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A Precautionary Approach to Welfare in Wild Fish Research and an Adaptable Framework to Inform Anaesthetic and Immobilisation Method Selection

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ABSTRACT

For researchers and professionals working with wild fishes, the decision to choose a particular anaesthetic or immobilization technique can be fraught with uncertainty and conflicting guidance over what method might be most appropriate for their research setting/context and the welfare of fish being handled. There are diverse perspectives relating to how researchers, ethicists, and other personnel invested in fish welfare approach this subject, including if/to what extent certain responses observed in fishes are informative about individual welfare states, or whether certain philosophical tools used in welfare-related decision-making (particularly the precautionary principle) are actually helping or hindering efforts to improve welfare from an evidence-based lens. Here, the authors provide overviews of some key elements of anaesthesia/immobilization technique selection and fish welfare more generally, and introduce a precautionary principle of the authors' own formulation relating to the complexities of refinement of research protocols involving wild fish. Next, the authors propose and describe a framework that can be used to help select the most suitable anaesthetic or immobilization technique for a given research effort in line with refinement of research methods. This framework also specifically incorporates consideration of complex realities inherent to many research projects on wild fish and allowances for critical context-specific considerations. The authors include discussions and examples largely from a North American (particularly Canadian) lens, but many of the core concepts illustrated are or may become applicable in other jurisdictions as scientific knowledge, laws, and ethics guidelines continue to evolve. The authors then explore the basis of the proposed precautionary principle and its connection to the framework in greater depth, along with some potential criticisms. The authors conclude with recommendations for future work relating to significant knowledge gaps and challenges relating to information access and consistency among researchers, governments, and other relevant participants or supporters of wild fish research.

KEYWORDS

Ethics; fish; handling; research; stress; welfare

Introduction

An overarching concept in animal research ethics guidelines in many jurisdictions is the so-called "Three Rs" of animal research, first put forth by Russell and Burch (1959): *replacing* sentient subjects with subjects that are non-sentient (or, potentially controversially, of "lower" sentience), *reducing* the number of sentient subjects used as much as possible, and *refining* research practices to minimize the negative effects of research on necessary sentient subjects. Curzer et al. (2016) proposed a fourth R, the proactive *refusal* to authorize or carry out a research project if the harm that is expected to occur is too high to justify doing the work. In many wildlife research scenarios, the replacement of a species is not possible because that species is directly necessary for the work (e.g., conservation/monitoring work, movement ecology). In such cases, efforts to minimize welfare harms in research design may therefore be focused more on the reduction of subjects (if upper sample size limits are not restricted by abundances or catchability) and refinement of methodologies.

In wild fish research, there is often a need to sedate, anaesthetize, or immobilize fish in projects

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involving extensive handling and/or invasive procedures. In line with the principle of refinement, anaesthetic or immobilization techniques selected for any given protocol are chosen primarily based on efficacy, safety (human, subject, and environmental), side effects, logistics of use, cost, and permissibility (Neiffer and Stamper 2009; Trushenski et al. 2013; Sloman et al. 2019). Debates on anaesthetic/immobilization technique selection typically include at least one, and often all, of those components. In particular, common elements of such discussions include logistical and "human-use" considerations (including legality and safety), fish welfare (see discussions below for definitions) during the administration of immobilization or anaesthesia, and fish welfare in the period following immobilization or anaesthesia. Some anaesthetics are more commonly used than others on account of being generally safe, effective, and legally approved in many – but not all – jurisdictions (e.g., tricaine methanesulfonate or MS-222, notwithstanding withdrawal periods; Topic Popovic et al. 2012). Yet, robust evidence of anaesthetic/analgesic efficacy and dose-response curves for many substances are still quite scarce and rarely have they been assessed in a wide range of fishes and under different environmental conditions (Chatigny et al. 2018a; Chatigny 2019). Alternatives to chemical anaesthesia such as electro-immobilization (a general term for practices involving the immobilization of fish using electric currents; Reid et al. 2019) and tonic immobility (immobilization inducible in many fishes by inverting them and holding them supine; Whitman et al. 1986) are also used in a number of settings. The variability and inherent subjectivity associated with method selection can therefore make it challenging for those looking to refine their practices to determine what method(s) may be most suitable for their particular context. In addition, those approaching research design from diverse disciplinary perspectives (e.g., applied wildlife research vs. ethics) may have very different approaches to deciding what method may be most suitable, or are/are not acceptable for a given set of circumstances.

In their review on fish research ethics, Sloman et al. (2019) discussed considerations relating to the selection and use of chemical anaesthetics, but electro-immobilization methods were only briefly mentioned. Specifically, Sloman et al. (2019) highlight electro-immobilization as controversial, first due to a paucity of data on the extent to which electric currents can render fish unconscious or induce analgesia, and second on the grounds that there is very high risk of injuring fish in the application of currents. The first point is true, though this could also be said for many chemical anaesthetics and future research may address such concerns for any immobilization technique as discussed below. The second point, however, relied on a single reference (Wargo Rub et al. 2014) that in turn concluded electro-immobilization was highly likely to injure fish based on only three other papers (Thompson et al. 1997; Redman et al. 1998; Gaikowski et al. 2001). Thompson et al. (1997) described injuries in response to electrofishing practices rather than electro-immobilization and, given when these works were written, none of them captured the substantial evolution of electro-immobilization equipment and practices over the past two decades. Indeed, some more recent works have found no evidence for elevated incidences of vertebral injuries following electro-stunning but encourage the tailoring of settings used to induce immobilization based on fish size and species to reduce risk of adverse effects (Vandergoot et al. 2011; Faust et al. 2017). Consequently, the topic of anaesthetic/immobilization technique selection in wild fish specifically is deserving of further review and discussion.

Here, the authors endeavor to provide a comprehensive approach for selecting anaesthesia and immobilization options in wild fish research *via* an adaptable decision-making framework, grounded in the use of ethics and empirical evidence to support identification, prioritization, and prevention or mitigation of welfare harms. The authors begin by briefly summarizing available options for the anaesthesia and immobilization of wild fish, reviewing conceptions of animal welfare, and the precautionary principle in this context. The authors propose their own formulation of a precautionary principle for the purposes of optimizing welfare outcomes *via* refinements in experimental design. Then, the authors present a framework for anaesthetic/immobilization technique selection, including a step-by-step exploration of the framework with reference to a variety of real-life examples and potential scenarios. Next, the basis and rationales for the authors' precautionary principle formulation are discussed in greater depth. The authors conclude with recommendations for future research and actions to facilitate knowledge sharing to help promote best practices in fish anaesthesia and immobilization. The authors do not provide in-depth syntheses for the use of any given anaesthetic agent or immobilization technique, as such efforts fall outside the scope of this work. The authors' synthesis of relevant information is instead focused on the types of information that should be considered in decision-making. Throughout this paper the authors identify potential syntheses that

could be conducted in the future (e.g., on the use of different techniques).

This work is intended to be accessible (and hopefully useful and actionable) to a diverse audience, including but not limited to research ethicists, fish and fisheries biologists, members of animal care and use committees including veterinarians and other aquatic animal health professionals, and wildlife management professionals. The authors realize that some perspectives will inevitably be missed, and that there is no "one-size-fits-all" solution to the challenges the authors explore below. The examples explored below are primarily based on scenarios encountered in Canada and the United States. The authors' perspectives and framework below are intended to be viewed as a step toward, and not a destination for, better welfare of wild fishes used in research. A core assumption here is that the authors start from a position of believing that animal research in general is necessary and can be performed in an ethically justifiable manner, a point contested by those subscribing to abolitionist views of animal use (i.e., that the use of animals, not just in research but in human society as a whole, is entirely morally unjustifiable and must stop; Wrenn 2012). The authors acknowledge too that there is debate surrounding the adequacy of current ethical standards and guidelines for animal research in many jurisdictions (Galgut 2015; DeGrazia and Beauchamp 2021), but leave those discussions for others. Also, it is important to differentiate between evaluating a project's justifiability in terms of its overall significance/benefits – relating to Curzer et al. (2016) concept of refusal – versus determining how a project deemed justifiable in general can best be designed and refined from a welfare lens. While these are both highly important considerations, this work is centered on the latter.

Broader considerations

Overview of available options

Many techniques for anaesthetizing or immobilizing fish are available after decades of research in these fields, and there is broad variation in the legal and ethical statuses of individual chemical agents or immobilization practices across jurisdictions (Ross and Ross 2009; Topic Popovic et al. 2012; Trushenski et al. 2013). Until recently, tricaine methanesulfonate (MS-222) was the only fully approved and listed anaesthetic or sedative in Canada for use on fishes that may be slaughtered for human consumption or released into the wild with the possibility of later consumption by humans. MS-222 approval status in Canada is technically only for salmonids (outside of research) and requires that fish be held for a minimum of five days in water at least 10 °C or warmer before release or slaughter (Health Canada 2010). MS-222 is also the only fully approved anaesthetic in the United States, where it is approved for aquatic ectotherms (including fishes); however, a 21-day holding period before slaughter or release is required (Trushenski et al. 2013; FDA 2023a). Aqui-S®, a 50% isoeugenol solution, has recently been approved in Canada for salmonids with a pre-slaughter/release period of 6 degree-days. MS-222, isoeugenol, and benzocaine are approved for fish anaesthesia in Norway (Norwegian Medicines Agency [Statens legemiddelverk] 2022). Researchers in Canada, the U.S., and elsewhere may also be permitted to use other chemical substances that are not fully approved or are under investigation for approval for research purposes (e.g., "Investigatory New Animal Drugs" [INADs] in the U.S.), although substance-specific requirements such as prerelease holding periods may still apply (Trushenski et al. 2013).

Electro-immobilization may be a viable alternative to chemical anaesthesia when the latter is not feasible, appropriate, or legal in field settings. There are two primary methods of implementing electro-immobilization, which the authors refer to as "electroanaesthesia" and "electrostunning" (reviewed in Reid et al. 2019). In electroanaesthesia, weak currents (~4-40 mA as a rough estimate, depending on species, size, and current type) are passed through a fish typically held supine and at least partially out of water with direct electrode contact, such that the muscles are relaxed but the fish is immobilized as long as the circuit remains intact. Virtually instantaneous restoration of equilibrium and motor control is normally observed following removal of the current (e.g., Ward et al. 2017; Slagle et al. 2020), and certain surgical procedures can be conducted under proper immobilization if the site is not obscured by the electrodes. In electrostunning, fish are usually placed in a stunning unit and briefly exposed to a strong current based on higher voltage settings (e.g., ~3-20 s and ~100-200 V; more similar to settings used in electrofishing). Stunning is followed by a prolonged recovery period without electric currents applied where fish are incapacitated for several minutes depending on the duration of exposure and current parameters (e.g., Rous et al. 2015; Faust et al. 2017; Trushenski et al. 2018). Although electro-immobilization methods have become more commonly used in field settings when chemical anaesthetics are not viable,

there remain welfare uncertainties with respect to the extent of potential analgesia or unconsciousness these techniques may provide (discussed below in more detail).

When inverted on their dorsal sides (i.e., put in a supine position), many fishes including chondrichthyans, sturgeons, and some teleosts will exhibit tonic immobility (Whitman et al. 1986), a reflexive and reversible state of immobilization likely arising from the inhibition of motor pathways in the brainstem and spinal cord in vertebrates (Gallup Jr & Rager 1996; Yoshida 2021). Yet, tonic immobility in vertebrates can manifest in various forms (e.g., rigid vs. relaxed muscles) with different triggers and, within the Chondrichthyes class, appears to be a vestigial ancestral trait that serves no apparent modern function and has been independently lost in least five distinct lineages (reviewed in Gayford and Rummer 2025). As a result, it is not necessarily a consistent immobilization method in fish in terms of occurrence, mechanism, and/or efficacy. In field settings, tonic immobility tends to be used only in scenarios where other options are not feasible, such as when working with very large fishes (Kessel and Hussey 2015; McLean et al. 2020). In cold-water and polar fishes where behaviour and physiological recovery processes may take much longer than temperate or tropical species (Egginton 1994; Campbell et al. 2008; Brijs et al. 2019) or in regions with high predator burdens where any level of impairment in an animal upon release may promote post-release predation, tonic immobility might also be the most viable method in some circumstances. Because this method involves no plausible anaesthesia or analgesia, it is otherwise considered controversial with respect to certain facets of welfare.

The authors do not discuss hypothermic shock or live chilling as an anaesthetic or immobilization technique for the purposes of this paper, as the authors are unaware of any evidence of this being used outside of aquacultural or laboratory contexts (where it is typically either used for euthanasia or pre-euthanasia immobilization, or for anaesthesia of zebrafish in the lab) in wild fish.

Elements of fish welfare

"In animal and research ethics, conceptions of "welfare" are diverse but usually fit within specific, non-mutually exclusive categories of welfare definitions. These categories are usually referred to as "nature-based", "function-based" (also called "functioning-based"), or "feelings-based", depending on what measures or aspects of animal welfare are emphasized (Fraser et al. 1997; Huntingford et al. 2006). Nature-based conceptions of welfare focus on an animal's capacity to live as natural a life as possible, including being able to display the full array of behaviours and "experiences" that this may entail (Fraser et al. 1997; Huntingford et al. 2006). Function-based conceptions of welfare focus on the biological health of the animal and concern physiological stress, behavioural impairment, and capacity to respond to stressors (Fraser et al. 1997; Huntingford et al. 2006). Feelings-based conceptions of welfare are centered on potential or assumed emotions, psychological states, and other intangible or unquantifiable aspects of consciousness comparable to conscious human experiences (Fraser et al. 1997; Huntingford et al. 2006).

Another conception of welfare that is relevant to conservation science is "population welfare". This idea of welfare is less thoroughly developed than those discussed above, but broadly relates to the health and well-being of populations rather than focusing on welfare at the individual level. These concerns may be linked, such as large-scale anthropogenic pressures causing blanket harms at the individual level, and corresponding harm reduction strategies provide benefits to individuals within a population (Stephen and Wade 2018). Yet, there may also be real or perceived tradeoffs where actions intended to benefit a population are done with less regard for, or at the disproportionate expense of, the welfare of individuals (Paquet and Darimont 2010; Stephen and Wade 2018). Individual welfare harms may be unavoidable even in conservation research (e.g., capture stress), and some harms are often justified on the grounds of greater benefits to the population as long as they can be limited as much as reasonably possible. When populations are imperiled, it is important to learn more about their biology and ecology (e.g., diet, growth rates, critical habitat, movement patterns), and those types of information frequently require capture, handling, and surgery on individuals from those populations. In such cases, researchers can at minimum focus on evidence-based best practices to minimize welfare harms based on both the ethics standards and guidelines they must follow and the context-specific demands of their work (Sekar and Shiller 2020).

In general, much of the debate on animal welfare definitions takes place in the contexts of captivity and holding conditions in agriculture and aquaculture, where humans have direct and long-term control over animals' quality of life, or in recreational and commercial fisheries where fish welfare is directly impacted during processes such as capture, handling, and retention. Different conceptions of welfare may conflict

with one another or lead to different conclusions on the welfare status of an animal, depending on whether the animal in question is farmed, held in captivity, or living in natural environments as is often the case with fishes (Diggles et al. 2011).

For research involving the capture, brief holding, and immediate or rapid release of wild fish, however, the direct interaction period between humans and fish may be very short, and specific activities or procedures (e.g., capture, surgery) likely have the greatest influences on short- and long-term welfare. As an example, an experienced team angling wild fish for intracoelomic transmitter implantation surgeries would expect to have a fish on the hook, anaesthetized or electrically immobilized, tagged, sutured, and released upon recovery in minutes, not hours or days. In this type of situation, the induction and recovery times of the chosen anaesthetic or immobilization method are probably the main factors that would prolong holding duration. Yet, each component of such procedures can and will alter the physiology, behaviour, and potentially the survival of fish in the period following release (Davie and Kopf 2006; Musyl et al. 2009; Donaldson et al. 2011; Wilson et al. 2017). The use of multiple metrics of welfare to provide a comprehensive assessment of the potential impacts of human activities on fish has been proposed as an optimal solution to measuring fish welfare (Huntingford et al. 2006; Sloman et al. 2019). Multi-metric approaches can allow us to quantify the impacts of research interventions on fish using different conceptions of welfare and make evidence-based assessments accordingly, provided that the metrics used can be measured empirically and interpreted unambiguously.

A controversial topic in fish welfare is the question of whether/to what extent fish may be capable of experiencing pain (Rose 2002; Sneddon 2003; Braithwaite and Boulcott 2007; Rose et al. 2014; and see Key 2016 and ensuing commentaries). A recent review and rigorous appraisal of the evidence and arguments made around the fish pain hypothesis falls outside the scope of this work; however, Mason and Lavery (2022) and Diggles et al. (2024) have done so recently and in great detail, and the authors (of this work) refer interested readers to these works for nuanced perspectives on the matter. Despite ongoing uncertainties and debate, the question of fish pain remains an inextricable part of the dialogue on refining current research practices in fish and fisheries research. Here, the authors attempt, with care, to place it within the broader question of how to minimize the negative effects of research (with particular focus on anaesthesia and electro-immobilization) on wild fish as one of many considerations with varying sizes and qualities of evidence bases that can be used to inform decisions. If the research community cannot agree on whether the question of fish pain has been answered satisfactorily (or if this can even be done), then the community is challenged to find a way to hold productive discussions on topics that will still inevitably relate back to this uncertainty.

Precautionary principles

The precautionary principle has been invoked in debates on potential consciousness and pain in various taxa (Bradshaw 1998, 1999; Jones 2016; Birch 2017). In essence, this is an advocation for a "better safe than sorry" approach to addressing uncertainty with respect to mutually exclusive, morally significant outcomes or scenarios; it is better to try to prevent possible consequences of the more negative or costly outcome (e.g., in terms of health, welfare, economics) than it is to assume such risks are not real unless "proven" to exist (Cameron and Abouchar 1991; Bradshaw 1999; Resnik 2003; see Appendix II in Sandin 1999 for more definitions). Theoretically, the precautionary principle may be useful when there is a plausible threat or negative outcome, and the precautions taken in response are reasonable (Resnik 2003; Petrenko and McArthur 2010). For example, many welfare harms to fish undergoing surgical procedures are plausible, if not inevitable. There are also many reasonable actions that researchers can take to minimize welfare harms, such as refinements to handling and surgery techniques (Harms and Lewbart 2000; Wagner et al. 2011). But, in many cases, the precautionary principle is often vague and criticized over concerns such as: uncertainty over how/when/to what extent it should be applied; whether it leads to the misdirection or wasting of time and resources; whether it enforces unreasonable burdens of proof or hinges on unfalsifiable hypotheses; who decides (and how) the way risks are evaluated and presented; what constitutes an appropriate degree of precaution or response to perceived risks; being ambiguous when risk tradeoffs and paradoxes cannot be eliminated; and, whether the principle itself actually offers any meaningful guidance whatsoever (discussed in Foster et al. 2000; Tubiana 2001; Van den Belt and Gremmen 2002; Resnik 2003; Sunstein 2003; Turner and Hartzell 2004, among others).

In political and regulatory contexts, debate exists over the use of the terms "precautionary principle" vs. "precautionary approach". This distinction stems from differences between the language preferred by regulators in the United States versus regulators in

the European Union (Peel 2004; Dinneen 2013), and this debate does not appear to be discussed in the literature on animal welfare research where "precautionary principle" seems to be used consistently (regarding occurrence, not definition or formulation). For the purposes of this work, the authors follow recommendations from Hartzell-Nichols (2013) where, rather than considering "the precautionary principle" as a single overarching or authoritative entity, more narrow and specific renditions – precautionary *principles* – can be crafted to provide guidance that can best be tailored to individual contexts or scenarios. In other words, the authors are not concerned with contrasting definitions of "*the* precautionary principle" vs. "precautionary approach". Instead, the authors employ a precautionary approach in general (meaning that the authors approach decisions in animal research and welfare through a precautionary lens that is adaptable and mindful of context-specific considerations). Precautionary principles, in turn, are nested within this broader precautionary approach as specific tools to help with decision-making. In other words, when the authors refer to precautionary principles in this work (including author), these are decision-facilitating tools created and used in a "bottom-up", rather than a "top-down", manner.

In consideration of the above, the authors have formulated a specific rendition of the precautionary principle to provide useful guidance for optimizing welfare outcomes in wild fish research. This precautionary principle underpins the anaesthetic/immobilization technique framework that the authors introduce below, and the authors explore the basis for this formulation in more detail later on. In this text, the authors refer to "the authors' precautionary principle" to refer to this specific formulation to clearly differentiate between it and "the precautionary principle" more generally. The authors' precautionary principle is as follows:

> If wild fish welfare will be adversely affected by a research intervention, wherein such impacts are known or plausible and capable of being mitigated based on current scientific evidence, experimental protocols must be refined with reasonable attempts to minimise the incidence or severity of unnecessary or avoidable negative welfare impacts, prioritising the reduction of impacts that most severely affect post-release welfare and the need to follow context-specific legal/jurisdictional, practical, and ecological considerations.

An adaptable framework

The framework (summarized in Figure 1) is adaptable to, and encourages, new developments in fish welfare research so that subsequent decision-making efforts can be better supported by more robust evidence bases. Fundamentally, the framework is structured as a series of questions to help refine (as part of the Three Rs framework) research projects from a welfare lens by guiding the selection of appropriate anaesthetic or immobilization techniques within the context of a given project. The five questions central to the framework concern: (1) novelty of the technique, (2) validation history, (3) analgesic or anaesthetic properties, (4) post-release welfare considerations, and (5) considerations of necessity. The authors explore the dimensions and considerations of each step of the framework below. As previously noted, this framework does not deal with broader considerations such as the ethical justifiability of a research protocol as a whole, nor does it purport to resolve all refinement questions or attempt to usurp the authority of ethics oversight bodies in determining what techniques may or may not be permissible. This framework is a tool to provide an organized and standardized approach to part of the overall refinement process and to highlight research needs and gaps deserving of further attention.

Running through the framework

Novelty of the technique (step 1)

Is the proposed anaesthetic or immobilization technique novel in the sense that it has not or seldom been tested on fish? Exploring novel options is key to identifying anaesthetics or techniques that overcome the shortcomings of currently available options. If the proposed work involves piloting or validating a new anaesthetic or immobilization technique not yet studied in fish, then it is recommended that the work proceed if there are reasonable grounds to suspect that the substance or technique may show promise (e.g., precedent as an anaesthetic in other vertebrates). Approval of such work should be conditional on the collection and detailed reporting of all relevant data concerning fish, substances/equipment, and doses/electric current parameters used; relevant environmental parameters such as water chemistry; responses measured, with reference to both intentional and unintentional effects (including reference to stages of anaesthesia if applicable); and any relevant safety concerns for fish and humans (e.g., compare reported and absent information in Iversen et al. 2003; Matsche 2011; Jones et al. 2012 for chemical anaesthetics, and in Hudson et al. 2011; Trushenski and Bowker 2012; Rous et al. 2015 for electro-immobilization). Such efforts should ideally

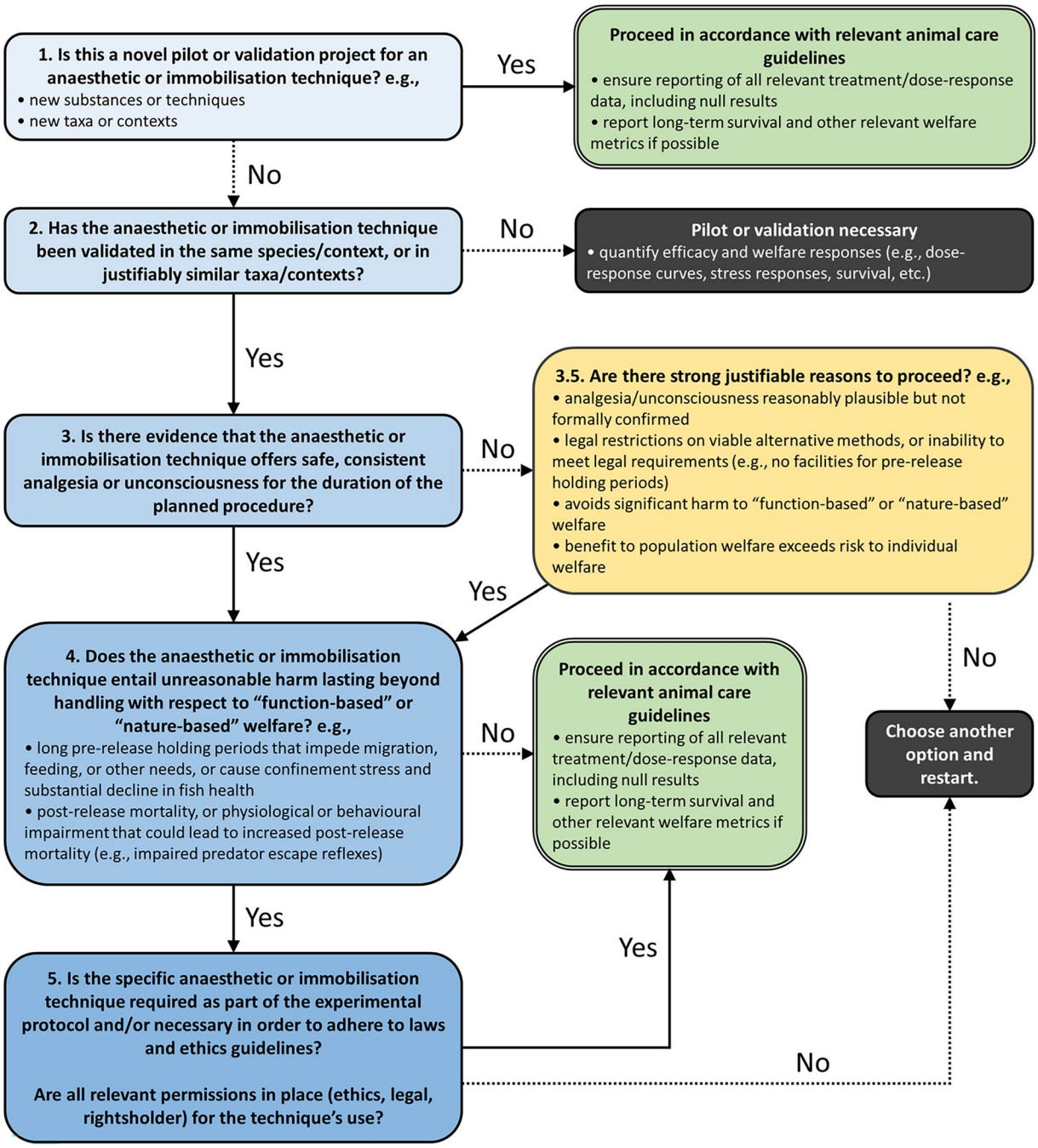

Figure 1. The proposed anaesthetic and electro-immobilization method selection framework, summarized. Steps begin in the top left ("1. Is this a novel pilot…"), branching to further steps or recommended guidance depending on the answers to each step. This framework is a refinement tool for research protocols that are (more broadly) ethically justifiable, subject to approval by an animal care committee; that is, projects in question cannot violate jurisdictional laws or ethics guidelines on the care and use of animals in research.

involve a pilot-scale experiment and have clear stopping rules should predetermined endpoints deemed overly adverse occur. Researchers should begin by consulting any available dose-response data from other taxa and similar substances/techniques, and proceed with novel drug/technique testing using as few animals as possible in the initial stages (Martins et al. 2019). It may be best to perform initial pilot work on species with well-studied stress responses under a variety of conditions (e.g., rainbow trout *Oncorhynchus mykiss*). Negative results should be reported as well; this is important to the research community and to reduce unnecessary replication by others.

Examples of novel techniques that would require validation at this step might be newly discovered chemical substances with anaesthetic potential, or new

electro-immobilization devices that administer currents to fish in new ways. If the proposed method is not novel, then persons working through the framework may move on to step 2.

Validation history (step 2)

Has the anaesthetic or immobilization technique been validated in the same or reasonably similar species and contexts? Is anything known regarding the mechanism of action (e.g., pharmacology for chemical agents) in the same or reasonably similar species and contexts? Any substance or technique selected to anaesthetized or immobilize fish should have some valid precedent in the form of evidence of effective use in similar taxa and contexts. By effective use, the authors refer here primarily to a method capacity to act as an anaesthetic or immobilizing agent; other concerns such as the physiological impacts of the method or jurisdictional constraints are covered in later steps. This question of validation history is related to the question of novelty in step 1, but differs from the latter in that it applies to substances that may be very well-studied and demonstrably effective in some cases (i.e., not novel per se) but that still require extra validation informed from previous knowledge when moving to new circumstances. For instance, clove oil has been used effectively in a number of species and there are good examples of dose-response curves at least for certain stages of anaesthesia for some fish such as rainbow trout *Oncorhynchus mykiss* (Keene et al. 1998), European sea bass *Dicentrarchus labrax* and gilthead sea bream *Sparus aurata* (Mylonas et al. 2005), and Atlantic salmon *Salmo salar* (Iversen et al. 2003). Effective doses for surgical-plane anaesthesia using clove oil can vary substantially and are commonly reported in the range of ~20-100 mg/l (Taylor and Roberts 1999; Kroon 2015; Priborsky and Velisek 2018), and lethal concentrations for a given species/life stage at a given exposure time may also be highly variable across species and possibly overlap with effective doses for other species (Taylor and Roberts 1999; Gullian and Villanueva 2009). Effective doses also may vary with environmental conditions, temperature, or salinity, for example. Additional validation may thus be required if the method is being applied in a sufficiently distinct context where currently available information on doses in tested taxa may not be confidently transferable.

Some chemical anaesthetics have been reviewed in sufficient detail to provide at least an overview of validated doses and, occasionally, additional details such as reported induction/recovery times for a number of species (e.g., Topic Popovic et al. 2012; Priborsky and Velisek 2018). At least for some common anaesthetics such as MS-222 or clove oil (or its derivatives), many effective doses for inducing anaesthesia appear to overlap, or at least do not differ substantially when exposure times can be easily adjusted, across reasonably diverse ranges of fish taxa (n.b. the vast majority of fishes studied here are teleosts, and other taxa are underrepresented in this literature). This overlap suggests that lower commonly used doses may likely translate well as starting points for many species on which those anaesthetics have not yet been tested. Researchers planning to work with taxa that are more physiologically unique and/or associated with atypical or extreme environmental factors (e.g., Channichthyidae, which have unique physiological adaptations for life in very cold Antarctic waters) should consider performing or integrating pilot evaluations of effective doses and exposure times, starting at lower concentrations and considering valid behavioural responses and physiological stress markers if possible, before proceeding with the main experiment or study. Long-term holding or monitoring is not always possible for wild fish and may be harmful, but at minimum there should be evidence of full behavioural recovery using standardized metrics (e.g., reflex action mortality predictors; Davis 2010; Raby et al. 2012) before release along with detailed reporting of symptoms and possible adverse effects associated with anaesthesia. If any physiological stress and/or longer-term welfare metrics can be reported, this should be highly encouraged.

For electro-immobilization, the same principle of validation applies but is somewhat more complicated due to a greater diversity of adjustable current types, settings, and methods/devices, as well as a generally poor understanding of how (and when) electro-immobilization techniques work on a physiological level. The authors' recommendations for validation of electroanaesthesia and electrostunning differ based on the unique considerations associated with each method.

Electroanaesthesia is typically performed using low voltage constant DC emitted from either a commercially developed device or an ad hoc setup made by researchers, increasing slowly from the lowest setting/no current (e.g., Hudson et al. 2011; Abrams et al. 2018; Reid et al. 2024a). Having current control in real-time is a significant advantage in that outputs can be easily adjusted or stopped at any time. Constant DC outputs are uniform in strength without variable parameters associated with pulsed DC (e.g., pulse width, frequency, and shape), and are therefore, in

theory, straightforward to apply and test on new species. One potential challenge with novel electroanaesthesia testing on some species is that existing setups (generally involving direct contact with electrodes in the form of conductive gloves or pads) are primarily used on fish with fusiform or elongated body shapes. Such setups may not be suitable for fish that are heavily compressed laterally or vertically, or otherwise differing greatly from fusiform or elongated shapes, as these fish may not physically fit well in the setup. It is also possible that non-fusiform or elongated fishes may not respond as expected to current administration if there are biologically relevant interactions between body shape and the paths currents take through fish. The authors cannot provide an evidence-based assessment of this concern as the research community lacks an up-to-date understanding of the underlying physiology of electroanaesthesia, but the possibility deserves consideration. Another challenge is the need to ensure that evaluations and validations are performed for methods that have jumped straight into practical use. As an example, transcutaneous electrical nerve stimulation (TENS) units are used as simple, low-cost alternatives to constant DC electroanaesthesia devices. TENS units emit a biphasic pulsed current rather than constant DC, and have been used in place of existing validated devices with apparent efficacy for surgeries (Reid et al. 2022; Izzo et al. 2023). Importantly, however, most experiments using TENS units in this fashion have not specifically evaluated the quantity and quality of immobilization offered while controlling for concurrent tonic immobility induced by holding fish supine. Only recently have there been direct attempts to quantify the immobilization provided by TENS units vs. tonic immobility alone (Reid et al. 2024b; 2025), and results from these few investigations have not supported the conclusion that TENS units improve or supplement immobilization attributable to tonic immobility. The implication is that without further research in this area, attempting to induce electroanaesthesia with TENS units may risk adding stress from exposure to currents with little to no conceivable benefits to fish or researchers. Or, perhaps TENS units may be useful in providing immobilization beyond tonic immobility alone, but their capacity to do so varies across species and other factors. The authors simply do not have enough data to address this in depth, and future research should prioritize filling these gaps through necessary validation steps.

Electrostunning is typically employed using brief exposure to pulsed DC at higher voltages than electroanaesthesia (e.g., Trushenski et al. 2012; Rous et al. 2015; Faust et al. 2017). There is little evidence to date on the incidences of internal injuries such as vertebral fracture and hemorrhaging along the spine during pulsed DC electrostunning, as there is not a wide body of literature in this field assessing injuries across many species, sizes, and current parameters. Moreover, accurate assessment of many of these injuries is highly invasive and costly, requiring euthanasia of fish for CT scans and/or visual inspection of the spinal cord. Nonetheless, rates of reported injuries following pulsed DC electrostunning have been found to range from negligible/indistinguishable from natural or congenital injury rates to highly probable (Gaikowski et al. 2001; Vandergoot et al. 2011; Faust et al. 2017). Risks of vertebral injuries and hemorrhaging from electrostunning may therefore vary as functions of species, size, current parameters (e.g., pulse specifications), and orientation of the fish with respect to the electric current, as they do with pulsed DC electrofishing (McMichael et al. 1998; Snyder 2003; Dolan and Miranda 2004; Culver and Chick 2015). Mortality rates following electrostunning tend to be very low or nil (Trushenski et al. 2012; Trushenski and Bowker 2012; Faust et al. 2017), suggesting that potential injuries are usually limited to sublethal impacts. In general, the authors recommend that electroanaesthesia be given preferrable consideration over electrostunning due to the latter's greater intensity. It is generally accepted that fishes with larger, stronger bodies and/or high numbers of vertebrae are likely more susceptible to injuries from electrofishing (Dolan and Miranda 2004; Clément and Cunjak 2010); it is reasonable to assume this principle applies to electrostunning as well. In the presence of better alternative methods, it may also be prudent to avoid performing electrostunning on fish that are likely at high risk of current-induced injuries (e.g., large anguillids; Reynolds and Holliman 2004).

If there is reliable evidence or valid justification that an anaesthetic or immobilization technique is suitable in inducing effective anaesthesia or immobilization, persons working through the framework may proceed to step 3. If the method cannot adequately immobilize or anaesthetize fish even in the absence of additional handling or surgical procedures, then it is not likely to be of much benefit to the researchers or fish and should not be used.

Evidence of analgesia or unconsciousness (step 3)

Is there evidence that the anaesthetic or immobilization technique induces analgesia or unconsciousness in a safe and reliable manner? Empirical evidence of

a chemical anaesthetic or electro-immobilization technique's ability to provide analgesia or induce unconsciousness is undoubtedly a good thing. There is little reason to prefer an option that has not been shown to provide unconsciousness or analgesia, if all other considerations are equal (e.g., safety for fish and humans, efficacy, legal status, etc.). It is important to assess (1) whether anaesthesia or analgesia is needed for a protocol, and if so, (2) the duration for which anaesthesia/analgesia (or even immobilization) can be maintained. Some procedures may simply require that a fish be kept still (e.g., taking body measurements) and an added anaesthetic or analgesic component is not necessary. If anaesthesia or analgesia is necessary, however, then the method must be capable of providing it consistently for as long as the procedure requires. An intracoelomic telemetry tag implantation surgery may take less than 3–4 min, while other surgeries such as those involving cardiac output work may require much longer periods of consistent anaesthesia (e.g., up to 1 h; Webber et al. 1998). Electrostunning options typically only immobilize fish for several minutes following the brief application of a stronger pulsed current, and so can likely be ruled out straight away for procedures where repeated exposures and/or interruptions of the procedure are intolerable.

At the same time, the quantity and quality of neurophysiological evidence to support conclusions on the anaesthetic or analgesic properties of many substances and electro-immobilization techniques is often suboptimal, especially in fishes. The lack of knowledge in this area is a common concern raised by researchers and ethics committee members when discussing electro-immobilization (and fairly so), but this concern appears to be shared less often for chemical anaesthetics that may have been assumed to render fish unconscious based on behavioural symptoms without confirming substance capacities to elicit analgesia or anaesthesia at particular doses. As more informative research methods become available to help us address knowledge gaps in fish welfare, it is important that all prospective methods be held to the same fair standards of investigation and approval.

The efficacy of anaesthetic or immobilization techniques in inducing analgesia or unconsciousness may be assessed in fishes using electrophysiological methods. For chemical anaesthetics where the mechanisms of action are relatively well-understood, there might also be insights from literature on anaesthetics tested in other taxa (e.g., amphibians) given that the requirements for nociception, analgesia, and anaesthesia are generally present and conserved in vertebrates (Walters and Williams 2019; Williams et al. 2019). For example, the anaesthetic properties of MS-222 and benzocaine have been validated through stimulus tests of sensory and motor nerve functions conducted under each anaesthetic or a non-anaesthetic paralytic agent in partially intact physiological preparations of *Xenopus laevis* tadpoles (Ramlochansingh et al. 2014). Machnik et al. (2018) evaluated the anaesthetic efficacy of MS-222, 2-phenoxyethanol, and Aqui-S® using electrophysiological recordings in Mauthner cells in the fish brainstem. Carefully chosen behavioural responses may be useful too; Jones et al. (2012) quantified dose-response relationships for the analgesic effects of morphine in rainbow trout using behavioural responses to electric shock stimuli.

Electroencephalography (EEG) is another method that can be used in fish for various purposes (e.g., Laberge and Hara 2003; Cho et al. 2017), and researchers may be able to use it to measure the success of anaesthesia stunning, or incapacitation by quantifying responses to sensory stimuli (e.g., Robb and Roth 2003; Lambooij et al. 2015). Sensory responses recorded through electrophysiological means such as EEG may be more reliable indicators of whether an anaesthetic effect has been achieved than outwardly observable indicators such as equilibrium loss, because sensory stimuli may still be processed in the central nervous system even if faculties such as motor control have been impaired (Kestin et al. 2002; Retter et al. 2018; Bowman et al. 2020). The changes in brain activity over the course of anaesthesia with MS-222, and the presumed "sleeplike" unconsciousness that seems to manifest based on the gradual cessation of neurological responses to a strobe light stimulus, have been detailed in rainbow trout by Bowman et al. (2019) using a noninvasive EEG setup.

Despite the value of insights from electroencephalography experiments in fish, EEG data must be interpreted with a degree of caution. The presence or absence of responses to sensory stimuli appears to be a reliable indicator of whether effective anaesthesia or unconsciousness has been induced, but fundamentally cannot tell us anything about what a fish might be "experiencing" or even to what extent a fish may be conscious or self-aware in the first place. Indeed, two major sticking points in welfare debates are (1) whether consciousness (however defined) and facets of consciousness such as pain exist in the taxa in question, and (2) if so, is it even possible to measure these in a robust and replicable manner, based on current understandings of consciousness and hypotheses of the physiological mechanisms underlying psychological pain (Michel 2019; Browning et al. 2020;

Melloni et al. 2021; Diggles et al. 2024). The few EEG experiments involving the administration of eugenol (the main active ingredient in clove oil) appear to support the hypothesis that eugenol can induce a sleeplike unconscious state (Cho et al. 2017; Shin et al. 2022). Clove oil itself appears to be at least an effective muscle relaxant in fish (Fujimoto et al. 2018); however, Sladky et al. (2001) observed more reactions to a needle puncture in fish anaesthetized with eugenol than MS-222 and, paradoxically, reaction incidences were more frequent at higher doses of eugenol (but less frequent at higher doses of MS-222) in red pacu *Piaractus brachypomus*. Anaesthesia with eugenol also elicited approximately threefold increases in heart rate in lake trout relative to a control or electroanaesthetised fish during exposure (Funnell et al. 2025). Barbas et al. (2021) administered clove oil to juvenile tambaqui *Colossoma macropomum* and recorded epileptiform brain activity patterns similar to those induced by pentylenetetrazole, a seizurogenic drug. The authors (of this work) cannot say whether these differences in the anaesthetic or excitatory potential of clove oil or its derivatives are products of dose-dependent or species-dependent effects, or ingredients present in clove oil apart from eugenol, but the possible implications for clove oil as an anaesthetic in fish are unclear. Fish exhibiting epileptiform brain activity are also considered to be unconsciousness (Lambooij et al. 2010) much as humans are unconscious during generalized epileptic seizures (Cavanna and Monaco 2009). Would an epileptiform type of unconsciousness from one anaesthetic truly be worse than a "sleeplike" state induced by another, if fish exposed to either option exhibit similar, transient physiological stress responses, no substantial injury rates or toxicological effects, and can resume normal behaviours and functions in short order?

Finally, dose and timing are critical components of anaesthesia and analgesia: how long does a drug require to take effect, and for how long does this effect last? These questions are relevant not only to general anaesthetics but to local anaesthetics or analgesics as well. Research on fish analgesia as a whole is limited, and the most well-understood fish analgesics in terms of efficacy and pharmacology are opioids such as morphine, which are highly unlikely to be distributed for field research on wild fish (Chatigny et al. 2018a). At least in Canada, some animal care committees (ACCs) are recommending or requiring that lidocaine, a local analgesic, be administered to fish subjected to invasive procedures under electro-immobilization. Yet, presentations of dose-response relationships for most local anaesthetics and analgesics (e.g., Jones et al. 2012) in fish are sparse or missing entirely, and there are currently no detailed, evidence-based references or guidelines that could allow researchers to make informed choices about what (if any) doses and times-to-effect may induce adequate analgesia in various species of wild fish under different conditions. Moreover, lidocaine may have untoward side-effects (e.g., numbing of swimming muscles) that must be balanced against its analgesic effects (Chatigny et al. 2018b). Therefore, use of lidocaine in field contexts would not be supported at this step within the framework.

For electro-immobilization techniques or anaesthetics that have not been proven to induce unconsciousness or analgesia (as opposed to simply immobilization or muscle relaxation), the question of what fish may or may not be capable of "feeling" remains open in the absence of reliable electrophysiological and neurological evidence indicative of analgesia or the absence of consciousness. The authors are currently unaware of any data on analgesia or alteration of conscious states in fish under any electro-immobilization protocols. While currents could very well be nociceptive stimuli in at least some cases, possible explanations for the mechanisms of electro-immobilization have been proposed relating to epilepsy where unconsciousness could certainly be plausible, precluding any potential psychological experiences (Sharber and Sharber Black 1999). Given these uncertainties, the authors (of this work) treat electro-immobilization techniques in a similar manner as potential anaesthetics whose anaesthetic or analgesic properties have not been tested or validated. The authors therefore refrain from making assumptions about whether or not specific current types and settings used in electro-immobilization have analgesic or nociceptive effects.

If an anaesthetic or immobilization technique is found to offer reliable, consistent analgesia or unconsciousness that is adequate for the purposes of a research project, then persons working through the framework may proceed to step 4. As discussed, however, there is a scarcity of evidence on this topic for many chemical anaesthetics and all electro-immobilization techniques. Also, analgesia or unconsciousness alone does not make an option suitable if it entails potentially serious risks to fish welfare beyond the immediate term. A lack of evidence for analgesia or unconsciousness should therefore not automatically lead to rejection of the method in question, but should reinforce the need to consider why that method is being preferred for a particular project and if another method might be a better choice (Figure 1, step 3.5).

While it may appear that this step is redundant in the framework as passing both 3 and 3.5 leads to the same considerations, the inclusion of 3.5 accounts for the possibility of some researchers choosing one particular method over others for legal or cultural reasons or when longer-term fish welfare is of equal or greater focus. Consider a situation where researchers are looking to perform a tagging surgery on some fish just before a critical migration, and where some of the fish might be caught and consumed by humans in the post-release period. If MS-222 is the only fully approved chemical anaesthetic in that jurisdiction and a drug clearance time like that of Canada (5 days) or the United States (21 days) must be applied, they may fail to pass step 4 if they do not have adequate facilities to hold anaesthetized fish in isolation at the field site and/or there is substantial risk of adverse welfare impacts in captivity. Captive fish may be unable or unwilling to feed, prone to injuries from repeated contact with holding tank or pen surfaces, more susceptible to infection and disease, and more likely to die in or following periods of captivity (Portz et al. 2006; Donaldson et al. 2011). A technique permitting the immediate release of fish (e.g., electroanaesthesia) might be optimal in terms of post-release welfare. But, what if a superior anaesthetic was approved in their jurisdiction that demonstrably induces unconsciousness, entails only mild, transient stress responses, is easily and safely prepared and disposed of, and would allow for immediate release of fish? In that case, any remaining differences would likely fall beyond fish welfare such as the need to respect and follow local laws and values. If there are no such obligations and the only advantage that electroanaesthesia might offer is speed and convenience without tangible benefits to fish welfare, that alone would not justify a preference for that method, and the researchers would fail to pass step 3.5. Note that this example is purely hypothetical, as in reality no such ideal anaesthetic has been identified/approved in most if not all jurisdictions, but some day this may change.

Post-recovery welfare (step 4)

Does a particular anaesthetic or immobilization technique entail significant harms to welfare after the fish has recovered? Research interventions on wild fishes will inevitably lead to at least some physiological stress. Virtually any capture method used in research will lead to the release of catecholamines and corticosteroids into the circulatory system, along with subsequent changes in secondary and tertiary stress indicators (Melloti et al. 1992; Pankhurst and Sharpels 1992; Chopin et al. 1996; Lawrence et al. 2018). Stress can be beneficial in the short term by allowing an organism to respond physiologically and behaviourally to potential threats and re-establish homeostasis once the threat has passed or resolved (Chrousos 1998; Barton 2002). Conversely, exposure to repeated or chronic stressors can be deleterious to an individual's health (e.g., energy reserve depletion, increased susceptibility to disease and infection), development, reproductive potential, and capacity to respond to subsequent exposures to stressors (Iwama 1998; Schreck 2000; Barton 2002). Stress in fish can be quantified using a variety of physiological and behavioural indicators, yet the utility and relevance of many stress markers vary depending on their specificity in if, when, and how they change in response to different types of stressors (reviewed in Sopinka et al. 2016).

There is a reasonably large body of literature evaluating electro-immobilization and fish welfare through changes in primary, secondary, and tertiary stress markers following immobilization that are often, but not always, similar to changes in the same stress markers following chemical anaesthesia (e.g., Trushenski and Bowker 2012; Balazik et al. 2013; Soltani and Mirzargar 2013; Prystay et al. 2017; Trushenski et al. 2018; Reid et al. 2022). The insights gleaned from experiments like these are valuable, but often not directly comparable because of potentially confounding differences in experimental methodologies and setups. For example, there may be other concurrent stressors such as surgeries; species, population, or strain differences; variation in water temperature, water quality, holding conditions, stress history, and shifting baselines; or, incomparable time courses used to measure responses. In addition, whether demonstrably effective anaesthetics can suppress physiological stress responses in fish, or if the anaesthetics themselves may induce physiological stress responses, may depend on the dose, exposure duration, timing of exposure with respect to other stressors (e.g., capture), the specific mechanism of action of the substance in question, and biological sources of variation such as species (Barton and Peter 1982; Thomas and Robertson 1991; Olsen et al. 1995; Small 2003; Zahl et al. 2010). For the purposes of this framework, attempting to provide full reviews of the stress literature for various anaesthesia and electro-immobilization would be neither practical nor particularly informative.

It is also beyond the scope of this work to provide a comprehensive evaluation of the strengths of evidence bases on the great number of possible

indicators of welfare in fish in response to the diverse range of anaesthetic and immobilization practices available today. Some welfare indicators can be easy to interpret (e.g., mortality, physical injuries), but many can be less straight-forward or informative about the welfare statuses of fish if measured alone and without context provided by other relevant indicators (e.g., heat shock proteins; Sopinka et al. 2016). In essence, how one assesses whether an anaesthetic or immobilization method will lead to significant harms post-recovery will depend on what metrics of harm are prioritized based on their reliability and evidence bases, and what harms are designated as significant. There is of course no one-size-fits-all approach, but the following is a non-exhaustive starting point of relevant welfare indicators and impacts quantifiable in pilot/validation projects and, potentially, field applications too:

- Mortality and/or permanent physical injury as a result of anaesthesia or immobilization;
- Severe or long-lasting physiological stress putting the fish at risk of further injury, infection/disease, or mortality upon release, or constituting high metabolic recovery costs that would cause a significant energetic penalty;
- Reflex and behavioural impairment lasting beyond a viable prerelease recovery time;
- Failure to feed, and changes in growth and condition in the period following exposure to the anaesthetic or immobilization technique.

Here, it is also important to consider the impacts of drug clearance times and mandatory prerelease holding periods in fish. Clearance times and holding requirements vary by substance and jurisdiction and can impact wild fish welfare due to stress and other harms associated with prolonged confinement (Portz et al. 2006; Donaldson et al. 2011). If laws or ethics guidelines require that a technique be followed by isolated holding time before release, then that holding period is intrinsically linked to the method of interest wherever those jurisdictional requirements apply. Any negative effects on fish welfare due to extended holding are therefore attributable to the choice of that method (Cooke et al. 2016). To date, there is little research on the added confinement stress of drug clearance periods in wild fishes that must eventually be released. Absence of this information is likely due to a lack of incentive or need, because ethics approval can often be obtained for alternative methods without such requirements (e.g., electroanaesthesia or immediate-release chemical options, gastric tagging of migrating fish without anaesthesia). Nevertheless, the authors raise this point as ethics guidelines and jurisdictional requirements continue to be updated, and circumstances might arise where researchers must attempt to hold wild fish for many days before releasing them. In such cases, expensive and/or complex ad hoc holding solutions will likely be required, and there will probably be little relevant data available beforehand to estimate how the fish might fare during prerelease holding.

Another type of situation may arise where the recommendations of ethics committees and jurisdictional requirements come into conflict with one another, if ethics approval hinges on researchers using a particular method that is associated with legal obligations that are ultimately detrimental to fish welfare (Cooke et al. 2016). Some of the authors of this work experienced this first-hand while attempting to get clear guidance for a project involving evaluating post-surgery stress responses in juvenile lake sturgeon *Acipenser fulvescens* following different anaesthesia or immobilization treatments (Reid et al. 2025). In short, the authors in Reid et al. (2025) intended to include a treatment with lidocaine administered locally to fish that were all intended to be released into the wild months later. After some back-and-forth with their institutional veterinarians and the federal government, the authors (of that work, not of this work) learned several interesting things: (1) despite common practice in field research, from the Canadian Food Inspection Agency (CFIA)'s perspective clove oil is apparently not an immediate-release option and requires a drug clearance time of 21 d; (2) any fish receiving any amount of lidocaine cannot be (re-)released into the wild and must be euthanized; and (3) the attending veterinarian would be held legally responsible for any violations "detected by the CFIA". The authors (of that work) elected to forego the lidocaine treatment because, as valuable as the associated physiological and behavioural data would have been, it would not justify their euthanasia. Although anecdotal, this example illustrates some very real problems likely unmentioned in the primary literature. Many of the rules and guidelines on drug use in wildlife may not be common knowledge or easily accessible, and it is possible (at least in principle) that ACC members could put themselves in legally precarious positions as a result of well-intentioned welfare recommendations. Additionally, there is a need for better communication and transparency between researchers, ethics authorities, and governments when research and scientific knowledge generation outpace the bureaucratic processes of reviewing and updating drug approval

statuses. Keeping with Canada as an example, Health Canada's list of drugs that are approved for fish in Canada does not appear to have been updated since 2010 at the time of writing (Health Canada 2010).

It is not possible to provide specific decision-making guidance on when the stress of a technique becomes unacceptable, because this is highly subjective and there are no clear thresholds for when changes in a given physiological stress marker become too much. As a guide, the answer to this step of the framework needs to be determined through consultation and agreement between the researchers and their animal care committee. One general way of approaching this is through the following question: "In considering avoidable and anticipatable impacts on the stress and survival of fish in the context of a given research project, do all fish have a strong likelihood of surviving the procedure and making a full recovery from imposed stress?". In other words, is a wild fish likely to be able to go back to their pre-capture "normal" state (if not prevented from doing so by necessary and justifiable aspects of the research protocol)? This is not to say that optimal outcomes must be observed for every single fish in protocols using dozens or hundreds of individuals – often that is not a realistic expectation even for work not involving invasive procedures – but each individual used in the research must have a strong likelihood of making such a recovery. Here is where insights from pilot and validation work on stress and survival (as discussed in steps 1 and 2) may be especially valuable in shaping the decision-making processes.

Necessity (step 5)

There may, however, be instances where a particular anaesthetic or immobilization technique is required even if it is not considered optimal under the above considerations. The authors include concerns about the necessity of particular methods here in the framework as this can be important even if other methods are available and better from a welfare lens. If an earlier experiment involving the use of a particular anaesthetic or immobilization method must be replicated (i.e., if there are concerns about the validity or integrity of the original experiment's data and findings), then that same method must be used as part of the replication effort if permissible under applicable jurisdictional laws and ethics guidelines. Researchers may also be required by law to use a particular method, either because it is the only permissible method in their jurisdiction, or because all other viable methods are not permissible in their case. If rightsholders and project partners have valid objections to the use of a particular anaesthetic (e.g., if Indigenous communities may not want to administer drugs to harvestable fish on their traditional territories), those objections are part of the considerations of necessity to be reflected on in this step.

A defence of the proposed precautionary principle & framework

Here the authors discuss the basis for their formulation of the precautionary principle and its links to the framework in greater depth.

Basic structure & rationales

There are often four key elements of precautionary principles: the "threat dimension", the "uncertainty dimension", the "action dimension", and the "command dimension" (Sandin 1999). The "threat dimension" refers to the existence and severity of a given threat or adverse outcome (e.g., "if [threat] can occur"). The "uncertainty dimension" reflects the degree to which one can or cannot say that a threat would or could occur (e.g., "which [may/could/will] happen as a result of […]"), based in turn on how (in)conclusive scientific evidence may be on the subject. The "action dimension" refers to which precautionary measure(s) is/are recommended in response to the threat in question, coupled with the "command dimension" (italicised in the following example) as a semantic measure of how strongly the action dimension is enforced or regulated (e.g., "[insert actions] *[must/should]* be performed"). These dimensions can be brought together to create specific formulations of the precautionary principle as a conditional statement, the strength and precision of which depends on the strength and precision of the language used in the weakest/vaguest dimension(s) (Sandin 1999).

Because there is no one right way to formulate a precautionary principle, there is no "best" version for any given context. Still, the authors argue that their formulation above is useful and defensible when applied toward experimental design and ethics approval processes. This precautionary principle is also flexible, as it is adaptable to new knowledge and evidence on welfare impacts and mitigation strategies that are currently poorly understood or have no unanimously accepted answers, but may in the future.

The framework presented herein was designed with the authors' formulation of the precautionary principle in mind, which contained the four dimensions described above in addition to a novel dimension the authors refer to as the "priority dimension":

Threat dimension: The threat dimension in the authors' formulation of the precautionary principle reads: "If wild fish welfare will be adversely affected by a research intervention". This is intentionally broad; as previously discussed, welfare can be described in multiple non-mutually exclusive ways, each deserving of due consideration given the state of available evidence and knowledge gaps. It is also assumed to be true given the near-ubiquitous nature of stress and potential for adverse events in any research project on wild fish involving the use of anaesthesia or immobilization. Fundamentally, the goal is to minimize negative impacts on fish, whether in the form of adverse physiological stress, behavioural impairments, risk of infections and disease onset, or other quantifiable metrics that fit into various conceptions of welfare. In addition, this is already in line with current best practices in experimental design, where focusing on identifying and proactively minimizing all negative welfare effects also benefits research and data quality by reducing potentially confounding effects (e.g., physiological stress from capture and non-lethal sampling influencing subsequent fisheries discard/release mortality estimates; Cooke et al. 2013).

Uncertainty dimension: The uncertainty dimension comes next: "wherein such impacts are known or plausible and capable of being mitigated based on current scientific evidence". This statement is somewhat open to interpretation, but the authors emphasize that only unrealistic welfare impacts can/should be eliminated in the context of a particular research intervention. For example, a snorkeling survey might disturb fish and elicit flight responses, with the adrenaline release and other transient physiological changes that naturally follow, but disturbed fish are unlikely at risk of acquiring an infection or having impaired reflexes merely from the passage of a snorkeller. In contrast, surgical procedures entail greater risks up to and including reduced chance of survival, in which case fewer impacts can be considered unrealistic. It is also important to specify that for the guidance that follows, the impacts must be somehow mitigable in a realistic way (otherwise the guidance is less useful). For instance, adrenaline release, which occurs in response to many stressful stimuli within seconds (Sopinka et al. 2016), could be considered an unmitigable impact because of its ubiquity and rapidity as a physiological response to potential stressors. On the other hand, there is an abundance of concrete empirical evidence on how fishes' physiological states, stress responses, and abilities to resume natural behaviours and survive can change following handling, anaesthesia or immobilization, and surgery, as well as how researchers can mitigate such changes to the benefit of quantifiable nature- and function-based welfare metrics (e.g., Chomyshyn et al. 2011; Wagner et al. 2014; Gutowsky et al. 2015; Jepsen et al. 2015).

Action + command dimensions: The action dimension and the command dimension (italicised) follow: "experimental protocols *must be* refined with reasonable attempts to minimize the incidence or severity of unnecessary or avoidable negative welfare impacts". Essentially, this part of the statement echoes the "refinement" element of the three Rs as a mandatory part of experimental design. The command dimension is strong as researchers designing and conducting experiments must bear the responsibility of refining their experimental approach (including anaesthetic/immobilization technique selection) as much as realistically possible. The authors deliberated considerably over the most appropriate language. "Must be" is clearly in line with existing language used in ethics guidelines in reference to the three Rs and related concepts (e.g., see CCAC (Canadian Council on Animal Care) 2005 §3.1) to emphasize the obligation that researchers have to animal welfare. At the same time, there was concern that the strength of a "must be" command dimension might be misinterpreted so as to unnecessarily preclude many research projects where certain negative welfare impacts cannot be helped, regardless of the recognition and guidance offered in the rest of the principle. Another option was "should be", which might be more flexible and better clarify the need to truly consider each proposal on a case-by-case basis, however that could be misconstrued as an effort to loosen the need to adhere to the three Rs framework.

When working with wild animals, some welfare impacts relating to capture, handling, and release are simply unavoidable – short-term increases in physiological stress markers and altered behaviour, for example (Sharpe et al. 1998; Brydges et al. 2009)

– while some are necessary as part of experimental treatments or study designs. Some impacts of anaesthesia or electro-immobilization qualify as necessary when these are required to reduce the stress of a research intervention, provide immobilization for the safety of fish and researchers, and/or are themselves the focus of the research. The use of the phrase "with reasonable attempts to minimize" is intentional. It is often impossible to identify and implement every single possible precaution to minimize the myriad ways in which welfare might be adversely affected during a research project, or the ways that welfare threat mitigation measures might conflict with one another. It is also not possible to provide an all-encompassing definition of what constitutes "reasonable attempts" at minimizing welfare harms and doing so would be counterproductive to the need to avoid vague or overgeneralized guidance; rather, this should be decided on a case-by-case basis through dialogue and understanding between researchers and their ethics committees. The action dimension above therefore aims to strike a balance between the need for researchers to do the best that they can and the need to recognize that, in wildlife research, many factors that can affect animal welfare are simply beyond researchers' control (e.g., nature). In other words: are researchers working with wild fish able to make the case that they have considered the most likely ways that fish welfare might be impacted as a result of their protocols, and have they taken reasonable steps to plan for the prevention or mitigation of those harms whenever possible given any potential context-specific constraints?

Priority dimension: The authors end with an extra dimension of the precautionary principle to address potential tradeoffs and conflicts between welfare mitigation strategies, which the authors call the priority dimension: "prioritizing the reduction of impacts that most severely affect post-release welfare and the need to follow context-specific legal/jurisdictional and ecological considerations." The added priority dimension aims to address concerns about precautionary principles leading to vague or contradictory guidance. This may be the case if feelings-based welfare concerns require solutions coming at the expense of nature- and/or function-based welfare, or vice versa. Consider a wild migrating fish about to undergo a telemetry transmitter implantation surgery, after which the fish must be released as soon as possible for the sake of its own health and reproduction success, and also data collection purposes. Prioritizing a feelings-based approach to welfare would likely lead to selection of a validated general anaesthetic (e.g., MS-222) for the duration of the surgery. Yet, handling time from capture to release should be minimized as much as possible because even without surgery, longer periods in captivity can lead to significant sublethal stress (which may reduce the probability of successful reproduction) and potentially lethal consequences to fish in such scenarios (Donaldson et al. 2011). For this reason, an anaesthetic requiring a long prerelease holding period such as MS-222 is impractical and potentially harmful in this type of research setting (Woody et al. 2002), and would conflict with nature- and function-based welfare considerations. Conversely, an option such as electroanaesthesia may entail greater uncertainty regarding feelings-based welfare during surgery, but ultimately lead to better outcomes in terms of fish health, survival, and reproductive output.

There is one final but critical component of the priority dimension above. It allows us to recognize that there can be significant legal, social, and/or management considerations specific to individual research projects that may conflict with blanket approaches to fish welfare recommendations. Again, the uncertainty is not *whether* researchers should try to improve their experiments from a welfare lens, but to what extent this can reasonably be done, and what exactly counts as "unnecessary or avoidable" with respect to the underlying research objectives and applicable "context-specific legal/jurisdictional and ecological considerations". For example, in Canada, Indigenous communities are increasingly restoring their capacities as fully self-governing and self-determining states in the wake of historical and ongoing colonialism and oppression, including greater participation and leadership in human research and research ethics (Hayward et al. 2021). Indigenous nations leading or participating in research projects on their traditional territories may very likely have their own perspectives on what is ethical and respectful to wildlife and nature, and could object to the use of chemical anaesthetics, for instance, on fish, for reasons

that could extend to food safety and sovereignty. Conflicting viewpoints on animal welfare between western animal rights perspectives and Indigenous communities are not new (e.g., Kymlicka and Donaldson 2015), but attempts to force Indigenous rightsholders in conservation and management to comply with official guidance of ACCs are immoral and likely illegal and/or unconstitutional (disregarding Indigenous sovereignty and inalienable rights protected under Section 35 of the Constitution of Canada), perpetuating ongoing legacies of colonialism and oppression. It is imperative that western animal ethics authorities are open to considering such nuances, and willing to make allowances for protocols that may include deviations from the standard recommended "best practices" if there are reasonable legal and/or ethical grounds to do so.

Linking the authors' precautionary principle & framework

In the context of anaesthesia and electro-immobilization, these methods themselves may pose possible threats (e.g., if a method can easily or unpredictably lead to overdosing) or have threat-mitigating properties (e.g., reduced stress). Both cases may be identified during validation or pilot projects (see steps 1 and 2). The inclusion of all facets of fish welfare in the authors' precautionary principle is incorporated in steps 3, 3.5, and 4 of the framework; elimination of an anaesthetic or immobilization at any of these stages would be in line with reasonable attempts to "minimize the incidence or severity of unnecessary or avoidable negative welfare impacts". The uncertainty dimension and the priority dimension underscore the possibility of continuing to step 4 through step 3.5, due to the importance of prioritizing known and measurable aspects of welfare that have greater implications for the overall health and ultimate fate of the fish over potentially obscure or currently unfalsifiable welfare concerns. The action dimension and the priority dimension both entail recognition for context-specific considerations, including what constitutes "reasonable attempts" at minimizing unnecessary welfare impacts (to be determined on a case-by-case basis through dialogue between researchers and their institutional ethics committees) and the need to adhere to certain laws or other essential obligations that might be at odds with welfare concerns but cannot be ignored (framework, step 5). Because the flexibility for case-by-case considerations is accounted for elsewhere in the authors' formulation of the precautionary principle, the command dimension ("must be") remains strong and underlies the need for firm cutoff points in the framework (e.g., choosing another option in the event of reaching and failing steps 3.5 or 5).

Final recommendations & conclusions

There are many opportunities for future research to improve the research community's understanding of fish welfare, including the filling of some longstanding knowledge gaps. As a starting point, the authors suggest that it may be beneficial to create a standardized set of guidelines or criteria for evaluating novel anaesthetic or immobilization techniques in fish, creating fair and even standards to which prospective methods may be held. Such guidelines may yield "scores" for how well individual anaesthetics or immobilization techniques are understood, with scoring metrics covering key topics such as efficacy, the extent to which dose-response curves have been studied in model species under controlled conditions, whether the underlying mechanisms of action have been established, and whether possible secondary effects (including the incidence and possible causes of adverse responses) have been explored. Even some relatively well-studied electro-immobilization methods and chemical anaesthetics do not necessarily have established mechanisms of action and/or pharmacokinetics in fish (e.g., Vibert 1963 vs. Sharber and Sharber Black 1999; Zahl et al. 2012), despite their fundamental importance to exploring safe and effective applications as well as anatomical and physiological differences that limit the translatability of pharmacology from other taxa (e.g., mammals) to fish (Toutin 2010). The authors are not aware of any jurisdictions with formal guidelines or approval statements in place for any form of electro-immobilization as a distinct practice from electrofishing, though this will be included in the upcoming edition of the Canadian Council on Animal Care's fish ethics guidelines. The authors hope that this subject becomes thoroughly and thoughtfully adopted in animal care guidelines as evidence bases continue to grow.

A centralized, open-access database of tested anaesthetics and electro-immobilization methods to which researchers can submit original or extracted data (e.g., dose(s)/parameters used, species tested, relevant water quality and chemistry data if available, induction and recovery times, behavioural symptoms if applicable, source publication, etc.) would also be beneficial. Again, the reporting of negative results is a crucial part of establishing a comprehensive evidence base.

The closest equivalent of which the authors are aware is the Phish-Pharm database (Reimschuessel et al. 2005; FDA 2023b). Phish-Pharm summarizes some pharmacokinetics data for various substances including some anaesthetics in fishes, but is not necessarily an ideal or comprehensive reference point for individuals looking for dose-response data or other information specifically relating to validation and efficacy of anaesthetic agents or immobilization techniques. A review or report delineating legal requirements and applications of various substances in certain jurisdictions with respect to relevant governing authorities may be valuable for researchers, ethics approval committees, and policymakers given the current lack of clarity regarding some guidelines on fish (e.g., whether clove oil or adjacent products can be used as an immediate release anaesthetic in Canada or not). Such work might also investigate opportunities to improve communication and logistics in drug review and approval processes for jurisdictions where the pace of drug reviews greatly lags behind the paces of scientific investigations into potentially viable anaesthetic options and drug approval processes in more efficient locales.

In summary, the authors have discussed some high-level elements of welfare and ethical discussions pertaining to scientific research on wild fishes. The authors have proposed a novel formulation of the precautionary principle in an attempt to reconcile the need for precautionary approaches to minimizing welfare harms and promoting best practices with at least some of the genuine concerns about potential challenges and undesirable outcomes that may result from incautious or overbroad invocation of precautionary approaches. The authors put forth a step-by-step framework to serve as an anaesthetic or immobilization method selection tool for researchers working with wild fish, based on the research community's best understanding of how to effectively and realistically refine research protocols so as to minimize the incidence and severity of avoidable welfare harms to fish. The adoption of this framework would help facilitate research into anaesthetic and immobilization techniques that in turn can provide feedback on their efficacy and help future users navigate through the workflow more easily. The authors did not attempt to address or contribute to broader ongoing debates surrounding many important topics that naturally fall outside the scope of the work and are best left to others (e.g., whether precautionary principles can/should be applied as moral versus epistemic versus rational rules; Petrenko and McArthur 2010). The ideas the authors proposed above are intended to generate helpful discussion and guidance, not to try and serve as "right/correct" solutions. Ultimately, the authors hope the ideas laid out in this work form an informative and useful step toward improving the welfare of wild fishes used in research.

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CRediT: **Connor Reid**: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing; **Jonathan Midwood**: Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing; **Christopher Vandergoot**: Conceptualization, Writing – original draft, Writing – review & editing; **Ernest Stevens**: Conceptualization, Investigation, Writing – original draft, Writing – review & editing; **Steven J. Cooke**: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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