American Eel Passive Integrated Transponder (PIT) Tagging

Best Practices Guide

Prepared by:
Dirk Algera
Sarah Rabideau
Jacqueline Pallard
Nicolas Lapointe

CANADIAN WILDLIFE FEDERATION

PHOTO CREDIT: S. LANDSMAN

Acknowledgements

This guide was the result of ongoing engagement with practitioners with expertise on eel handling, surgery, and tagging, funded by CWF and in part by the Ontario Ministry of Environment Conservation and Parks Species at Risk Stewardship Program (Project #18-19-CWF).

We benefitted greatly from the participation, guidance, advice, and feedback of many people throughout this process — this publication would not have been possible without their support.

We would like to thank Steve Woods for conducting initial interviews with practitioners, as well as to all the practitioners that participated in interviews, submitted completed questionnaires. We thank Steven Cooke, Taylor Creaser, Angela Deny, Alex Haro, Aaron Henning, Michelle Lavictoire, Molly Leblanc, Bob Mueller, Andy Narenzy, Josh Newhard, Kathleen Sevigny, and Sara Turner for their participation and invaluable input at the workshop. Finally, we extend our thanks to Carleton University for providing facilities to host the workshop.

Canadian Wildlife Federation

350 Michael Cowpland Drive

Kanata, Ontario K2M 2W1

Telephone: 1.877.599.5777 | 613.599.9594

CanadianWildlifeFederation.ca

Suggested Citation:

Algera, D., S. Rabideau, J. Pallard, and N. Lapointe. 2024. American Eel Passive Integrated Transponder (PIT) Tagging: Best Practices Guide. Canadian Wildlife Federation. Ottawa, Ontario, Canada. 18 pp.

Cover Photo: © Sean Landsman

Table of Contents

1.0 Introduction

1.1 Context and Rationale

The American Eel (*Anguilla rostrata*) is a panmictic, catadromous species with a wide distribution range extending from the Sargasso Sea (where spawning occurs) into many freshwater systems throughout Greenland, North America, and the northern portion of South America. American Eel populations have dramatically declined over the past few decades, resulting in the species being listed as Endangered on the International Union for Conservation of Nature (IUCN) Red List, Threatened in Canada by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), and Endangered under the *Ontario Endangered Species Act*.

Researchers and wildlife practitioners from academia, (non-) governmental agencies, industry, and consulting firms have conducted fundamental and applied research projects using biotelemetry technologies to monitor biological outcomes, mitigate the effects of anthropogenic disturbances, and assess recovery efforts for American Eel. Common biotelemetry technologies used in research projects have included radio, acoustic, and passive integrated transponder (PIT) tags. The choice of biotelemetry technology used depends on project objectives, study design, and costs. Relative to other telemetry technologies, PIT tags are a cost-effective tool to track eel movements, assess population sizes, fill knowledge gaps on growth and survival rates, and assess various hazard mitigation and monitoring actions (Rudershausen et al. 2019; Camhi et al. 2021).

Methods used to implant PIT tags into American Eel have varied amongst practitioners and project objectives. Furthermore, handling and holding after capture, anesthetics used, tag size, fish body size, tag placement on the body, and the implantation method used are known to affect fish injury, tag retention, and detection rates. American Eel are considered an imperilled species in multiple jurisdictions, thus maximizing harm reduction, data acquisition, and data quality are imperative when working with the species.

Best practices have been developed and published for implanting PIT tags into various finfish, most predominantly juvenile salmonids (e.g., PTAGIS 2014; Liedtke et al. 2014). Practitioners implant PIT tags into juvenile salmonids captured streamside and at traps/weirs, but most PIT tags are implanted in fish reared in a hatchery environment and subsequently released for population enhancement purposes. This controlled hatchery environment produces a relatively similar body size among fish, consequently enabling routine tasking, mass marking or batch processing capabilities, and the development of standard operating procedures for PIT tag implantation. Best practices developed for juvenile salmonids aim to reduce injury and mortality of tagged fish (e.g., through reduced holding and handling times), while promoting consistency in data collection and quality (e.g., high tag retention rates, reduced human error during data collection). In contrast, most American Eel that are implanted with PIT tags are

wild caught and vary considerably in body size. American Eel aquaculture rearing programs exist whereby juvenile eels (i.e., glass eels) are captured and grown to adulthood, but these fish are sold to markets as food. Practitioners have implanted PIT tags into cultured American Eel for research projects, but standard operating procedures like those developed for juvenile salmonids are not directly applicable to American Eel. In this guidance document, evidence-based research and expert knowledge from experienced practitioners and researchers were used to inform the development of more generalized guidance and best practices for implanting PIT tags into American Eel.

1.2 Purpose and Applicability

The purpose of this document is to provide practitioners with objective, evidence-based guidance and best practices for implanting PIT tags into American Eel. Guidance and best practices for equipment, anaesthetics, handling, tagging, recovering, and releasing fish are included to increase tag retention and detection rates while minimizing stress, injury, and mortality to fish. Guidelines and best practices promote consistency in data acquisition and can enhance data quality for practitioners working with American Eel. These guidelines and best practices may be applicable to similar congeners such as European Eel (*Anguilla anguilla*), given their physiological and morphological similarities to American Eel.

1.3 Evidence and Support for the Development of Guidance and Advice

The guidance outlined in sections 2.0 (Best Practices) and 3.0 (Other Advice) was developed based on empirical evidence compiled through a scientific literature review and expert opinion obtained through structured interviews with experienced practitioners and researchers (henceforth "experts"), an online questionnaire, and an in-person workshop on implanting PIT tags into American Eel (see Appendix A for a brief description). A separate report provides a complete account of the evidence, rationale, and references supporting this document, as well as the literature review, expert interviews, online questionnaire, and workshop outcomes (Rabideau et al. 2020). Citations are provided here only for additional evidence not included in that report.

A decision-making framework was used to identify the Best Practices and Recommendations in section 2.0 (see Appendix B Table B1). Several additional topics were identified for which empirical evidence and expert knowledge were available; however, the decision-making framework was not applied to develop Best Practices or Recommendations for them. Supplementary advice is provided for these additional topics in section 3.0.

1.4 Definitions – Best Practices, Recommendations, and Insufficient Evidence

Formal terms used throughout this document reflect the degree of consistency in evidence and support obtained through the decision-making framework (See Appendix B for more details), and are defined as follows:

- "**Best Practices**" were provided for practices that were demonstrated to be effective (**USE**) or harmful/counterproductive (**AVOID**) according to empirical evidence in the methodological research (i.e., literature review) and a large majority of experts. When Best Practices were identified, no evidence summaries were provided and no alternative methods were suggested.
- "**Recommendations**" were suggested for practices that had limited empirical evidence or involved inconsistent evidence or expert support. For a Recommendation, some (but not necessarily all) empirical evidence in the methodological research indicated that a practice was effective (**USE**) or harmful/counterproductive (**AVOID**) and most (but not necessarily all) experts agreed with this conclusion. Evidence summaries were provided and key alternative methods were suggested for Recommendations.
- Practices were categorized as having **"Insufficient Evidence"** when there was limited or no research, or the research was contradictory in the literature, and/or when experts were split in their advice or provided no advice. In these cases, a strong rationale was not provided to indicate that these practices should be used or avoided. Additional research would be required to establish a Best Practice or Recommendation for these practices.

1.4 How the Guidance and Advice Should be Used

The guidance and advice in this document were developed to complement applicable institution-specific animal care criteria and protocols, as well as regulatory or permitting requirements, while providing evidence-based guidance and advice specific to American Eel.

NOTE: When discrepancies occur between guidance in this document and a permit or an institution's animal care protocols under which tag implantation work is conducted, this guidance document could be provided to the Animal Care Committee as rationale for approval to use methods that differ from their accepted protocols.

Detailed equipment checklists and prescriptive step-by-step procedures for implanting PIT tags into American Eel were beyond the scope of the guidance in this document because these were not consulted on or covered in the review process for developing the guidance. Moreover, equipment lists and procedures were not included to avoid being overly prescriptive or conflicting with existing institutional protocols. Examples of general protocols for tagging fish are freely available online, including:

- General guidelines for fish (CCAC 2005)
- Anesthetics for fish (Ackerman et al. 2005)
- Marking and tagging of finfish (DFO 2004)

2.0 Best Practices

2.1 Tag Types and Readers

PIT tag types include half duplex (HDX) and full duplex (FDX) technologies, which vary in tag size, operation frequency, signal strength, read rate, and read range detectability (i.e., detection distance). HDX tags contain capacitors, which increase the tag size relative to FDX tags, but allow for greater signal strength and read ranges than FDX tags. Readers used for HDX technologies have reduced detection rates relative to FDX because HDX readers repeat a cycle of transmitting a signal then stopping transmission to receive tag signals, whereas FDX systems constantly transmit and receive signals.

Tags and readers from different manufacturers are generally interoperable within FDX and HDX technologies; however, HDX and FDX are not expressly compatible technologies and there are PIT tag readers that detect only HDX, only FDX, or both (i.e., universal). Furthermore, some PIT tag manufacturers produce encrypted (e.g., proprietary) tags and readers which are not universally compatible among all systems. Evidence from the literature and expert knowledge indicated that the choice between using HDX, FDX, and encrypted tags/readers will largely depend on project objectives and experimental design considerations.

Best Practice(s)

✔ **USE** the following PIT tag types and readers (not ranked):

- HDX
- FDX-B
- Universal handheld tag reader

✖ **AVOID** using the following PIT tag types and readers:

- Encrypted tags and readers

NOTE: There may be valid applications for using encrypted tags and readers, but usage of encrypted tags/readers should be limited to research in closed systems because American Eel disperse and migrate over long distances and can be recaptured by others. Use of encrypted tags and readers creates risks of implanting more than one tag into specimens, or lost opportunities to collect data from multiple capture events.

Recommendation(s)

✖ **AVOID** using the following PIT tag types:

- FDX-A tags

There was limited methodological research on FDX-A tags in the literature and most experts lacked experience with using FDX-A tags. The main operational differences between FDX-A and FDX-B tags include read frequency (125 Hz versus 134.2 Hz), ID characters (may contain letters and numbers versus numbers only), maximum ID length (up to 10 versus 15 characters), and universal reader compatibility (not compliant with International Organization for Standards (ISO) standards versus ISO standard compliant).

Insufficient Evidence

None – All practices reviewed produced a Best Practice or Recommendation.

Box 2.1 – Summary of Best Practices, Recommendations, and Insufficient Evidence for PIT tag types and readers.

USE	AVOID
Best Practice(s)	
• HDX tags • FDX-B tags • Universal handheld tag readers (ISO compliant)	• Encrypted tags • Encrypted tag readers
Recommendation(s)	
• N/A	• FDX-A tags
Insufficient Evidence	
• N/A	

2.2 Body Size and Tag Size

Tag size (or volume) relative to fish size is one of the most important factors to consider when choosing a tag because tags that are too large are known to cause sublethal effects (e.g., behavioural impairments) or lead to mortality. Evidence from the literature and expert knowledge suggested that PIT tags should be implanted into American Eel with a minimum body length of 200 mm. Implanting PIT tags into eels with a body length of less than 200 mm increased the risk of mortality from heightened sensitivity to handling, holding, and surgery processes. Furthermore, even when using the smallest PIT tags available, tagging fish less than 200 mm increased the risk of sublethal tagging effects such as behavioural impairments (e.g., reduced swimming capabilities), which may compromise data quality and the reliability of inferences drawn from the data analysis. Eel girth and mass were also noted as important factors related to fish size; however, fish girth and mass at a given body length varies among specimens and thus was difficult to formulate consistent guidance upon. Consequently, girth and weight were not considered further as criteria for inclusion for guidance in this document. The guidance reported herein focused on the minimum body length of 200 mm because the risk of tagging-related morality or behavioural impairments continually diminished as body size increased above 200 mm.

According to evidence from the literature and expert knowledge, common PIT tag sizes for American Eel range from 8 to 32 mm. Operation frequency and signal strength capabilities varies among tag sizes (and tag type technology, see section 2.1 Tag Types and Readers); therefore, tag size can affect read range (i.e., detection distance). Larger tags typically emit stronger signals and have an improved probability of being detected further away from a tag reader than smaller tags. Experts advised that the choice of tag size will largely depend on project objectives and experimental design considerations. For example, if project objectives involved tagging juveniles, then smaller tags would be required for these individuals to minimize mortality risk, tagging effects (e.g., behaviour, injury), and tag expulsion. Conversely, if a project involved tagging out-migrating adult eels, a larger tag capable of emitting stronger signals to maximize read range and detection probabilities may be considered.

Best Practice(s)

✔ **USE** 8 mm PIT tags with a minimum body length ≥ 200-250 mm

✖ **AVOID** tagging American Eel < 200 mm

Recommendation(s)

✔ **USE** 11 mm and 12 mm PIT tags with a minimum body length ≥ 250 mm

There was limited literature and expert knowledge supporting that 11 mm PIT tags are safe for use with eels < 250 mm, and the empirical evidence suggested that expulsion rates are high for this combination of eel and tag size. Empirical evidence supported the use of 12 mm PIT tags with a minimum body length of 250 mm, but there was not sufficient consistency in expert knowledge to establish this as a Best Practice. Since mortality and tag expulsion are rare when implanting 12 mm tags in ≥ 250 mm eels, 11 mm PIT tags would also be appropriate for use with eels ≥ 250 mm.

Insufficient Evidence

- Using tags > 12 mm

There was limited research in the literature on advantages/disadvantages of using tags > 12mm and experts had limited experience using tags > 12 mm to warrant discussion on usage or avoidance of these tags.

Box 2.2 – Summary of Best Practices, Recommendations, and Insufficient Evidence for PIT tag sizes and minimum body lengths.

USE		AVOID	
Best Practice(s)			
Body length	**Tag size**	**Body length**	**Tag size**
• ≥ 200-250 mm	• 8 mm	• < 200 mm	• All tags
Recommendation(s)			
• ≥ 250 mm	• 11 mm	• < 250 mm	• 11 mm
• ≥ 250 mm	• 12 mm	• < 250 mm	• 12 mm
Insufficient Evidence			
• Tags > 12 mm			

2.3 Anesthetics

A fish is typically considered anesthetized when a loss of equilibrium is exhibited. Evidence from the literature and expert knowledge indicated that water temperature and chemistry, body size, holding density, stress levels, and desired sedation level are key factors that can influence a fish's dosage response and induction time. Best Practices were not developed for specific anesthetic dosages because each of these factors affect dose-response and induction time; however, additional advice based on research in the scientific literature and from expert experience is provided in section 3.1 (Anesthetic Dosages).

Chemical-based anesthetics including clove oil (mixed with ethanol) and tricaine methanesulfonate (MS-222; buffered with sodium bicarbonate) were commonly reported in the literature and used by experts for effectively anesthetizing American Eel. Alternative chemical anesthetics including benzocaine, metomidate, and 2-phenoxyethanol were not commonly reported in the literature or used by experts. Non-chemical methods included electro-sedation, ice baths (i.e., induces hypothermia) and no anesthetic (i.e., wearing cotton gloves for animal restraint). These non-chemical methods were not commonly reported in the literature or used by experts.

NOTE: Anesthetic approval varies among institutions and regulators. Acceptable anesthetics must be verified before use regardless of the guidance provided in this document.

Best Practice(s)

✔ **USE** the following anesthetics (not ranked):

- Clove oil (mixed with ethanol)
- MS-222 (buffered with sodium bicarbonate)

Recommendation(s)

✔ **USE** the following anesthetics:

- Eugenol

✖ **AVOID** the following anesthetics:

- Benzocaine
- 2-phenoxyethanol
- Ice water

Eugenol had strong literature support and moderate expert support; however, Eugenol is not authorized for use by many institutions and therefore experts had limited experience using it. Eugenol has comparable characteristics, advantages, and disadvantages to clove oil because it is a derivative of clove oil. There was a Recommendation to use Eugenol with American Eel because, like clove oil, it has an induction time of less than three minutes, has a wide margin of safety between sedation and lethal dosage, and reduces stress during handling and/or surgery.

There was limited evidence in the literature supporting the use of ice water (i.e., to induce hypothermia) as an anesthetic and some experts provided rationale against using this ice water because rapid temperature drops at immersion can induce cold shock (a stressor) or cause acidosis. Furthermore, immersion in ice baths would be ineffective in sedating eels when ambient water temperatures were already cold.

Limited literature evidence supported using benzocaine and 2-phenoxyethanol, and experts lacked practical experience with these anaesthetics. As a precaution, the Recommendation was to avoid benzocaine and 2-phenoxyethanol because they are known to possess one (or more) disadvantageous characteristics including: a low safety margin between an inductive and lethal dose (2-phenoxyethanol); retention by fish of some muscle response while anesthetized (benzocaine, 2-phenoxyethanol) and; the inability to block stress response (2-phenoxyethanol) (Ackerman et al. 2005).

Insufficient Evidence

- Metomidate
- Electro-sedation
- No anesthetic

The scientific literature indicated that metomidate can be as effective as Eugenol for anesthetization, but experts lacked practical experience using it. Metomidate is known to have a low safety margin between an inductive and lethal dose and fish retain some muscle response while anesthetized with metomidate (Ackerman et al. 2005).

Electro-sedation and no anesthetic garnered limited literature and expert support. One rationale that was provided for using these non-chemical alternatives was that they have shorter handling and recovery times relative to chemical anesthetics; however, no methodological research was available on the effectiveness and animal safety implications of these non-chemical alternatives. Following the workshop, one researcher reported positive outcomes from the limited use of clove oil to initiate partial anaesthesia, followed by electro gloves during surgery (S. Cooke, Carleton University, pers. comm.).

Box 2.3 – Summary of Best Practices, Recommendations, and Insufficient Evidence for anesthetics used for PIT tag implantation.

USE	AVOID
Best Practice(s)	
• Clove oil • MS-222	• N/A
Recommendation(s)	
• Eugenol	• Benzocaine • 2-phenoxyethanol • Ice water
Insufficient Evidence	
• Metomidate • Electro-sedation • No anesthetic	

2.4 Tag Implantation Methods and Instruments

Surgery and injection were the most reported methods for safely and effectively implanting PIT tags into American Eel in the literature and by experts. The surgical method involves making an incision with a scalpel and subsequently implanting the PIT tag into the chosen location by hand (i.e., without a tool) or using a tool. With injection, a needle is used to puncture the skin and a tag is implanted using an injector tool. Commercially produced injector tools expressly designed for PIT-tag insertion are commonly used (e.g., multi-tag injector gun, single-use tag injectors). Alternatively, a standard hypodermic needle can be used to puncture a hole in the skin and the tag can then be subsequently implanted by hand. Some PIT tag types are designed to be compatible for implantation using an appropriately sized standard hypodermic needle and syringe, but this practice was not reviewed or included in this document.

Best Practice(s)

✔ **USE** the following implantation methods and instruments (not ranked):

- Multi-tag injector gun
- Single-use tag injector
- Scalpel incision and tag placement by hand

Recommendation(s)

✔ **USE** the following implantation methods and instruments:

- Needle puncture and tag placement by hand

✖ **AVOID** the following implantation methods and instruments:

- Separate needles for puncture and tag insertion

Needle puncture and subsequent placement of tags by hand received considerable expert support, but received no support in the scientific literature due to a lack of research on this specific practice. It was postulated that this method could be used in the absence of readily available injector tools or when an injector tool's needle becomes too dull, but there is another suitable needle on hand.

Using an empty needle to puncture a hole and subsequently implant a tag with a different tool (i.e., multi-tag injector gun) received limited research and workshop practitioner support. The premise behind this practice is that a separate needle might be used if it was sharper than a multi-tag injector gun needle. This method results in the use of two different tools, increasing the risk of injury or infection.

Insufficient Evidence

None – All practices reviewed produced a Best Practice or Recommendation.

Box 2.4 – Summary of Best Practices and Recommendations for tag implantation methods and instruments.

USE	AVOID
Best Practice(s)	
• Multi-tag injector tool • Single-tag injector tool • Scalpel incision and placement by hand	• N/A
Recommendation(s)	
• Needle puncture and placement of tag by hand	• Separate needles for puncture and tag insertion
Insufficient Evidence	
• N/A	

2.5 Wound Closure

Implantation wounds occur regardless of the tagging method and tools used. Wounds from implanting PIT tags can potentially lead to tag loss, infection, or other adverse effects. Wound-management practices reported in the literature and by experts included leaving the wound open to heal and using sutures (mainly for incisions) and medical-grade adhesives for wound closure. The main reasons to close wounds were to minimize infection risk, healing time, and tag expulsion.

Leaving small (e.g., < 1 cm) wounds open to heal after surgical and injection tag implantation was commonly reported in the literature and by experts because wounds resulting from PIT-tag implantation methods are relatively small (and superficial with intramuscular injection) with minimal risk of the wound not healing timely or properly. Wounds were closed to reduce the risk of tag expulsion; however, evidence from the literature and provided by experts indicated that surgical and injection wounds heal rapidly when the wound was left open and tag retention was similar or higher relative to using sutures (which are a foreign body) or adhesives. Furthermore, American Eel were found to bite the wound area or rub along tanks or structures to attempt to remove sutures or adhesives, aggravating the wound area, delaying healing, and increasing the risk for infection and tag expulsion.

Best Practice(s)

✔ **USE** the following wound closure option:

- Leaving the wound open if small

✖ **AVOID** the following wound closure options:

- Sutures
- Adhesives

Recommendation(s)

None – All practices reviewed produced a Best Practice.

Insufficient Evidence

None – All practices reviewed produced a Best Practice.

Box 2.5 – Summary of Best Practices, Recommendations, and Insufficient Evidence for wound closure practices.

USE	AVOID
Best Practice(s)	
• Leave wound open	• Sutures • Adhesives
Recommendation(s)	
• N/A	• N/A
Insufficient Evidence	
• N/A	

2.6 Animal Recovery and Release

Recovery and release endpoints are important post-operative care considerations to reduce animal stress by minimizing handling and holding time. Juvenile eels have heightened sensitivity to handling and holding, and though adults are more robust, handling and holding remains stressful. Releasing eels as quickly as possible after recovery contributes to reduced behavioural impairment and downstream displacement, improved survival potential, and maximizing the potential for useful data generation.

Resumption of normal swimming behaviour was commonly used as a recovery endpoint in the literature and by experts. Normal swimming behaviour was confirmed by the display of equilibrium, swimming vigour, avoidance or escape responses to reflex tests (e.g., tail pinching). Resumption of normal swimming behaviour and capabilities were highlighted as particularly important recovery endpoints for experts working with American Eel expected to circumnavigate hydropower facilities because upstream migrating juveniles must be capable of swimming up eel ladders (or other fish passage infrastructure), and adults migrating downstream must behave normally to objectively study their passage routes and outcomes.

Best Practice(s)

✔ **USE** normal swimming behaviour as an indicator for release

✖ **AVOID** holding eels for extended periods of time

NOTE: There may be some exceptions for holding eels for extended periods of time such as for transport (e.g., conducting truck and transport programs) or experimental design (e.g., testing tag retention rates, etc.,) purposes.

Recommendation(s)

None – All practices reviewed produced a Best Practice.

Insufficient Evidence

None – All practices reviewed produced a Best Practice.

Box 2.6 – Summary of Best Practices, Recommendations, and Insufficient Evidence for animal release practices.

USE	AVOID
Best Practice(s)	
• Release eels when normal swimming behaviour is resumed	• Holding eels for extended periods of time
Recommendation(s)	
• N/A	• N/A
Insufficient Evidence	
• N/A	

3.0 Other Advice

The practices outlined in this section were identified in the methodological research, in the questionnaire results, or were discussed during the workshop; however, the full decision-making framework was not applied to them. Advice on practices in this section is informal and was based on evidence in the literature review and expert experience.

3.1 Anesthetic Dosage

To remain consistent with the anesthetics recommended for use in section 2.3, anesthetic dosage expert advice was provided only for clove oil, Eugenol (e.g., Aqui-S 20E), and MS-222. Furthermore, the expert experience-base was limited to these anesthetics and this advice is therefore specific to American Eel, and not based on evidence or experience with congeners.

The desired sedation level is an important consideration when selecting anesthetic dosage. Implanting PIT tags is a relatively minor procedure and requires minimal sedation levels compared to more extensive surgical procedures requiring sutures. When PIT tags are implanted along with other transmitters such as acoustic telemetry tags, a deeper sedation level may be required and would necessitate a higher anesthetic dosage. Other key considerations in determining anesthetic dosage included body size, water temperature, and holding density. When uncertain, experts advised that a conservative approach to anesthetic dosage application should be used initially because more anesthetic can subsequently be added to achieve a desired sedation level.

NOTE: Institutions may have their own dosage and induction protocols, which should take precedent over the anesthetic dosage advice in this document.

Clove oil

- Mix clove oil with ethanol 1:10 (e.g., 1 mL clove oil added to 9 mL ethanol).
- 100 mg/L is considered a standard dose.
- A higher dose may be required at water temperatures below 15°C.
- Clove oil will agglomerate in very cold water even when emulsified in ethanol.

Eugenol

- No mixing or buffering agent is required.
- 30 mg/L is considered a good option.

MS-222

- Buffered with sodium bicarbonate.
- 150 mg/L is considered a standard dose.
- Water temperature affects dilution capability and sedation time and level – sedation level should be monitored closely, and more MS-222 may be added to maintain it.
- May inhibit olfaction if dosage is too high.
- Known human carcinogen – wear proper personal protective equipment.

Box 3.1 – Range of clove oil, Eugenol, and MS-222 dosages used when implanting PIT tags into American Eel, with associated length ranges when available. The data are specific to American Eel, with sources provided in Rabideau et al. (2020).

Anesthetic	Dosage (mg/L)	Length range (mm)
Clove oil	30	Not indicated
	40-80	> 200
	100-120	200-370, 200-500
	200-250	> 250
Eugenol	30	Not indicated
	90	< 750
	120	> 750
MS-222	75	< 130
	100	> 250
	150	Not indicated
	240	115-210
	250	> 250
	300	> 130
	400	> 90

3.2 Tagging Location

Evidence in the literature and from expert advice identified four locations where PIT tags are implanted into American Eel: behind the head, in the dorsal musculature anterior to the dorsal fin, in the dorsal musculature above the cloaca, and in the intracoelomic cavity. Some tagging locations were common in a particular geographic region/jurisdiction, and many experts used the same location consistently among their own studies. Additional considerations for selecting a tagging location included eel size and whether other tags or transmitters were being concurrently implanted. Examples of when experts may need to account for these additional considerations include:

- Intracoelomic tag implantation is suitable for eels of any size, and is often selected when incisions for larger acoustic transmitters are already available.
- Smaller eels (250 mm or less) should be tagged under the skin if not using intracoelomic tag implantation.
- Tags are implanted behind the head to reduce risk of human consumption (where this is of concern) and when radio transmitters are being implanted because they may interfere with or restrict PIT tag detection range.

3.3 Handling, Holding, and Operative Care

All handling, holding, and operative care practices produce a stress response in fish, but some preparations, techniques, and considerations can substantially reduce or minimize stress levels. Generalized advice applicable to American Eel from experts with extensive fish handling, holding, and operative care experience included:

- Use ambient water temperature throughout the process.
- Use yoga mats (that do not contain scents or anti-microbial properties) as padding. Avoid transferring pathogens by cleaning the padding with Virkon between tagging sessions.

- Keep "field clean" with Betadine as a disinfectant for surgical tools.
- Avoid using antibiotics or analgesics.
- Avoid using antiseptics on wounds.
- Ensure that holding trap (if used) mesh size does not permit escape, which may cause injury or mortality to eels.
- Reduce light levels in the holding tank or provide structure (e.g., PVC pipe) for eels to hide.
- Closely monitor anesthetic endpoints (e.g., opercular movements, respiration).
- Create fresh baths of anesthetic after a low number of American Eels have been sedated. It is good practice to record how many eels or batches of eels (and approximate number per batch) were anesthetized per bath.

3.4 Data Collection, Quality Assurance, and Sharing

Implanting PIT tags in American Eels provides an opportunity to gather data on the species, both during implantation and when recaptures occur. Useful American Eel traits that experts advised to consider measuring and recording included life stage, age, maturity phase (e.g., silver eel, yellow eel, glass eel), sex, lateral line development, colour contrast, swim bladder parasites, and injuries. For data quality assurance and sharing, experts also advised to:

- Use an electronic data logger and avoid transposing by hand (to mitigate human error).
- Record geographic data, but obscure when publicly reporting data (e.g., 1 km resolution for a map grid).
- Register with tracking networks that exist to report PIT tag numbers and locations and serve as a data repository (e.g., Ocean Tracking Network).
- Report any American Eel captured with existing PIT tags to the tracking networks.

4.0 References

Ackerman, P. A., J. D. Morgan, and G. K. Iwama. 2005. Anesthetics. In: CCAC guidelines on the care and use of fish in research, teaching and testing. Available online: https://ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Fish_Anesthetics.pdf (Accessed 05 Jan, 2023).

Camhi, M., M. Bednarski, J. LaBelle, and J Waldman. 2021. Abundance and Distribution of American Eel in a Heavily Dammed Urban River. North American Journal of Fisheries Management. 41:1131-1140.

CCAC [Canadian Council on Animal Care]. 2005. CCAC Guidelines on the care and use of fish in research, teaching and testing. Available online: https://ccac.ca/Documents/Standards/Guidelines/Fish.pdf (Accessed 05 Jan, 2023).

DFO [Fisheries and Oceans Canada] 2004. 6.0 Marking and Tagging of Finfish. Available online: https://ccac.ca/Documents/Education/DFO/6_Marking_and_Tagging_of_Finfish.pdf (Accessed 05 Jan, 2023).

Liedtke, T.L., J. W. Beeman, and L. P. Gee. 2012. A standard operating procedure for the surgical implantation of transmitters in juvenile salmonids. U.S. Geological Survey Open-File Report 2012-1267, 50 p. Available online: https://pubs.usgs.gov/of/2012/1267/pdf/ofr20121267.pdf. (Accessed 05 Jan, 2023).

PTAGIS [Columbia Basin PIT Tag Information System]. 2014. PIT Tag Marking Procedures Manual. Prepared by: PIT Tag Steering Committee. Version 3.0. Available online: https://www.ptagis.org/content/documents/2014-mark-procedures-manual.pdf. (Accessed 05 Jan, 2023).

Rudershausen, P. J., L. M. Lee, S. M. Lombardo, J. H. Merrell, and J. A. Buckel. 2019. Survival and Habitat of Yellow-Phase American Eels in North Carolina Tidal Creeks. Transactions of the American Fisheries Society. 148: 978-990.

5.0 Appendices

5.1 Appendix A – Additional Information

Participants for the structured interviews, online questionnaire, and workshop included representatives from environmental consulting firms, government, academic, and non-governmental researchers, and industry stakeholders from Canada and the United States.

Literature Review

A literature review of the peer reviewed scientific literature was conducted (November 2019) to determine common PIT tagging methods and synthesize comparisons of methodologies for PIT tagging eels. No attempts were made to locate articles not freely available via our subscription base. 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. Over 550 articles were screened (title/abstract/keywords) from a targeted search of online scientific literature databases (Web of Science Core Collection) using the following screening criteria:

- *Anguilla* species was used in the study
- Satellite, data-storage, VIE, coded wire, PIT, acoustic, or radio tags used in study
- Mark/recapture studies (because PIT tag use is common in these)

Relevant data were extracted from 43 articles published from 2000 to 2019. The data were used as inputs for question formulation in the questionnaire (see Section 2.3).

Practitioner Interviews

Structured interviews were conducted (Summer and Fall 2018) with 18 practitioners. Interview subjects were chosen for their experience working with American Eel. There is some overlap between the literature review and interview results because many of the practitioners conduct and publish scientific research on American Eel. The interview format allowed for practitioners to discuss their PIT tag implanting procedures and provide more detailed rationale for their chosen methods —information that is often omitted from studies published in scientific journals. Such rationale provides valuable insight into why certain methods were used.

Online Questionnaire

The literature review and structured interview results were synthesized and formed the basis for an online questionnaire. The purpose of the questionnaire was to present practitioners with the literature and interview results, assess their support (or disagreement) for the literature review/interview results, and obtain their advice for Best Practices and Recommendations. The questionnaire was distributed (January 2020) to 32 practitioners, and 12 responses were received. Questionnaire results were synthesized and formed part of the evidence presented to practitioners at the workshop.

Practitioner Workshop

A workshop was held at Carleton University (February 2020) with the expressed purpose of generating consensus on PIT tagging Best Practices and Recommendations. Attendees (via in-person and teleconference) discussed the evidence and expert knowledge gleaned from the literature review, structured interviews, and online questionnaire. The workshop moderator generated a proposition based on the discussion, and attendees voted (yes, no, abstain) to indicate the overall level of support (i.e., consensus) for a given practice. The level of support for establishing a "Standard" ("Best Practice" in this document) or an "Evidence Summary" were determined using a decision-making framework ratified by all participants at meeting onset. See Appendix B for the decision-making framework.

5.2 Appendix B – Decision Framework

Best Practices were developed by applying a decision framework (Table B1). Best Practices were established when there was strong empirical evidence found in the scientific literature or expert agreement that a given practice was either effective or counterproductive. Recommendations were developed using a combination of empirical evidence in the literature and expert knowledge that did not meet Best Practices thresholds, but nonetheless provided evidence that a practice was likely effective or counterproductive. This included situations where there was limited empirical evidence or inconsistent evidence or expert knowledge.

Table B1 – Decision framework used to determine Best Practices and Recommendations in this American Eel PIT tagging best practices guide.

Outcome	Empirical Evidence	Workshop Support	Rationale
Best Practice	Methodological research demonstrates that one method has greater effectiveness	>50% in favour of that same method	Decision is based on empirical evidence and a basic majority
	Limited empirical research available on the effectiveness of alternate methods	>75% in favour of one method	Insufficient empirical evidence, but a strong majority agrees
	Methodological research demonstrates that one method has greater effectiveness	>90% in favour of a different method	Likely reflects expert knowledge that outweighs evidence from one study (e.g., study was flawed or not widely applicable)
Recommendation	Limited empirical research available on the effectiveness of alternate methods	<75% in favour of one method	Insufficient empirical evidence and agreement to support a firm standard
	Methodological research demonstrates that one method has greater effectiveness	<50% in favour of that same method	Likely reflects expert knowledge that outweighs evidence from one study (e.g., study was flawed or not widely applicable)