REVIEW

On the Electroimmobilization of Fishes for Research and Practice: Opportunities, Challenges, and Research Needs

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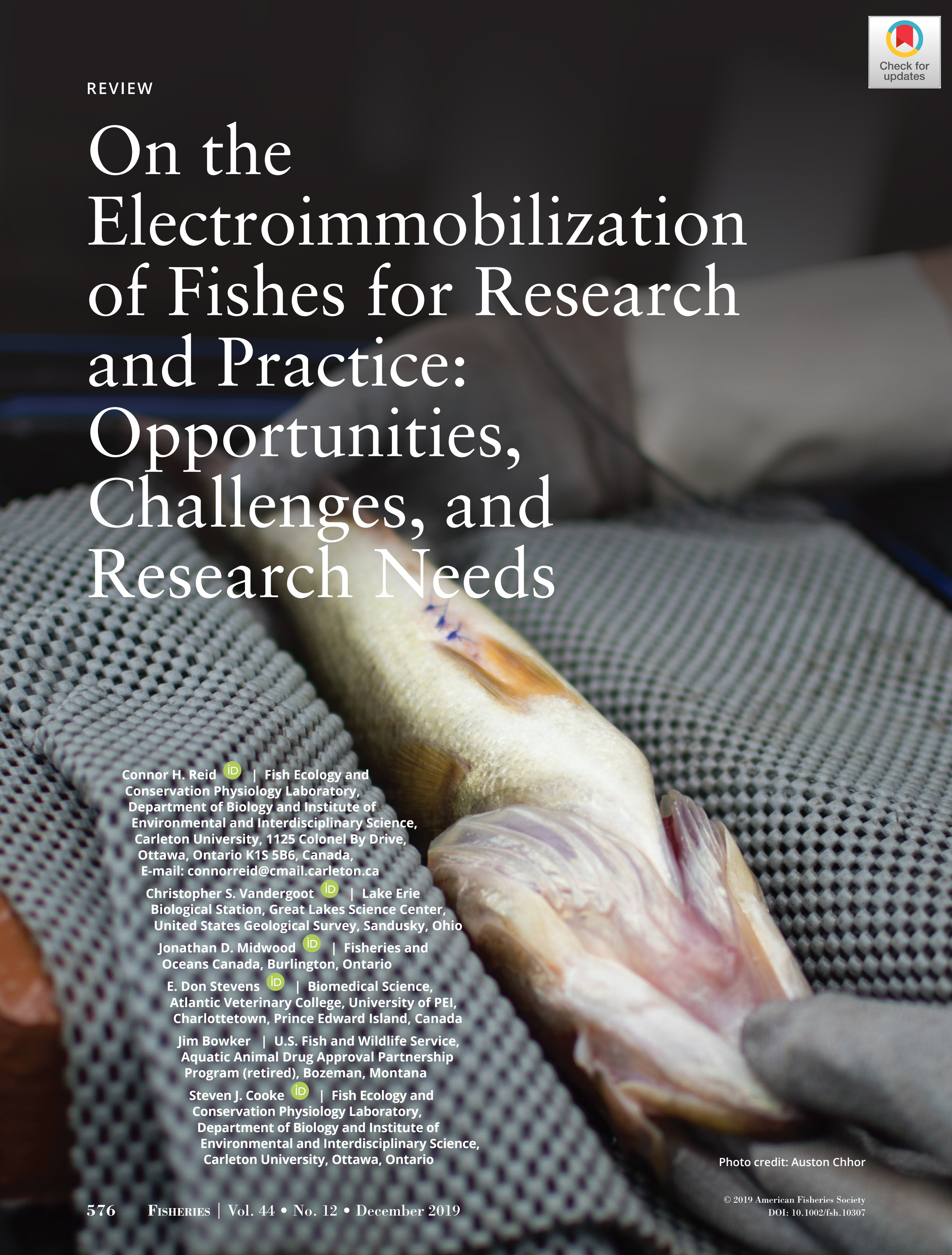

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DOI: 10.1002/fsh.10307

As a result of growing demand for immediate-release sedatives in fisheries research, electroimmobilization has been receiving increasing attention due to its superior induction and recovery times and practicality, particularly under field conditions. However, a review of electroimmobilization and its role in fisheries science has not been previously conducted. Here we describe and differentiate the various forms of electroimmobilization and attempt to standardize relevant terminology. We review the known efficacy of electroimmobilization of fish and summarize the current available knowledge on this topic while identifying major knowledge gaps. Although more information is needed to determine optimal forms and settings for different species, life history stages, and environmental variables, electroimmobilization is a useful tool for fish handling that equals or surpasses the capabilities of chemical sedatives without exacerbating (and sometimes reducing) the negative consequences associated with chemical sedatives and fish handling practices more generally.

INTRODUCTION

Data collection in fisheries science frequently requires the safe acquisition and handling of live fish as well as the use of both invasive (e.g., surgical implantation of transmitters, nonlethal tissue sampling) and non-invasive sampling procedures (e.g., measuring body size). These activities can induce stress (e.g., from capture and/or handling) and cause injury (e.g., if a fish is dropped), which individually or collectively can have negative consequences for the physiology, health, and welfare of fish (e.g., Barton and Iwama 1991). Immobilization techniques (prevention of movement, typically utilizing various chemical compounds) are frequently employed to help minimize the negative consequences of handling fish (Trushenski et al. 2013) and ensure researcher safety (particularly when working with large fishes).

Traditionally, chemical sedatives/anesthetics such as MS-222 (tricaine methanesulfonate), carbon dioxide, or clove oil have been used to facilitate fish handling (reviewed in Ross and Ross 2008). These techniques are still frequently used, despite the assorted logistical challenges associated with their use, which include: lengthy handling times while waiting for drugs to take effect; stress on experimental subjects associated with the metabolic effects of anesthetics; enforcement of proper chemical handling practices; lack of clarity or understanding regarding the legal status of use; and extended post-exposure recovery and substance withdrawal periods (Trushenski et al. 2012b, 2013). In recent years there has been increased demand for immediate-release approaches that allow fish to be safely released or consumed immediately following recovery (i.e., also referred to as “zero withdrawal;” Schnick 2006; Trushenski et al. 2013). Electroimmobilization techniques are an appealing substitute with potential advantages over chemical sedatives that will be explored here in further detail.

Electroimmobilization (see Box 1 for a thorough description) has been considered a potential alternative to chemical anesthesia for many years (e.g., Kynard and Lonsdale 1975). However, a review of the efficacy and safety of electroimmobilization, as well as an effort to summarize current knowledge and identify knowledge gaps on this topic, has not been previously conducted. The purpose of this review is to: (1) assess the effectiveness of electroimmobilization techniques for the safe handling of live fish in fisheries science; (2) compare the behavioural, physiological, and fitness impacts as well as the logistics of electroimmobilization techniques with those of chemical sedation; and (3) summarize current available research on electroimmobilization techniques while identifying knowledge gaps and areas of future research. We begin by providing a brief overview of how low doses of electricity have been used in humans and non-fish vertebrates to emphasize the work on the topic and precedence for using such tools in clinical and therapeutic contexts. We acknowledge that the terminology associated with research in this area has been inconsistent, so we also define and differentiate key terms (Box 1).

INSIGHT FROM HUMAN AND NON-FISH VERTEBRATE APPLICATIONS

Electrosedation, electroanesthesia, and electrotetany (see Box 1) have been used in humans and other non-fish vertebrates for medicinal purposes and in the farming of livestock. Research on the use of electrosedation on humans as replacements for chemical anesthetics during surgeries and as therapeutic treatments was common throughout much of the 20th century, but declined in the 1970s and 1980s due to major developments in chemical anesthetics (Francis and Dingley 2015). The electrical stimulation of nerves for analgesia (“pain” relief) is seen in modern physiotherapy with mixed results using devices such as transcutaneous electrical nerve stimulation (TENS) units (DeSantana et al. 2008; Vance et al. 2014; available from pharmacies without prescription) and in dentistry for pain management and muscle spasm relief (Quarnstrom 1992). There are also procedures such as deep brain stimulation (Arsenault et al. 2015) that attempt to use electricity for the treatment of various neurological disorders. Beyond use in humans, electricity of various forms has also

BOX 1

Terminology: In fisheries science the terms “sedation,” “anesthesia,” and “narcosis” have occasionally been used synonymously and/or inconsistently (Trushenski and Bowker 2012), and explicitly stated definitions for these terms are often not provided. The terminology recommended in this review is roughly based on symptomatic definitions supplied by Ross and Ross (2008), wherein anesthesia is defined as, “a reversible, generalized loss of sensory perception accompanied by a sleep-like state induced by drugs or by physical means,” and sedation as, “a preliminary level of anesthesia, in which the response to stimulation is greatly reduced and some analgesia is achieved but sensory abilities are generally intact and loss of equilibrium does not occur.” The terms “narcosis” and “anesthesia” have been considered synonymous. This review will use the terms electrosedation and electroanesthesia (as with the respective terms from Ross and Ross 2008; the former may be considered to be a lesser or preliminary stage of the latter). Electrosedation is a state of immobility from muscle relaxation due to low-voltage electricity, characterized by partial loss of equilibrium and reactivity to other stimuli. At higher voltages, full loss of equilibrium and reactivity to other stimuli is observed; this we refer to as electroanesthesia. Further increases in voltage may induce electrotetany, a state of immobility from contracted muscles. Electrosedation, electroanesthesia, and electrotetany are typically associated with very fast recovery times. Alternatively, fish may be immobilized for a prolonged period with initial tetany and a delayed, sleep-like recovery period potentially spanning several minutes (Cowx and Lamarque 1990). This non-instantaneous recovery from tetany distinguishes this more intense form of immobilization from electrotetany, and so we refer to it herein as electrostunning. In short, electrosedation, electroanesthesia, electrotetany, and electrostunning are all forms of electroimmobilization that occur along an increasing gradient of voltage (though depend on current type/parameters as well).

been used in veterinary practices for both pain management and therapeutic applications, though this has also seen mixed results in part due to lack of knowledge of appropriate electrotherapy parameters and protocols across a wide array of real life scenarios (Baxter and McDonough 2007). Complete immobilization via electricity has been used to facilitate the slaughter of livestock (Grandin 2013) although its use in mammals remains controversial (see American Veterinary Medical Association 2008). In other words, while many people tend to view electricity as a hazardous or noxious stimulus, this is not always the case, and there is no reason to believe that humans or non-human mammals are the only animals capable of experiencing beneficial effects from the safe application of electricity in a controlled setting and for specific purposes.

BACKGROUND AND THEORY OF ELECTROIMMOBILIZATION

Electricity in its various forms has been used for many decades on wild fishes and at aquaculture facilities. Aquaculturists use electroimmobilization to stun (i.e., to inducing unconsciousness; Robb and Roth 2003) fish prior to slaughter or spawning, whereas field biologists typically use it to temporarily immobilize fish during handling procedures. Despite its widespread use, the exact means by which various forms and strengths of electricity affect fish are not well-understood. The biological mechanisms by which electric currents can sedate, tetanize, or stun fishes is presumably based on the same mechanisms underlying electrofishing. However, no recent work has been conducted in this area. The following is a brief summary of the relevant physiology as examined in detail by Cowx and Lamarque (1990). This information is often cited in the electroimmobilization literature, however some of the physiological explanations offered by research cited therein (e.g., Vibert 1963) are erroneous and merit re-evaluation by modern experts. When exposed to constant direct current (cDC; constant polarity), the nerve elements are facilitated (“activated”) if the anode (+) is positioned at the body cell end of a fish’s nerves (i.e., head facing the anode) or inhibited if the cathode (-) is positioned at the body cell end of a fish’s nerves (in general, taxonomic- and orientation-dependent effects of electric currents on neurons seem to exist more broadly in animal nervous systems; Müller 1970). When a fish faces the anode, electricity will induce (in order of increasing voltage) electrosedation, electroanesthesia, electrotetany, and then electrostunning. When a fish faces the cathode, electrosedation is not observed, and electrotetany and electrostunning occur at lower voltages than when the fish faces the anode (n.b., author observations corroborate the existence of some orientation-dependent variation in responses to cDC, though the full extent of this phenomenon should be elucidated in future work). Exposing a fish to pulsed direct current (pDC) may yield less predictable outcomes because of the immense variation in potential pDC settings (e.g., waveform, pulse frequency, and pulse duration). Electrosedation and electroanesthesia are unachievable with this pulsed current due to the intermittent nature of pDC that precludes chronic muscle relaxation. Instead, pDC induces electrotetany (at much lower voltages than those required by cDC) and, through more intense exposure, prolonged incapacitation, probably through synaptic fatigue and post-tetanic potentiation (lowered activation threshold lasting for a variable but prolonged time after the current ceases). Alternating current (AC) will induce electrostunning at sufficiently high voltages, but, because of the continuous anode/cathode switching, will not yield any polarity-induced effects (e.g., anodic/cathodic galvanotaxis).

A summary of the known benefits and challenges of electroimmobilization has been provided in Table 1. Currently, pDC and cDC are the most commonly employed current types for electroimmobilization. Once commonplace, use of AC has been largely abandoned after being found to be more hazardous towards fish than direct current (Ackerman et al. 2005).

CURRENT KNOWLEDGE AND GAPS

Known Effects of Electroimmobilization on Fish

Physiological Alterations

Most investigations of the physiological alterations of electroimmobilization concern blood chemistry and stress physiology. Plasma cortisol was not shown to differ significantly from a control when Bluegill *Lepomis macrochirus* were exposed to electroanesthesia (cDC) or electrostunning (pDC), though in Largemouth Bass *Micropterus salmoides* plasma cortisol was significantly higher among electroanesthetized individuals (Abrams et al. 2018). This suggests that taxonomic variation does not necessarily permit extrapolating cortisol responses from one species to another, even within the same family. Grass Carp *Ctenopharyngodon idella* electrostunned (pDC) at different voltages and durations of current exhibited

Table 1. Identified benefits and challenges of electroimmobilization based on the available literature. Much of the benefits concern advantages over chemical sedation, while the challenges may be shared with chemical sedation (e.g., “dosage” concerns) or may be unique (e.g., positioning).

Benefits	Challenges
Unlike chemical sedatives, no concerns over expiration/degradation or proper handling and disposal protocols	Failure to maintain equipment in proper working condition may lead to failure or risk of injury/death for fish and humans
Significantly shorter induction and recovery times than chemical sedatives; fish may be released immediately following removal from electric stimulus	Inappropriate application of electricity may result in insufficient electricity for desired effect, or internal injuries (e.g., spinal damage, hemorrhaging)
Better real-time control over application of electricity compared to chemical methods (easy to adjust “dosage”)	Fish must be positioned properly in electroimmobilization apparati (i.e., proper orientation, facing anode, adequate distance from electrodes, etc.)
May significantly reduce fish handling times, particularly during short (<5 min) sampling procedures	Lacking knowledge of appropriate electrical settings (e.g., current, voltage) for different fish species, life history stages, etc. and environmental variables (e.g., temperature, salinity)
A single electroimmobilization device is reusable many times over (as opposed to chemical sedatives that must be purchased regularly).	Certain electroimmobilization devices (e.g., electrostunning units) have greater initial “start-up” costs relative to chemical sedatives.

quick, short-lived increases in plasma cortisol and lactate, a gradual net increase in glucose, and mild variations in haematocrit and osmolality (Bowzer et al. 2012); similar findings were reported in Sunshine Bass *Morone chrysops* × *M. saxatilis* (Trushenski and Bowker 2012). Electric fish handling gloves designed for low-voltage electrosedation or electroanesthesia (cDC) yielded no significant differences in blood glucose, lactate, pH, or haematocrit compared with non-electric handling or control groups (Ward et al. 2017).

Side-by-side comparisons of blood chemistry profiles between electroimmobilized and chemically sedated fish have been conducted for a number of species, though there are visible inconsistencies across these studies' results. A decrease of plasma cortisol and increase in blood glucose relative to a control group was detected in Crucian Carp *Carassius carassius* that either underwent prolonged electroanesthesia (cDC; 1 h) or were anesthetized with MS-222 (100 or 200 mg/L for 1 h), though the decrease in cortisol was greater in the fish treated with MS-222 (Gao et al. 2014). On the other hand, an experiment on electroanesthetized (cDC) juvenile Atlantic Sturgeon *Acipenser oxyrinchus* found no significant differences in plasma cortisol levels between electroanesthetized and MS-222-sedated individuals (Balazik et al. 2013). This may be attributable to the difference in exposure time; the 1-h exposure in Gao et al. (2014) being much longer than what would be required in virtually any field procedure. Interestingly, Japanese Eels *Anguilla japonica* electrostunned (AC or pDC) at high (≥240V) voltages exhibited lower plasma cortisol within 3 min of immobilization than conspecifics sedated with MS-222 or 2-phenoxyethanol (Chiba et al. 2006). It is unclear whether this apparent reversal in relative plasma cortisol trends between electroimmobilized and chemically sedated fish is due to the type of current or level of electroimmobilization used, the species tested, environmental factors, or an interaction of these variables. A comparison of Sunshine Bass that were electrostunned (pDC) or sedated with CO_2, benzocaine, eugenol, or MS-222 found that all treatments exhibited a generalized stress response visible in haematological profiles, and the observed blood chemistry changes subsided within 6 h (Trushenski et al. 2012a). Johnson et al. (2016) reported higher plasma osmolality (associated with stress responses) in electrostunned (pDC) Pallid Sturgeon *Scaphirhynchus albus* than conspecifics sedated with MS-222 or eugenol, but, as with Trushenski et al. (2012a), most variation in tested individuals' haematological profiles dissipated within 6 h. Therefore, while the haematological response to electrostunning via pDC is typically consistent with that of a generalized stress response, the same response may not be elicited through electrosedation or electroanesthesia via cDC. Ward et al. (2017) failed to detect an effect of electroanesthesia (cDC) on secondary stress markers (blood glucose, lactate, pH, and haematocrit) in Largemouth Bass, whereas higher haematocrit levels (associated with stress responses) were observed in clove oil-sedated Common Carp *Cyprinus carpio* compared to electroanesthetized (cDC) individuals (Monsef Rad et al. 2016). Variation in the relative physiological effects of electroimmobilization compared with chemical sedatives therefore appears to be influenced by a number of factors including species and the form of electroimmobilization used, as well as the concentration of employed chemical sedatives.

Fewer studies have been conducted on the physiological effects of electroimmobilization using non-haematological metrics. The prolonged electroanesthesia (cDC) of Crucian Carp resulted in oxidative stress detected through elevated gene expression for several mitochondrial respiratory chain genes, antioxidant enzymes, and heat shock proteins (Gao et al. 2014). Conversely, electrostunned (pDC) adult Zebrafish *Danio rerio* did not exhibit symptoms of oxidative stress or significant differences in metabolic rate and mitochondrial performance with respect to a control group (Teulier et al. 2018). Because of the differences between these two experiments (species, electroimmobilization type, metrics used), it is difficult to pinpoint the exact cause of this apparent contradiction. Duryea (2014) investigated healing rates of a ventral incision in Gopher Rockfish *Sebastes carnatus*, which did not significantly differ between electrostunned (pDC) and chemically sedated fish.

Behavioral Impairments

Arguably the most commonly studied behavioural effects of electroimmobilization concern induction and recovery times. As the time required to induce immobilization and allow for recovery of fish is critically important from an animal welfare perspective, this is understandable. The diverse representation of fish taxa, life history stages, and electroimmobilization parameters in studies on induction and recovery times can make direct comparisons across studies difficult. It is generally agreed that induction of immobilization appears to occur immediately in fish at all levels of electroimmobilization (i.e., sedation, anesthesia, tetany, and stunning; e.g., Balazik et al. 2013; Keep et al. 2015; Abrams et al. 2018). Recovery times are less predictable, varying with species and electrical parameters (pulse, voltage, exposure time, etc.). For instance, higher voltages and longer exposure times were associated with longer recovery times in juvenile Sunshine Bass (Trushenski and Bowker 2012), but this relationship was not visible in Grass Carp tested using similar testing protocols and exposed to the same voltages and similar exposure times (Bowzer et al. 2012). The recovery times of Crucian Carp from low-voltage electroanesthesia (cDC) observed by Gao et al. (2014) were non-instantaneous (ranging from 3–7 s), possibly due to the fact that the fish experienced chronic symptoms from exposure to the electric current for 1 h (again, a much longer time than necessary or relevant for virtually any field procedure).

A relatively large body of research in electroimmobilization either focuses on or includes comparisons of induction and recovery times between electroimmobilization techniques and fish drugs. This body of research clearly establishes superior induction and recovery times as one of the primary advantages of electroimmobilization over chemical sedation. Faster induction and recovery times for electroimmobilized fish have been documented in Pallid Sturgeon (electrostunned [pDC]; Johnson et al. 2016), Atlantic Sturgeon (electroanesthetized [cDC]; Balazik et al. 2013), Gopher Rockfish (electrostunned [pDC]; Duryea 2014), Striped Bass *M. saxatilis* (electroanesthetized [cDC]; Jennings and Looney 1998), and Walleye *Sander vitreus* (electroanesthetized [cDC]; Vandergoot et al. 2011). It should be noted that recovery times may not differ significantly between electroimmobilized and chemically sedated fish in cases where electrostunning is induced at high voltages (e.g., Vandergoot et al. 2011 [pDC]; Prystay et al. 2017 [pDC]).

The six stages of anesthesia defined by Summerfelt and Smith (1990) currently serve as the primary reference for determining the desired level of "anesthesia" (typically Stage IV; total loss of equilibrium, muscle tone, spinal reflexes with slowed opercular rate) in many studies involving electroimmobilization (e.g., Vandergoot et al. 2011; Bowzer et al. 2012; Balazik et al. 2013; Kim et al. 2017). As a result, many electroimmobilization studies report induction times that are not instantaneous because the authors defined "induction time" to mean the time taken to reach Stage IV anesthesia rather than the time required to immobilize the fish. For example, the Grass Carp electrostunned (pDC) by Bowzer et al. (2012) were immobilized immediately; however, because induction criteria were based on the stages of anesthesia described by Summerfelt and Smith (1990) the reported induction times are described as averaging 0.6 min before "Stage IV" anesthesia was visually confirmed.

The effects of electroimmobilization on other behaviours are not as well documented, though there has been some focus towards impacts on migratory behaviours. The time required for electroanesthetized (cDC) Lake Sturgeon *A. fulvescens* and Shortnose Sturgeon *A. brevirostrum* to exhibit positive rheotaxis did not differ significantly from non-immobilized individuals (Henyey et al. 2002). Similarly, spawning migrations of Atlantic Sturgeon were not significantly affected or delayed by electroanesthesia and surgical telemetry tag implantations (Balazik 2015), nor were migrations of electroanesthetized Chinook Salmon *Oncorhynchus tshawytscha* and Coho Salmon *O. kisutch* significantly different from conspecifics sedated with CO_2 (Keep et al. 2015). A study of the effects of intracoelomic telemetry tagging with electrostunning (pDC) on migratory behaviour in Walleye found a significant increase in downstream travel time in more recently tagged fish, but this difference was not determined to be ecologically relevant nor was it specifically attributable to capture (electrofishing), electrostunning, or the surgical procedure (Wilson et al. 2017). It therefore appears that electroanesthesia is at least as safe as other techniques used in research on anadromous fish during their migrations. It is, however, important to validate the effects higher levels of electroimmobilization on these and other migratory species and to conduct further field evaluations of post-recovery behaviour to assess electroimmobilization's safety in conducting field research on migratory fishes.

Injury, Growth, and Survival

The risk of injury when exposing fish to electric current depends on current type and intensity, fish species and life history stage, and environmental (i.e., water quality) variables (Henyey et al. 2002; Ackerman et al. 2005; Zydlewski et al. 2008). Injuries (e.g., fractured vertebrae, haemorrhaging) tend to occur more often and more severely with AC (Ackerman et al. 2005), and in larger fish that move with greater body oscillations (Duryea 2014). Electrotetany and electrostunning are more likely than electrosedation and electroanesthesia to result in injury (Dolan and Miranda 2004). Overall, reported rates of injury from electroimmobilization are inconclusive and need to be investigated further. No injuries attributable to electric current were observed in the application of electrostunning (pDC) or electrotetany (cDC) to Walleye (Vandergoot et al. 2011) or in the application of electrostunning (pDC) to Lake Trout *Salvelinus namaycush* (Faust et al. 2017). Conversely, higher electricity-induced injury rates have been reported in electrostunned (pDC) Chinook Salmon (e.g., Zydlewski et al. 2008). In a simulated electrofishing experiment where fish were exposed to similar risk of injuries, significant differences in haemorrhaging and spinal injury across different taxa were observed (Dolan and Miranda 2004). Therefore, care should be used to select appropriate settings to minimize the risk of injury from electroimmobilization, particularly in those taxa sensitive to injury due to strong muscles and high body undulations (e.g., eels and salmonids).

Fewer studies have monitored the long-term effects of electroimmobilization on growth and there is insufficient long-term information to make any conclusive arguments. Short-term growth (i.e., 1 month post-treatment) of juvenile Rainbow Trout *O. mykiss* was not affected by electrosedation (cDC; Kynard and Lonsdale 1975), however the presence or absence of vertebral injuries that could have impeded growth was not evaluated. The growth of the offspring of migrating adult Chinook Salmon was found to be uninfluenced by their electrostunned (pDC) parents (Zydlewski et al. 2008). One approach to better assess the potential effects of electroimmobilization on growth would be to conduct long-term monitoring (e.g., analyzing lateral radiographs from x-rays [Duryea 2014]) on injured and non-injured juvenile fish as they grow into adulthood, an effort that might be best conducted under controlled laboratory conditions.

When appropriate protocols are used, short-term (~24 h) survival after electroimmobilization is high. 100% survival rates post-immobilization were observed in electrostunned (pDC) Common Carp, Bluegill, and Brown Bullhead *Ameiurus nebulosus* (Kim et al. 2017), Largemouth Bass (Trushenski et al. 2012b; Kim et al. 2017), Gopher Rockfish (Duryea 2014), Walleye (though one mortality was observed during the application of pDC; Vandergoot et al. 2011), and Grass Carp (minor injuries reported; Bowzer et al. 2012). One mortality was observed out of 90 electrostunned (pDC) Sunshine Bass (Trushenski and Bowker 2012). Long-term survival is rarely monitored, though a 100% survival rate after 22 days was reported in two-stage (i.e., two different currents applied consecutively) electrostunned (pDC) Lake Trout (Faust et al. 2017). Jennings and Looney (1998) also report 100% survival during a 14-day period post-electroanesthesia (cDC) in Striped Bass.

Variation in Instrumental Parameters, Fish, and Environmental Effects

Variation in voltage and duration of exposure is necessary and dependent on the nature of the experimental subject (i.e., species, size, etc.). Proper immobilization of fish with pDC requires a thorough understanding of the appropriate pulse frequency, duration, and duty cycle (fraction of time that pulses are being emitted, given as a percentage; calculated as 100 × frequency × duration/1000 ms with frequency and duration given in Hz and ms, respectively; Miranda and Dolan 2004). Higher duty cycles have been recommended for safer, less injurious electrofishing (Dolan and Miranda 2004) but information concerning the effects of duty cycles in other applications of electroimmobilization is lacking. Similarly, there is a need for an improved understanding of the short- and long-term lethal and sublethal effects of pulse frequency and duration (Vandergoot et al. 2011).

Fish size may affect the success and times of induction and recovery, though the ability to detect significant effects of fish size may depend on the metric used. For example, effects of fish size may be found to be a factor when measured as length (e.g., Prystay et al. 2017) or mass (e.g., Trushenski et al. 2012a). Fish volume, however, is a better predictor of required power to induce tetany than size or length (Dolan and Miranda 2004), and so might be a more appropriate size metric for variables such as induction and recovery times. Fish orientation is also important when electrostunning fish situated between an anode and cathode plate, with optimal induction achieved when the fish is oriented perpendicular to the plates (Rous et al. 2015). Tetanus appears to be observed at lower voltages when the fish is oriented facing the cathode compared to if the fish were facing the anode (Lamarque 1989). It is therefore recommended that fish be oriented facing the anode during electroimmobilization, allowing for increased versatility and control over immobilization.

Environmental parameters such as water conductivity and temperature will influence the efficacy of electroimmobilization (Ackerman et al. 2005). Salinity may prevent adequate or successful electroimmobilization, depending on the equipment used (Balazik et al. 2013). More studies examining the effects of water quality variables at finer scales (e.g., conductivity, temperature, salinity, pH) on various electroimmobilization apparati at fixed settings are required. This type of assessment would be greatly facilitated by reporting the exact electroimmobilization settings (e.g., current type, voltage; when applicable, duty cycle and pulse frequency/duration). Other relevant parameters that are reported in electrofishing surveys (e.g., water temperature, conductivity) should also be provided.

Other Knowledge Gaps/Identified Questions

To help direct future work, we have identified key research questions or topics that have yet to be addressed comprehensively in the literature. Knowledge gaps exist throughout the topics of policy, theory, applications, and impacts of electroimmobilization. To attempt to cover this diverse array of problems in brief, the identified questions/topics have been divided into two general categories: (1) the "human" perspective (encompassing theory and applicability), and (2) the "fish" perspective (primarily concerning the effects of electroimmobilization). Insofar as we know, few jurisdictions have explicit policies regarding electroimmobilization procedures and essentially all will permit its use in field studies for collecting wild fishes.

The Human Perspective

As mentioned, the list of stages of anesthesia given in Summerfelt and Smith (1990) is the reference of choice for nearly all electroimmobilization studies when describing the desired level of effect. These stages are based on the typical symptoms elicited by chemical sedatives; problematically, other symptoms caused by electrical currents (e.g., tetany) are absent despite their obvious relevance. We therefore propose that a similar table be designed to reflect the "stages of electroimmobilization" that can combine the unique symptoms of electrical exposure with symptoms common to both (e.g., muscle relaxation, changes in opercular rate). Ideally, this will also be able to reflect which responses are available with different types of current. A preliminary attempt to develop such a table has been provided (Table 2).

The efficacy of electroimmobilization is negatively influenced by saline water (Balazik et al. 2013). Russian scientists have claimed success in the use of marine electrotrawling in immobilizing fish and increasing catching success, but these claims are highly questionable, often based on anecdotal observations and reporting raw data without statistical analyses (e.g., Maksimov et al. 1987). Though controversial, electric trawling is being considered for shrimp harvesting in the North Sea despite major remaining knowledge gaps concerning broader impacts on marine animals (Soetaert et al. 2015). Electroimmobilization would likely only occur in marine waters if the fish come into contact with the electrodes, and if differences did exist between catch rates of electric and normal trawls they would sooner be attributable to electrotaxis rather than electroimmobilization. Future work is needed to pinpoint any thresholds in salinity, water temperature, pH, and

Table 2. A preliminary list of the "Stages of Electroimmobilization," describing the permitting currents, general characteristics and injury risk of each stage (ordered by increasing voltage), and a comparison with Summerfelt and Smith's (1990) stages of anesthesia.

Stage	Permitting Currents	General Description	Overall Injury Risk	Versus Summerfelt and Smith's (1990) Stages of Anesthesia
0. No Effect	All	Normal equilibrium and reactivity to other stimuli	NA	"*Normal*" stage—identical
1. Electrosedation	AC, cDC	Muscles relaxed, normal opercular movements; slight loss of equilibrium and reactivity to other stimuli; very fast recovery	Low	"*Light sedation*"—slight loss of reactivity but normal equilibrium
2. Electroanesthesia	AC, cDC	Muscles relaxed, normal opercular movements; full loss of equilibrium and reactivity to other stimuli; very fast recovery	Low	"*Deep sedation*"—near-total loss of reactivity but equilibrium is still normal
3. Electrotetany	AC, cDC, pDC	Muscles relaxed or contracting weakly, opercular movements may be irregular; full equilibrium and reactivity loss; fast recovery	Moderate	"*Partial loss of equilibrium*"—erratic swimming, near-total loss of reactivity, still some equilibrium
4. Electrostunning	AC, cDC, pDC	Muscles contracting, opercular movements cease; prolonged full loss of equilibrium and reactivity to other stimuli; prolonged recovery	Moderate to high chance of spinal injury, haemorrhaging	"*Total loss of equilibrium*"—total loss of spinal reflex, muscle tone, equilibrium "*Loss of reflex reactivity*"—total reactivity loss, very slow opercular movements and heart rate
5. Adverse Effects	All	Intense muscle contractions, injuries, respiratory failure, and death; full loss of equilibrium and reactivity to other stimuli	Certain	"*Medullary collapse*"—opercular movement ceases, followed by cardiac arrest

other environmental parameters that make electroimmobilization ineffective. In addition, determining potential options for circumventing these limitations such as using low-voltage electrodes in direct contact with the fish or temporarily introducing marine fish to freshwater for immobilization, would be beneficial for species from in saline waters.

The Fish Perspective

We have described electrosedation, electroanesthesia, electrotetany, and electrostunning as different levels of electroimmobilization, though determining exactly where the boundaries lie between each stage would greatly enhance the accuracy and utility of this classification. Controlled studies with individual gradients in voltage and current (type and intensity) for different species could provide valuable, policy-relevant insight on predicting appropriate settings to use in a given research effort. As an immediate-release technique, electroimmobilization can be more appealing than drugs when handling fish during or shortly before spawning periods (especially in migratory species). Quantitative comparisons concerning the reproductive success between fish exposed to electroimmobilization or chemical anesthetics prior to spawning are currently lacking despite the clear value of this knowledge to selecting the most appropriate handling protocol in spawning or pre-spawning fish.

All of the research discussed herein has examined ray-finned fish (Actinopterygii). The efficacy and value in using electroimmobilization to facilitate handling procedures on other fishes (i.e., lobe-finned [Sarcopterygii], cartilaginous [Chondrichthyes], and jawless [Agnatha] fish) should be evaluated in future work. In line with the above note on salinity, a better understanding is needed on the effects of moving a marine species briefly into freshwater for electroimmobilization (Balazik 2015) and whether or not any induced stress response is detectable over that associated with general handling. It would also be interesting to examine the efficacy of electroimmobilization on electric fishes (e.g., *Electrophorus electricus*) and their post-handling physiological and behavioural responses.

The underlying physiology of the different stages of electroimmobilization deserves further exploration, especially from an institutional animal care and use committee (IACUC) welfare perspective on how electric currents of various types and intensities may influence nociception in fish. For example, is there any evidence of analgesia/sensory loss or, conversely, increased nociception as a result of electroimmobilization? Due to established evidence of nocifensive responses (behavioural responses to noxious stimuli) in fishes (Chatigny et al. 2018), is there any evidence that chemical anesthetics/analgesics alter behavioural responses during and after electroimmobilization-facilitated surgical procedures? Furthermore, although studies have demonstrated electroimmobilization's association with a typical generalized stress response (Bowzer et al. 2012; Trushenski and Bowker 2012), is it possible to differentiate between the physiological stress attributable to general handling procedure as compared with the stress induced from the electric stimulus itself? These are the types of questions that are being asked of researchers by IACUCs and thus need to be addressed with some expediency. The Canadian Council on Animal Care follows a precautionary approach in assigning categories of invasiveness to various procedures, and it is recommended that electrofishing be considered "Category D" invasiveness ("Experiments that cause moderate to severe distress or discomfort;" Griffin et al. 2007). There is no recommendation for other examples of electroimmobilization, but these would presumably fall into the same category. Given the evidence for rapid recovery and high survival of electroimmobilization, however, this assignment may need revision.

CURRENT AND POTENTIAL APPLICATIONS

Electroimmobilization Equipment

Electroimmobilization units may be uniquely designed and built by researchers (e.g., Jennings and Looney 1998; Hudson et al. 2011) or adapt gear such as TENS units, which typically cost less than US$50 (2019; see Vandergoot et al. 2011). The procedure requires the basic necessities for delivering current (i.e., power supply, anode/cathode plates, holding tank) in addition to personal protective equipment and adequate knowledge of the appropriate physical dimensions and electricity settings for different species, life history stages, and environmental conditions. Alternatively, such units can be found commercially made and designed (e.g., Portable Electroanesthesia System [PES] or low-voltage Fish Handling Gloves [FHG], both developed by Smith-Root [Vancouver, Washington]). The PES can emit cDC or pDC for all levels of electroimmobilization but is most commonly used to induce electrostunning with pDC. The FHG emit cDC for low-voltage electrosedation or electroanesthesia and have modified the anode and cathode plates of traditional apparati into mesh gloves for direct contact of the electrodes on the fish. Larger, industrial scale units have recently been developed for mass processing in fisheries such as the Humane Stunner Universal developed by Ace Aquatec (Dundee, Scotland). Figure 1 shows the active use of both the FHG (A) and PES (B). Regardless of the how the unit is built or designed, an understanding of the potential effects and relative efficacy of the equipment (as well as suitable equipment and protocol for operator safety) is critical to its appropriate application. Another potential setback is start-up cost; a 48-quart cooler PES unit costs roughly $9,500 (2019).

Current Applications

Electrostunning from pDC is used for lasting immobilization for an extended length of time following the cessation of exposure to the electric current (often referred to as the "recovery period"). Surgeries and other data collection procedures are frequently performed during the recovery period of electrostunned fishes. Alternatively, cDC may be used to subdue fish through electrosedation or electroanesthesia during less invasive sampling procedures while allowing for a nearly instantaneous recovery once the fish is removed from the electric current. Given current evidence suggesting that low-voltage electrosedation and electroanesthesia do not appear to result in ecologically relevant changes in post-release behaviour (e.g., Henyey et al. 2002; Wilson et al. 2017) or physiological alterations that differ significantly from a generalized stress response (which is also exhibited in chemically sedated fish; e.g., Ward et al. 2017; Abrams et al. 2018), invasive procedures may be safely undertaken while cDC is actively passing through the fish, provided that handlers follow appropriate safety protocols. This distinction could make cDC more suitable for procedures demanding precise duration times and immediate recovery. Recent work has begun to examine the

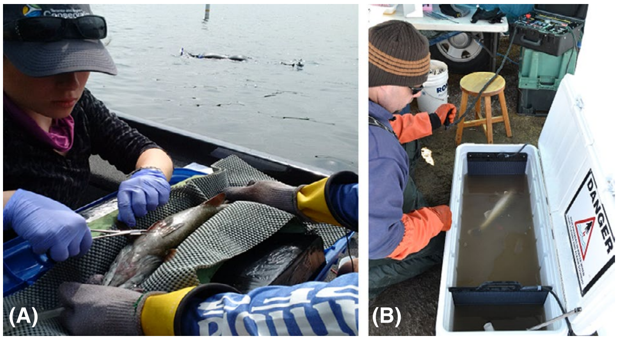

Figure 1. (A) A Smallmouth Bass *Micropterus dolomieu* is immobilized using low-voltage electrosedation (constant direct current) gloves. The Smallmouth Bass was angled from a nest and put in suppine position on a surgery table. A water hose was inserted into its mouth and then electricity was applied to immobilize the fish for surgical implantation of a biologging tag (procedure time = 5 min). The fish was released immediately following the procedure and resumed parental care duties within 1 min (the background snorkeller defended the nest in the nesting male's absense) and subsequently raised its brood successfully (see Prystay 2018). (B) A Walleye *Sander vitreus* is immobilized using a portable electrostunning unit. The fish was exposed to 35 V pulsed direct current for 3 s prior to being placed on a surgery table for implantation of an acoustic telemetry transmitter. The mean duration of the surgical procedure was 142 s at which time fish were held for ~5 min in individual water-filled totes prior to release and subsequent tracking as per Hayden et al. (2014).

potential for multi-stage electroimmobilization, involving the successive application of several different electric waveforms (e.g., Faust et al. 2017).

Additional Applications

With rapid induction and recovery times, electrosedation, electroanesthesia, and electrotetany may be used to facilitate handling fish for unpredictable/extended times without the dosage and safety issues associated with chemical sedatives. This could be particularly useful in testing novel methods designed to provide non-lethal means of collecting data that traditionally necessitated intensive surgeries or lethal sampling (e.g., sex determination in many non-reproductive stage fishes). For example, Matsche (2013) used electroanesthesia to assist with a non-lethal method of sex determination of sexually immature and developing Largemouth Bass.

In Canada, MS-222 is the only approved chemical sedative for fish and requires that treated fish be held for a minimum of 5 days in water temperatures above 10°C (Health Canada 2010); in the United States, where MS-222 is the only FDA-approved anesthetic for field applications on fish, this holding period is extended to 21 days (Trushenski et al. 2013). AQUI-S®20E (10% eugenol), available as an Investigational New Animal Drug with U.S. Fish and Wildlife Service approval, allows for immediate release under certain conditions but has a 3-day holding period for hatchery fish (Trushenski et al. 2012b; Silbernagel and Yochem 2016). Because electroimmobilization allows for immediate release (e.g., Jennings and Looney 1998), it can prove a superior alternative to chemical sedatives in cases where immediate release is critical to the wellbeing of treated fish and/or soundness of experimental methodology (e.g., telemetry tag implantation in migrating fish).

CONCLUSIONS

Based on the available evidence to date, electroimmobilization appears to be an effective tool for use in fisheries research and practice. Once the equipment is obtained and settings are optimized it can be used without causing significant stress or injury and high rates of survival can be expected. Notably, recovery of reflexes appears to be particularly rapid and there is no chemical "hangover" or withdrawal concerns as occurs when using chemical anesthetics (see *Additional Applications*; Table 1). Therefore, electroimmobilization serves to maintain the welfare state of fish while also addressing broader ecological and human health concerns. We identified a number of research gaps that once addressed will further refine use and operating guidelines for the tools used in electroimmobilization. It has been our observation that electroimmobilization has rapidly grown to be a common tool. Although some perceive it to be inconsistent with fish welfare (e.g., potentially causing nociception) the research to date suggests fish rapidly resume natural behaviours (e.g., migration, spawning, parental care), which supports the continued use of electroimmobilization. There are examples of electroimmobilization variably embraced and rejected by IACUCs in Canada and the United States with the most common concern for rejection being lack of certainty regarding whether the immobilization itself induces stress and "pain" or mutes discomfort that may be experienced from procedures (e.g., blood withdrawal, laparotomy) conducted while fish are immobilized. There will undoubtedly be more research on electroimmobilization in the coming years that will clarify and optimize the ways in which it is used to support fisheries research that maintains or enhances fish welfare.

ACKNOWLEDGMENTS

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Cooke is supported by NSERC, the Ocean Tracking Network, and the Canada Research Chairs Program. This work was partially funded by the Great Lakes Fishery Commission by way of Great Lakes Restoration Initiative appropriations (GL-00E23010). This paper is Contribution 65 of the Great Lakes Acoustic Telemetry Observation System (GLATOS). We would like to thank an anonymous reviewer for providing feedback during manuscript preparation. There is no conflict of interest declared in this article.

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