapointe: difficult to determine why, is it deliberate or for transport reasons?
- Lavictoire: sooner the better!
- Cooke: release ASAP

Antiseptic Practices

- Lapointe: applying antiseptic to wound or not. If leaving open, suggest no antiseptic application to wound area. Focus on items that will be going internal (e.g., tag, tools)
- Mueller: no antiseptics needed for wound. (Newhard agrees).
- *Lapointe: Betadine as an effective antiseptic is a clear recommendation. Internal contact is focus for sterility/antiseptic*
- *Lapointe: No antiseptic on wound as a BMP recommendation. Check with Cooke for citations to back recommendation if endorsing Betadine, otherwise rely on expertise, or make no specific antiseptic recommendation*

Important Surgery Process Considerations for American Eel

- General guidance RE: relative density of Eels – how many is too many?
- Leblanc: traps in overnight, mesh diameter is close to their girth then fish can escape or injure themselves. Light conditions also important, reduce stress. Gloves imperative because no anesthetic. Mesh nets with pool noodle to float, temporary solution (short term, 30 minutes).
- Lapointe: Anesthetic endpoints? Cease of opercular movements and response.
- Newhard: Respiring, pick up fish and see response. One mort from overdose (but likely from being shocked multiple times), only mort in 9 years.
- Lapointe: No overdosing, but many underdosing, maybe air on the side of caution and go heavier?
- Newhard: fresh bath of anesthetic (e.g., AQUI-S) after 3 or 4 eels. Good practice to pay attention to how many batches per bath.

Figure A4.1: Decision-making framework proposed for assessing the outcome of votes held during the workshop.