

## Behavioural impacts of MS-222 and electro-immobilization on wild fish assessed using a whole lake telemetry system

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## Abstract

To immobilize fish for the surgical implantation of electronic tags, tricaine methanesulfonate ('MS-222') and electrical currents ('electro-immobilization') are commonly used. Despite the abundance of literature examining the physiological side effects of these immobilization methods, few studies have examined if using MS-222 or electro-immobilization during tagging alters the behaviours of fishes in the wild. To do so, we used a whole-lake telemetry system to compare recently tagged largemouth bass (*Micropterus nigricans*) and northern pike (*Esox lucius*) immobilized with either MS-222 or transcutaneous electrical nerve stimulation (TENS) to previously tagged (i.e., recovered) controls. Despite species-specific alterations in behaviour for the first 72 h after tagging, the behaviours of both largemouth bass and northern pike were similar to controls within ~100-200 h of tagging, regardless of the immobilization technique used. Our results support the censoring of data from at least the first week post-tagging to avoid bias from the tagging process, as well as the use of TENS as a viable alternative for MS-222 given the similarity of recovery patterns among treatment groups.

## Keywords:

Electronic tagging, sedation, electro-immobilization, acoustic telemetry, MS-222, TENS.

## Introduction

Over the past several decades, electronic tagging and tracking tools (e.g., biotelemetry and biologging) have revolutionized our ability to study the behaviour and movement of fishes in the wild (Cooke et al. 2013; Hussey et al. 2015). Although tags can be applied to fish in various ways (Bridger and Booth 2003; Cooke et al. 2013), the most common approach for long-term tracking studies involves the surgical implantation of tags (Matley et al. 2024a). Due to the relative invasiveness of this technique and need for fish to remain still during the procedure, fish are generally sedated or immobilized during surgical procedures (Jenkins et al. 2014).

Tricaine methanesulfonate ('MS-222') is one of the most widely-used sedatives for fishes worldwide and is capable of maintaining sedation for prolonged periods, allowing for complex surgical procedures (Ross and Ross 2009; Topic Popovic et al. 2012). Generally administered through immersion in a buffered solution, MS-222 blocks ion channels to prevent the generation and conduction of nerve impulses (Frazier and Narahashi 1975; Matthews and Varga 2012). Currently, MS-222 is among few chemical sedatives fully approved for use in food fish in Canada and the United-States (Food and Drug Administration 2024; Health Canada 2010). However, due to its slow metabolic clearance, fish sedated with MS-222 cannot be consumed for a period of 5 days in Canada (Health Canada 2010) or 21 days in the United States (Food and Drug Administration 2024). This makes the use of MS-222 undesirable for field studies where tagged fish may be harvested by people given that such prolonged holding periods are usually not logistically feasible.

As an alternative to chemical anesthetics, electrical currents have been used to immobilize fish by generating electrotetany (muscle contraction) or electronarcosis

(unconsciousness and muscle relaxation), collectively referred to as ‘electro-immobilization’ hereafter (Barham et al. 1987; Summerfelt and Smith 1990). Electro-immobilization has been demonstrated as an effective alternative to MS-222 in immobilizing fishes (e.g., Prystay et al. 2017; Trushenski et al. 2012a; Trushenski et al. 2012b) and offers the advantages of its ease of use, lack of chemical hazards for researchers, rapid induction and recovery times, and for allowing fish to be released and consumed by humans immediately (e.g., Chiba et al. 2006; Jennings and Looney 1998; Trushenski and Bowker 2012).

Although the mechanisms by which electric currents immobilize fish are presumably the same as those of electrofishing, the ways in which different types and intensities of electrical currents affect fish are not well-understood (Reid et al. 2019). Furthermore, vertebral column fractures and relatively high rates of mortality have been reported with the use of some electro-immobilization techniques (e.g., Gaikowski et al. 2001; Redman et al. 1998; Walker et al. 1994), although these reported rates of injury are inconclusive and not fully understood (Reid et al. 2019). Moreover, it is unclear whether such methods depress the neurological system (thus providing anesthetic properties) (Reid et al. 2019). Finally, there is lack of knowledge on the potential latent behavioural consequences of electro-immobilization. As such, there has been some reluctance from animal care committees in approving the use of electro-immobilization.

Both MS-222 and electro-immobilization can be effective in mediating physiological stress associated with handling and tagging (Bowzer et al. 2012; Durhack et al. 2020; Prystay et al. 2017; Trushenski and Bowker 2012; Trushenski et al. 2012a, 2012b). However, MS-222 and electro-immobilization both have physiological effects. MS-222 is known to alter plasma biochemistry, blood gases, hematological profiles, and stress biomarkers, with effects often persisting for days following exposure (reviewed in Priborsky and Velisek 2018). MS-222 forms

an acidic solution with a pH as low as 2.8 in water (Ohr 1976), which may expose fish to a toxic acidic environment without adequate buffering (Carter et al. 2011). Although electro-immobilization is also associated with changes in hematological profiles and stress biomarkers, these physiological consequences appear quite variable in duration and severity across studies and species (reviewed in Reid et al. 2019).

Behaviour can be a useful indicator of stress and physiological injury in fishes (Beitinger 1990; Campbell et al. 2010). Despite an abundance of literature examining the physiological side effects of sedatives and immobilization techniques, relatively few studies have examined if MS-222 and electro-immobilization alter behaviours over extended periods (i.e., days-weeks). Rainbow trout (*Oncorhynchus mykiss*) reduced feeding behaviors during the first 24 h after sedation with MS-222, but the swimming performance of both rainbow trout and largemouth bass (*Micropterus nigricans*) (in addition to other behavioural metrics) was not impaired following sedation with MS-222 in lab or mesocosm environments (Anderson et al. 1997; Pirhonen and Schreck 2003; Prystay et al. 2017). Similar to MS-222, there is evidence that electro-immobilization has no short-term (< 24 h) impacts on largemouth bass swimming performance, although electro-immobilized fish may actually be more active post-immobilization than both control fish and those sedated using MS-222 (Abrams et al. 2018; Prystay et al. 2017). A few studies have also examined how tag implantation using electro-immobilization impacts migratory behaviors, with results appearing species specific. For example, while electro-immobilization did not appear to influence migration timing in Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) (Balazik 2015), walleye (*Sander vitreus*) implanted with tags using electro-immobilization had longer downstream travel times than fish tagged in previous years (Wilson et al. 2017). Post release condition and nest abandonment in smallmouth

bass (*Micropterus dolomieu*) were similar between fish treated with MS-222, transcutaneous electrical nerve stimulation (TENS) and controls, but not those electrostunned using a portable electroanesthesia system (Reid et al. 2024).

Of the limited work examining the behavioural effects of these two immobilization techniques, much has done so by examining relatively simple metrics in laboratory environments, which likely does not represent the true complexity of the recovery process. For example, while some behavioural metrics (e.g., swimming speed) appear to return to normal in zebrafish (*Danio rerio*) just 30-minutes after sedation (Nordgreen et al. 2014), cognitive impairments have been reported for young-adult zebrafish for 48 hours following sedation with MS-222 (Fontana et al. 2021). Fish behaviour is driven by complex environmental stimuli and social interactions that cannot be fully recreated in laboratory studies (Fahlman et al. 2020; Magnhagen 2012). Therefore, there is a need to assess post-tagging recovery using immobilization techniques in natural environments.

Given the physiological impacts of these immobilization techniques and the scarcity of information regarding post-immobilization behaviours, many electronic tagging studies censor data from the first days to weeks post-tagging (e.g., Krause et al. 2020; Wright et al. 2019), assuming that behaviours were altered by tagging procedures. In addition to significant losses of data, this may not be an option for studies concerned with short-term behaviours such as the consequences of catch-and-release angling, stocking hatchery-raised fish, or migratory behaviours. To address this issue, we used a whole-lake telemetry system to compare recently tagged largemouth bass (*Micropterus nigricans*) and northern pike (*Esox lucius*) immobilized with either MS-222 or TENS to previously tagged (i.e., fully recovered) individuals of each species. We aimed to evaluate the time required for fish to re-establish normal behaviour

following transmitter implantation, and if differences in recover patterns occurred between fish tagged with either MS-222 or TENS.

## Methods

### *Study System*

This study was conducted at Lindsay Lake (44.5373°N, 76.3897°W), a ~16 ha inland lake at the Queens University Biological Station in eastern Ontario, Canada (Fig. 1). The shoreline surrounding Lindsay Lake is largely composed of rock, timber and thick macrophytes, with soft substrates and sparse amounts of macrophytes along the bottom of the majority of the lake. The lake has a maximum depth of ~10 m and supports a predatory fish community of largemouth bass and northern pike. Prey fish consist of more abundant pumpkinseed (*Lepomis gibbosus*) and bluegill (*Lepomis macrochirus*), as well as less abundant yellow perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), and various minnow species. The lake has a small, shallow (<1 m deep) channel that connects it with Poole Lake, another small private lake at the Queens University Biological Station. These lakes are on private land and fishing by the general public is not permitted.

In May 2023, Lindsay Lake was equipped with an array of 33 Innovasea HR3 high residence receivers, with an additional HR3 receiver placed at the mouth of Poole Lake to detect emigration from Lindsay Lake (Fig. 1). Each receiver was placed approximately halfway in the water column, with a maximum distance of ~100 m between adjacent receivers. Receivers were retrieved and redeployed at approximately 6-month intervals. Stationary tags were also deployed at 5 locations in May 2023 to synchronize the internal clocks of the receivers and act as reference

tags within the receiver array, with a V6-4x (nominal delay of 6-10 s) and V3-1x (nominal delay of 25-35 s) tag placed at each location (Fig. 1).

### *Fish Tagging*

All research detailed here was conducted in accordance with the Canadian Council on Animal Care (approved by Trent University) and under a scientific collection permit issued by the Ontario Ministry of Natural Resources and Forestry. Between May 2023 and May 2024, a total of 48 northern pike and 54 largemouth bass were collected via angling using a variety of lure types, with landing times limited to less than 60 s (Table 1). After capture, fish were placed in submerged holding bags (0.31 m diameter x 1.22 m length; Dynamic Aqua Supply Ltd., Surrey, BC, CA) and transported via boat to a tagging station on the shore. Prior to surgery, tags were tested to verify that the correct codes were being transmitted and were sterilized via immersion in an iodine solution (as were surgical instruments) before being rinsed with distilled water. Fish in trials using MS-222 were placed individually into an aerated holding tank (0.5 m width x 0.5 m depth x 1.5 m length) filled with lake water and 100 mg L<sup>-1</sup> buffered MS-222 (Syndel, Nanaimo, B.C., Canada, <https://syndel.com>). The water bath was replaced for every four fish that were sedated. Once a fish reached stage 5 anesthesia (total loss of equilibrium, slowed opercular rate, and no response to stimuli), fish were placed in a wetted surgical sling. In electro-immobilization trials, TENS units (TENS 7000; Middleburg Heights, OH, U.S.A., <https://tens7000.com>) were used with a constant pulse rate (150 Hz) and pulse width (300 µs). The electrode leads were placed in the surgical sling such that the anode was placed toward the head and the cathode near the tail of the fish. Electrical intensity was increased incrementally until fish were immobilized and unresponsive to tactile stimulation, at which point surgery commenced.

Surgical methods in both trial types followed standard approaches in the field of fish telemetry (e.g., Wagner et al. 2011). Once supine in the wetted surgical sling (Fig. S1), their gills were continuously irrigated with aerated lake water. Acoustic tags were then inserted intraperitoneally through a 5-10 mm incision in the abdominal wall anterior to the pelvic girdle and ~10 mm off the central midline. Fish tagged in the fall of 2023 were implanted with V6 tags (1.2 g in air; 19.3 mm length x 6.3 mm diameter; 151 dB output, 14-18 s nominal delay). Since 307 kHz V6 tags were discontinued by the manufacturer halfway through the study, fish tagged in the spring of 2024 were implanted with V3 tags (0.3 g in air; 15 mm length x 4 mm diameter; 141 dB output, 10-14 s nominal delay). The incision was closed with a single 3-0 monofilament absorbable suture (Ethicon PDS II Plus polydioxanone, Ethicon US, [www.ethicon.com](http://www.ethicon.com)) using a 3-2-2 surgeons knot. The sample sizes and fork lengths of the largemouth bass and northern pike in each treatment group are summarized in Table 1.

### *Analyses*

The Vemco Positioning System (VPS) was used to produce fine-scale positioning estimates and horizontal position error (HPE) from telemetry data. HPE is an error estimate for each calculated position (Smith 2013) and was calculated for each tagged fish and for static V6 ( $n = 5$ ) and V3 ( $n = 5$ ) reference transmitters. All statistical analyses were conducted in version 4.3.2 (R CoreTeam 2024). Fish positions with HPE values  $> 3$  were removed from the analysis, as an *in situ* analysis of the reference transmitters indicated that mean estimated positional error was ~1 m for positions with an HPE of less than 3. Any fish with VPS positions consistently at the same location for at least two weeks were assumed to have died or lost their tags and were removed from the analysis.

Analyses were divided into two time bins for each species: 0–72 h and 0–14 days after surgery. These time bins were chosen to describe both short- and intermediate-term behavioral responses to sedation and tagging. Behaviours were not evaluated beyond 14 days, as we were unable to confirm the absence of angling in the system beyond this period (which could affect behaviours in our tagged fish). To compare previously tagged controls to newly tagged individuals, a centered timestamp was created for each sampling session, defined as the time between first and last releases of tagged fish. This timestamp was deemed the “time 0” for hours post release in control fish. Although these fish are not true “untagged” controls, tag burden (i.e., tag:body mass ratios) was estimated to be a maximum of 0.25%. Therefore, we assumed that the effects of the tag itself were negligible and that the short-term behavioural effects of tagging subsided in the 6+ months since tagging. The use of controls allowed us to account for whole lake alterations (changes in water temperature, clarity, etc.) that may have induced behavioural changes.

First, we analyzed movement speeds by calculating the distance between consecutive VPS positions divided by the difference in time between positions. To reduce the possibility of underestimating movement speed due to missing detections, movement speeds were only calculated using steps where the elapsed time between positions was less than 10 minutes. For the first 72 hours after tagging, movement speeds were calculated from  $n = 309,256$  positions for northern pike and from  $n = 212,264$  positions for largemouth bass. For the two-week period after tagging, movement speeds were calculated from  $n = 1,551,865$  positions for northern pike and from  $n = 955,099$  positions for largemouth bass. We fit generalized additive mixed models (GAMM) with movement speed as the response variable using the *bam* function from the *mgcv* package (Wood 2011). Predictor variables included smoothing parameters for hours post release

(grouped by treatment) and time of day, as well as treatment, tagging season and total length as parametric terms. Individual fish ID was included as random effect to account for the repeated measures for each individual. Since fish were tagged with V6 tags in the fall and V3 tags in the spring, including season as a covariate allowed us to control for seasonal differences in behaviour and any differences in detection efficiencies between tag types. Models were fit with fast restricted maximum likelihood (fREML) methods with a gamma distribution and log link function. Tagging season and total length were removed in cases where they did not improve model fits ( $p > 0.10$ ). Model fits were evaluated visually and using the function *gam.check* in the *mgcv* package. Recovery was assessed visually by examining where the 95% confidence intervals of the GAMM curves for each treatment group began to overlap with those of the controls.

Next, we analyzed movement types (MT). VPS positions were first separated into 5-minute segments; this timeframe was chosen to create a large number of segments while maintaining a high enough resolution to capture changes in MTs. We then used the function *as.itraj* from the package *adehabitatLT* (Calenge 2006) to define segments based on the following metrics: 1) sum of total distance travelled, 2) turning angle (relative angle), 3) movement speed and the 4) linearity ratio, defined as the distance between the first and last locations of a segment divided by the total distance (i.e., sum of each of the steps) (Heupel et al. 2012). Segments missing values for any of these four variables were removed from analysis, as well as segments with less than 3 detections. A correlation plot was produced using the *corrplot* function from *corrplot* package (Wei and Simko 2024) and was used to remove redundant variables with a correlation of  $> 60\%$ . Due to high levels of collinearity between turning angle and linearity ratio, and between movement speed and distance traveled, we clustered movement

types based solely on movement speed and linearity ratios. Over the 72 h post-tagging period, this created  $n = 22,051$  segments for northern pike and  $n = 17,284$  segments for largemouth bass. Over the two-week post-tagging period,  $n = 111,032$  segments were created for northern pike and  $n = 86,807$  segments were created for largemouth bass.

We then used the *kmeans* function (R CoreTeam 2024) to partition bursts into clusters using methods similar to those outlined by Bergen et al. (2022). To choose the number of clusters (i.e.,  $k$ ), the average silhouette widths (i.e., how close a datapoint is to datapoints of its own cluster relative to datapoints of the closest neighboring cluster) were bootstrapped with replacement from the original data set; we used  $B = 100$  bootstrap samples each of size 1000 each and averaged the silhouette widths for each value of  $k$ . *K-means* clustering was then performed with 3 clusters with 10 randomly chosen initial cluster assignments.

We modeled the proportion of time fish spent exhibiting each MT using GAMMs. Predictor variables initially included smoothing parameters for hours post release (grouped by treatment) and time of day, treatment, tagging season and total length as parametric terms, as well as individual fish ID as random effect. Tagging season and total length were again removed in cases where they did not improve model fits ( $p > 0.10$ ). Models were fit with fast restricted maximum likelihood (fREML) methods with a binomial distribution and logit link function. Recovery was again determined by visually examining where the 95% confidence intervals of the GAMM curves for each treatment group began to overlap with those of the controls.

## Results

Overall, two northern pike ( $n = 1$  MS-222;  $n = 1$  TENS) were assumed to have died or lost their tags in the two-week period after tagging. These events were estimated to have

occurred at ~54 h (MS-222) and ~120 h (TENS) after tagging and were identified by VPS positions being consistently detected in the same location for greater than 2 weeks.

### *Movement speed*

In all groups, movement speeds changed significantly during the first 72 h after tagging (Fig. 2; see supplement for GAMM outputs). During this 72 h period, northern pike tagged using TENS ( $0.053 \pm 0.108 \text{ m s}^{-1}$ ) (mean  $\pm$  standard deviation) were marginally slower ( $t = -2.506$ ,  $p = 0.012$ ) than previously tagged controls ( $0.064 \pm 0.116 \text{ m s}^{-1}$ ), whereas pike tagged with MS-222 ( $0.061 \pm 0.137 \text{ m s}^{-1}$ ) were not ( $t = -0.847$ ,  $p = 0.397$ ; Fig. 2a). Model fits indicated that the northern pike tagged using TENS return to similar movement speeds as control fish at ~30 h post-tagging (Fig. 2a). Control largemouth bass were significantly faster ( $0.086 \pm 0.142 \text{ m s}^{-1}$ ) than those tagged with both TENS ( $0.081 \pm 0.144 \text{ m s}^{-1}$ ) ( $t = -2.564$ ,  $p = 0.01$ ) and MS-222 ( $0.061 \pm 0.105 \text{ m s}^{-1}$ ) ( $t = -4.856$ ,  $p < 0.001$ ) for the first 72 h after tagging (Fig. 2b). While model fits indicate that the largemouth bass tagged using TENS return to movement speeds similar to controls at ~40 h post-tagging, bass tagged using MS-222 remained slower throughout the entire 72 h post-tagging period (Fig. 2b).

Movement speeds also changed significantly during the first two weeks after tagging across all treatment groups (Fig. 3). During this period, movement speeds were similar between control northern pike ( $0.070 \pm 0.129 \text{ m s}^{-1}$ ) and those tagged with MS-222 ( $0.070 \pm 0.140 \text{ m s}^{-1}$ ) ( $t = 0.430$ ,  $p = 0.667$ ) or TENS ( $0.070 \pm 0.159 \text{ m s}^{-1}$ ) ( $t = 0.333$ ,  $p = 0.739$ ; Fig. 3a). Movement speeds of control largemouth bass ( $0.087 \pm 0.156 \text{ m s}^{-1}$ ) were not statistically different than those tagged with TENS ( $0.139 \pm 0.194 \text{ m s}^{-1}$ ) ( $t = 0.498$ ,  $p = 0.619$ ) or MS-222 ( $0.105 \pm 0.178 \text{ m s}^{-1}$ ) ( $t = -1.440$ ,  $p = 0.150$ ) during the first two weeks after tagging when accounting for the strong influence of season ( $t = 3.806$ ;  $p < 0.001$ ; Fig. 3b).

### *Movement types*

Although the exact values in each MT differed between species (see supplement for specific values for each species as well as GAMM model outputs), behaviours were consistently clustered into three MTs. First, MT1 was classified by high movement speeds and low–moderate linearity ratios, representing fish that were burst swimming. Second, MT2 was classified by very low movement speeds and linearity ratios, presumably representing stationary fish. Finally, MT3 was classified by low–moderate movement speeds and high linearity ratios, representing sustained swimming or cruising behaviours.

The proportion of time fish spent exhibiting each of the three MTs displayed changed significantly during the first 72 h after tagging (Fig. 4). The MTs displayed by northern pike tagged with TENS or MS-222 were not significantly different from controls for 72 h after tagging (Table S2; Fig. 4 a–c). In exception to MT1 (burst swimming) in TENS fish, all recently tagged largemouth bass exhibited significantly different ( $p < 0.05$ ) MTs from controls for the first 72 h after tagging (Table S3; Fig. 4 d–f). In general, recently tagged largemouth bass spent less time burst and sustained swimming compared to controls, spending more time stationary (Fig. 4 d–f). Model fits indicate that while the MTs of largemouth bass tagged using TENS began to return to control levels at ~48 h after tagging, the MTs of largemouth bass tagged using MS-222 remained different than controls for the entire 72 h period (Fig. 4 d–f).

For the two-week post-tagging period, the proportion of segments classified as MT3 (sustained swimming) in northern pike sedated with TENS was marginally lower than that of controls ( $t = -2.057, p = 0.040$ ; Fig. 5 c). Based on model fits, these differences only appeared to have occurred between ~100–150 h after tagging (Fig. 5 c). The proportion of segments classified as MT2 was higher ( $t = 2.917, p = 0.004$ ) in largemouth bass sedated with MS-222

compared to controls, and the proportion of segments classified as MT3 was lower in bass sedated with both MS-222 ( $t = -2.146, p = 0.032$ ) and TENS ( $t = -2.347, p = 0.019$ ) compared to controls for the first two weeks after tagging (Fig. 5 e–f). Model fits indicate that the MTs of recently tagged largemouth bass return to control levels around 150 h after tagging, regardless of treatment (Fig. 5 e–f).

## Discussion

Using acoustic telemetry and wild fish, we found that the procedure of intracoelomic tag implantation can have substantial impacts on fish movement during the first 72 h after tagging. The short-term effects (i.e., < 72 h after tagging) of tag implantation appeared to be species specific. Northern pike only had marginally lower movement speeds in fish sedated with TENS compared to controls, whereas largemouth bass sedated with either MS-222 or TENS exhibited substantial alterations in movement speed and MTs. Interspecific differences were expected given the taxonomic variation that exists in the physiological responses of fishes to sedatives, immobilization and tagging (reviewed by Cooke et al. 2011; Reid et al. 2019). However, it is notable that the movement speeds and behaviours of largemouth bass and northern pike immobilized with either method were broadly similar to one another for the first 72 h after tagging. It was instead the controls that differed between species, which was expected given that northern pike are a classic “sit and wait” ambush predator (Casselman and Lewis 1996; Craig 2008), whereas largemouth bass tend to be more active, opportunistic foragers (Hodgson and Kitchell 1987). Thus, recovering largemouth bass were more readily distinguished from more active controls, whereas recovering northern pike likely appeared similar to control fish that were likely ambushing prey (i.e., remaining stationary). Future experiments using acoustic tags with

acceleration sensors could help further separate ambushing behaviours from those of recovering fish.

Despite the short-term impacts of tagging on movements, model fits suggest that both largemouth bass and northern pike recovered within ~100-200 h (4-8 days) of tagging, regardless of the tagging method used. In largemouth bass, there was a greater proportion of segments classified as stationary rather than as sustained swimming during the initial ~100-150 h after tagging. As similar behavioural clusters as MT3 have been proposed as searching behaviours (Landry et al. 2019; McLean et al. 2014), these trends may represent a transition back to typical largemouth bass foraging behaviour (i.e., actively searching for food).

Overall, recovery patterns were similar between tagging methods. As the physiological effects of electro-immobilization in fishes are often similar to MS-222 (e.g., Durhack et al. 2020; Trushenski et al., 2012a, 2012b), the similarity in recovery patterns between treatments is not surprising. However, many of the physiological side effects of these treatments subside within 72 h of exposure (Gomulka et al. 2008; Phuong et al. 2017; Reid et al. 2022; Trushenski et al. 2012a; 2012b), which does not explain the prolonged recovery periods we observed. While some research has shown lingering physiological effects of exposure to MS-222 for up to 168 h (Matsche 2013), it is most likely the case that fish were rather recovering from the stress of being captured, handled, tagged, and released as opposed to the type of immobilization technique having an important effect. Although the role of electro-immobilization in inducing analgesia/sensory loss is still unknown, the evidence of similar survival and recovery patterns of fish tagged while immobilized with TENS vs. MS-222 should be considered by animal care committees.

The physiological effects of intracoelomic tag implantation have been studied extensively (reviewed in Cooke et al. 2011; Matley et al. 2024b), with the effects of tagging being highly variable in both severity and duration. In comparison, very little work has been done to examine the time required to recover from tag implantation in the wild. In one study, common bream (*Abramis brama*) travelled further than previously tagged fish for the first 5 days after tagging, with no differences occurring 6–10 days after tagging (Gardner et al. 2015). While some studies have justified shorter recovery periods (i.e., less than 24 h) based on the recovery of equilibrium and typical behaviours in captivity (e.g., Pon et al. 2009), fish behaviour cannot be fully recreated in captivity (Fahlman et al. 2020; Magnhagen 2012). Although the consistency in recovery times between Gardner et al. (2015) and ours suggests that 100–200 h (~4–8 d) recovery periods may be sufficient, substantial taxonomic variation exists in the physiological and behavioural responses fishes to sedatives, immobilization techniques and tagging (Cooke et al. 2011; Reid et al. 2019; Topic Popovic et al. 2012). Therefore, we caution against the extrapolation of these behavioural responses to other species, even those in similar taxonomic groups. More work is needed assessing recovery from tag implantation in the wild using different tag sizes, species, and environments before such conclusions can be drawn.

In summary, our results indicate that intracoelomic tagging produces short-term (< 72 h after tagging) alterations in the movements of both northern pike and largemouth bass, regardless of being sedated or electro-immobilized. Despite these initial effects, both species appeared to recover to baseline movement patterns approximately 100–200 h after tagging. Electro-immobilization offers the advantages of its ease of use, lack of chemical disposal, rapid induction and recovery times, as well as allowing fish to be released and consumed by humans immediately (e.g., Chiba et al. 2006; Jennings and Looney 1998; Trushenski and Bowker 2012).

Given the evidence that recovery patterns of electro-immobilized fish are similar to those sedated with MS-222 in the wild, our work supports electro-immobilization as a viable alternative for MS-222. We also support further investigation of electro-immobilization as a viable alternative for MS-222 from animal care committees.

### **Acknowledgements:**

We would like to thank Leah Howitt, Imogen Bellinger, Christian Bihun, Erin Stewart, Sam Chasse, Carolina Koebel, Reace Murphy, Zach Jones, Erin Ritchie, Benjamin Hlina, Brooklyn Cars and the Howell family for their assistance in animal collections, logistics and tagging. Thanks to Dale Webber and Colleen Burluk for providing input on experimental design. Thanks to Aaron Zolderdo and the Queen's University Biological Station for logistical support.

### **Funding:**

This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Alliance Grant (to Graham D. Raby, Aaron T. Fisk and Steven J. Cooke), as well as funding and equipment from Innovasea Systems Inc and the Real-Time Aquatic Ecosystem Observation Network (RAEON).

### **Data availability:**

Data generated during this study are available in the following repository: Shorgan et al. (2025). VPS data - Behavioural impacts of MS-222 and electro-immobilization on wild fish assessed using a whole lake telemetry system [Dataset]. figshare.

<https://doi.org/10.6084/M9.FIGSHARE.28577471>

**Author contributions:**

Mitchell B. Shorgan: data generation, data analysis, and manuscript preparation. Bradley E. Howell: data generation, manuscript revision, and editing. Luc Laroche: data generation, manuscript revision, and editing. Steven J. Cooke: study conceptualization, resources, manuscript revision, and editing. Aaron T. Fisk: study conceptualization, resources, manuscript revision, and editing. Christopher S. Vandergoot: study conceptualization, resources. Graham D. Raby: study conceptualization, resources, data generation, manuscript revision, and editing.

**Competing Interests Statement:**

The authors declare there are no competing interests.

**Works Cited**

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1 **Tables:**2 **Table 1:** Tagging period, number of individuals and total length (mm) of largemouth bass3 (*Micropterus nigricans*) and northern pike (*Esox lucius*) tagged per experimental treatment.

4 Control fish represent fish tagged months prior that were assumed to have fully recovered from

5 tagging. Control fish for the Sep 28 – Oct 2, 2023 tagging period were tagged between May 04–

6 06, 2023 (immobilized using TENS). Control fish for the May 01 – 07, 2024 tagging period were

7 treatment fish tagged between Sep 28 – Oct 2, 2023. Fish in each tagging period were pooled for

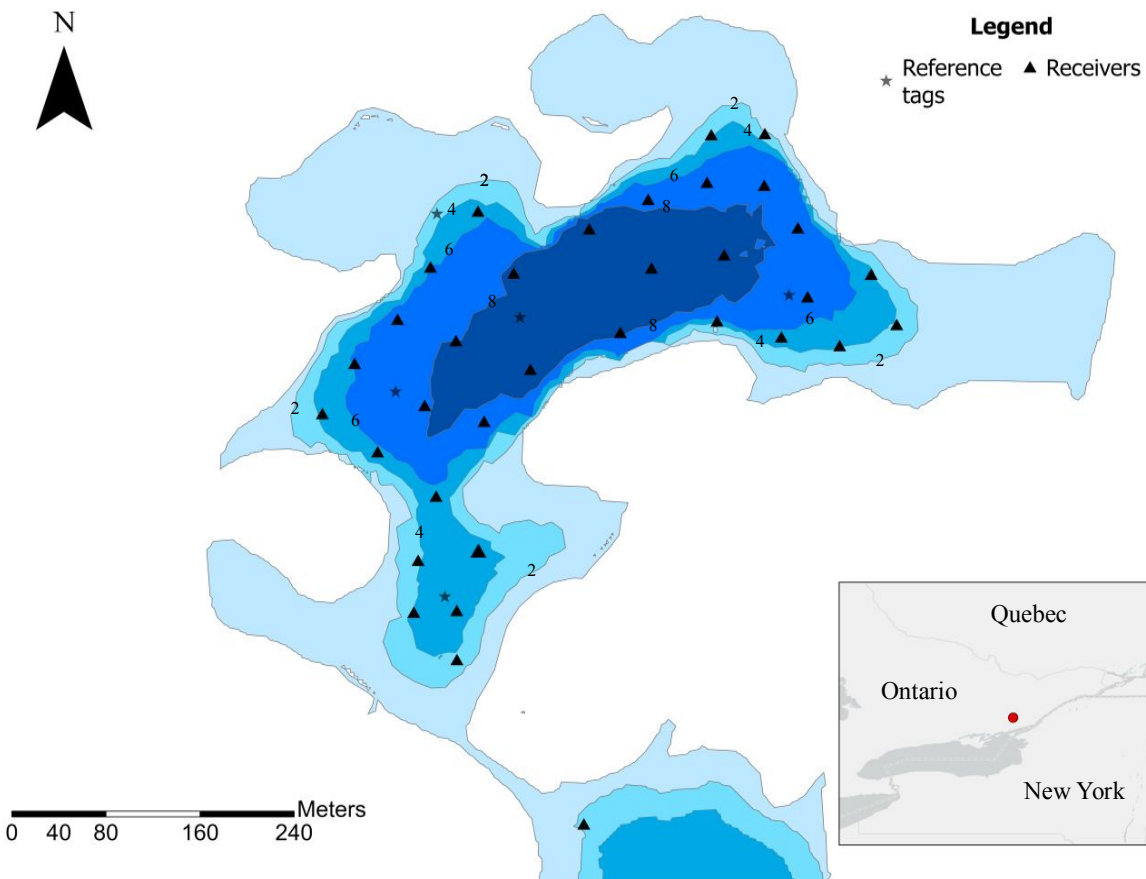
8 analysis.

9

Tagging period	Species	Treatment	Total length (mm), mean $\pm$ SD (range)	Number of individuals
Sep 28 – Oct 2, 2023	Northern pike	Control	644 $\pm$ 63 (545–735)	15
		TENS	624 $\pm$ 62 (515–665)	5
		MS-222	628 $\pm$ 49 (568–691)	5
	Largemouth bass	Control	430 $\pm$ 33 (376–481)	15
		TENS	421 $\pm$ 24 (387–445)	5
		MS-222	406 $\pm$ 58 (315–488)	6
May 01 – 07, 2024	Northern pike	Control	625 $\pm$ 55 (515–691)	9
		TENS	688 $\pm$ 32 (654–740)	7
		MS-222	660 $\pm$ 47 (595–705)	7
	Largemouth bass	Control	402 $\pm$ 39 (315–445)	9
		TENS	418 $\pm$ 11 (396–435)	11
		MS-222	425 $\pm$ 45 (346–477)	8
Total	Northern pike	Control	637 $\pm$ 60 (515–735)	24
		TENS	662 $\pm$ 55 (515–740)	12
		MS-222	647 $\pm$ 48 (595–705)	12
	Largemouth bass	Control	420 $\pm$ 37 (315–481)	24
		TENS	419 $\pm$ 15 (387–445)	16
		MS-222	417 $\pm$ 50 (315–488)	14

10

1 **Figures:**



2

3 **Fig. 1** Bathymetry of Lindsay Lake (44.5373°N, 76.3897°W) and the positions of Innovasea

4 HR3 receivers and reference tags used to study post-tagging behaviours of largemouth bass

5 (*Micropterus nigricans*;  $n = 54$ ) and northern pike (*Esox lucius*;  $n = 48$ ) from May 2023 – May

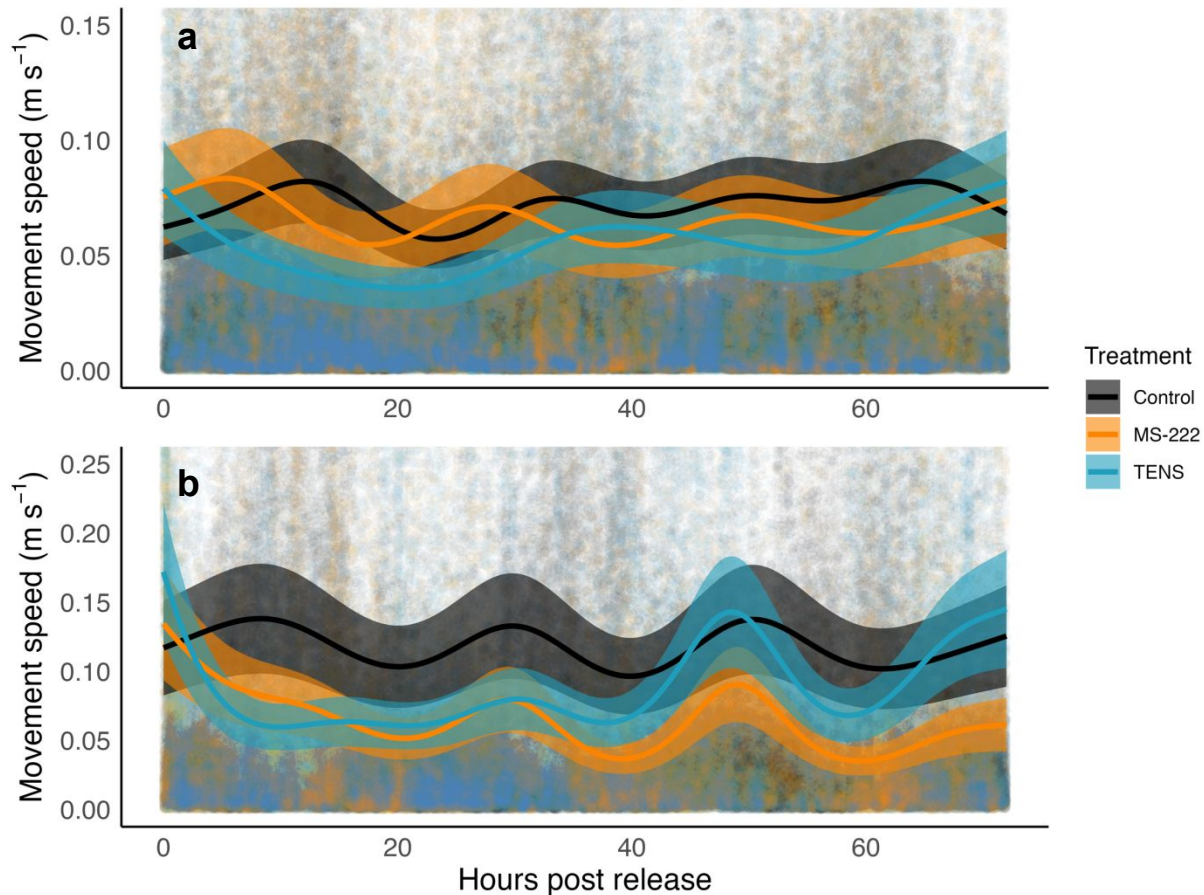
6 2024. Numbers represent contour lines (depth in meters). The map uses the World Geodetic

7 System 84 (WGS84) datum, with the WGS84 projection.

8

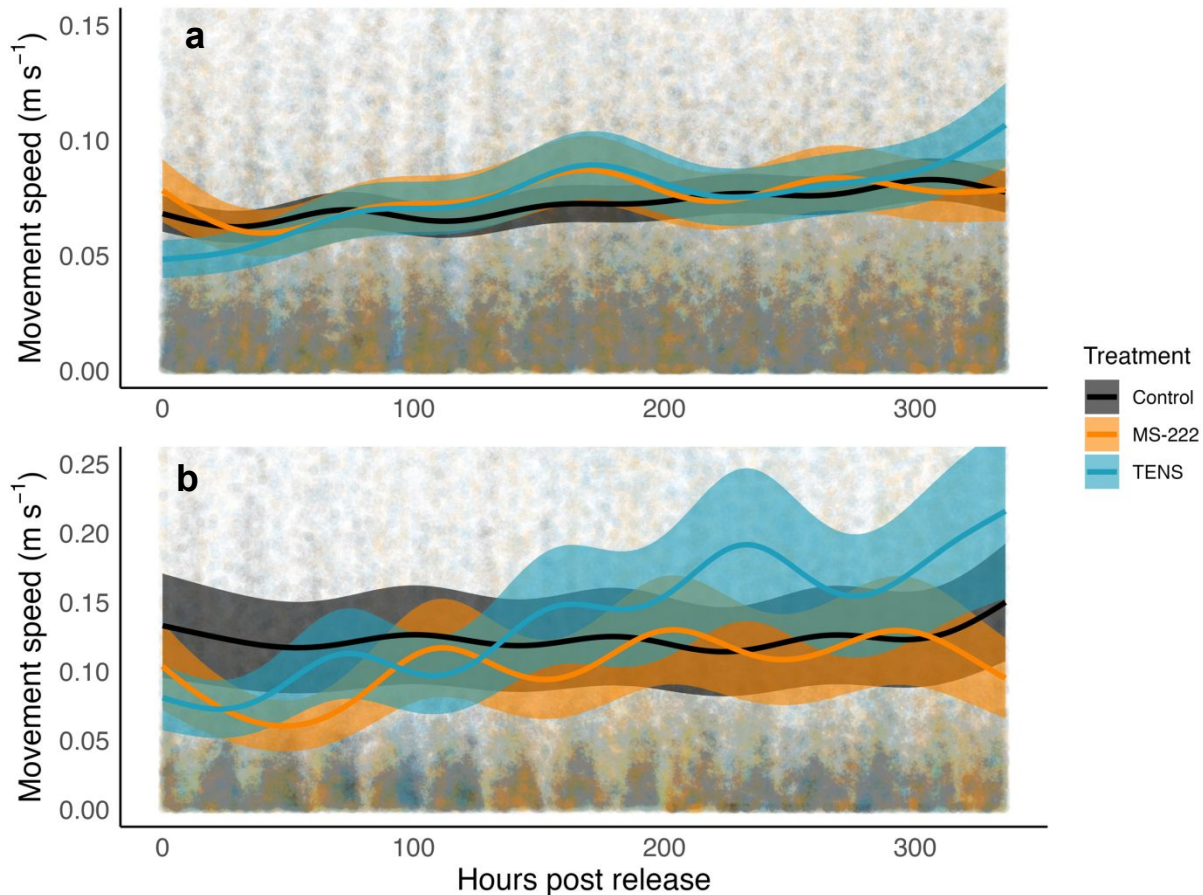
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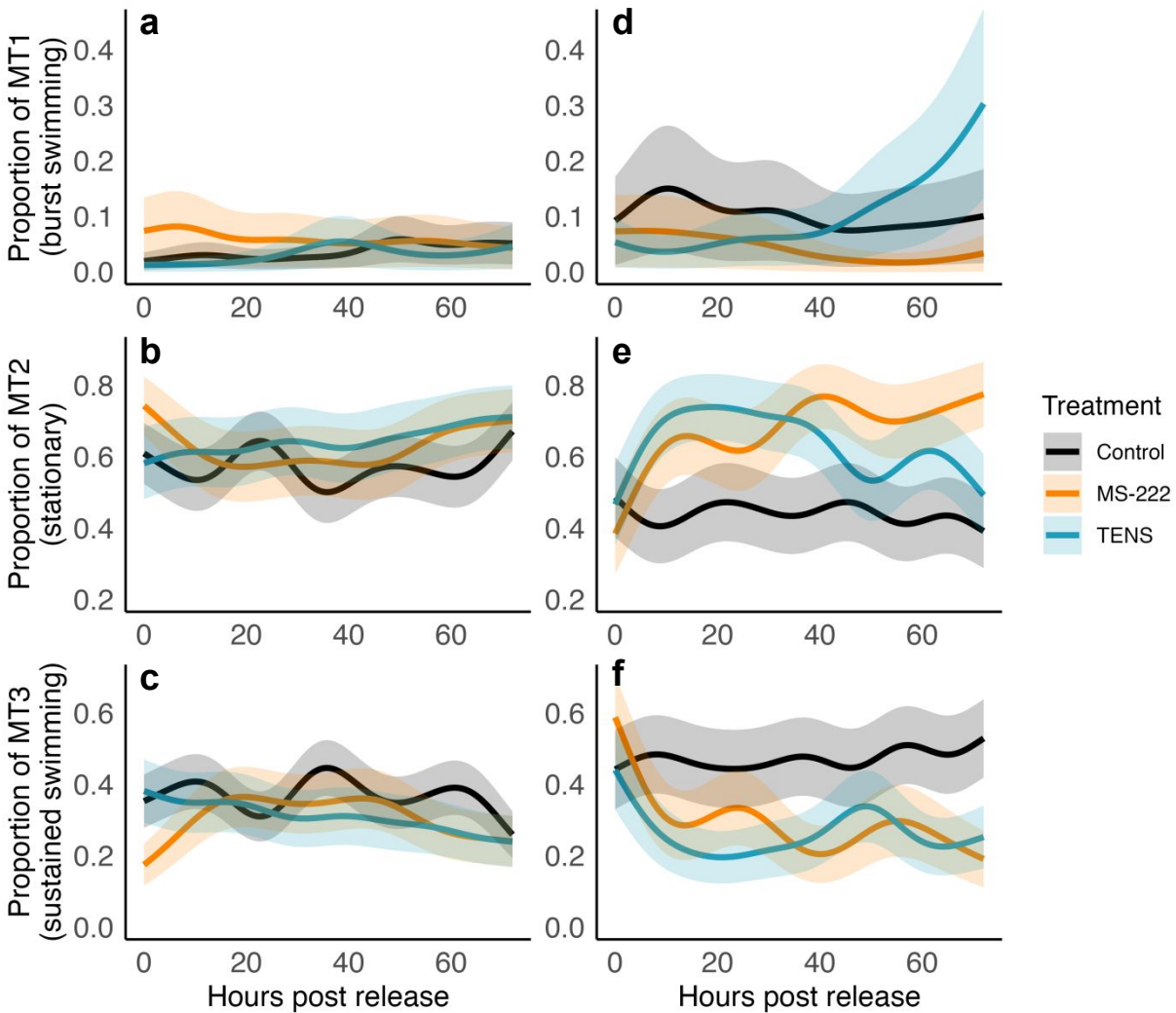
11 **Fig. 2:** Movement speed of a) northern pike (*Esox lucius*;  $n = 48$ ), and b) largemouth bass  
 12 (*Micropterus nigricans*;  $n = 54$ ) for 72 hours after intracoelomic tagging via sedation with MS-  
 13 222 or electro-immobilization with TENS. Control fish were tagged ~6 months prior and were  
 14 assumed to have fully recovered from tagging. Overlaid means  $\pm$  95% confidence intervals  
 15 represent model fits from generalized additive mixed models (Table S1). Movement speeds  
 16 exceeding 0.15  $\text{m s}^{-1}$  (a) and 0.25  $\text{m s}^{-1}$  (b) were omitted from plotting to visualize model fits.

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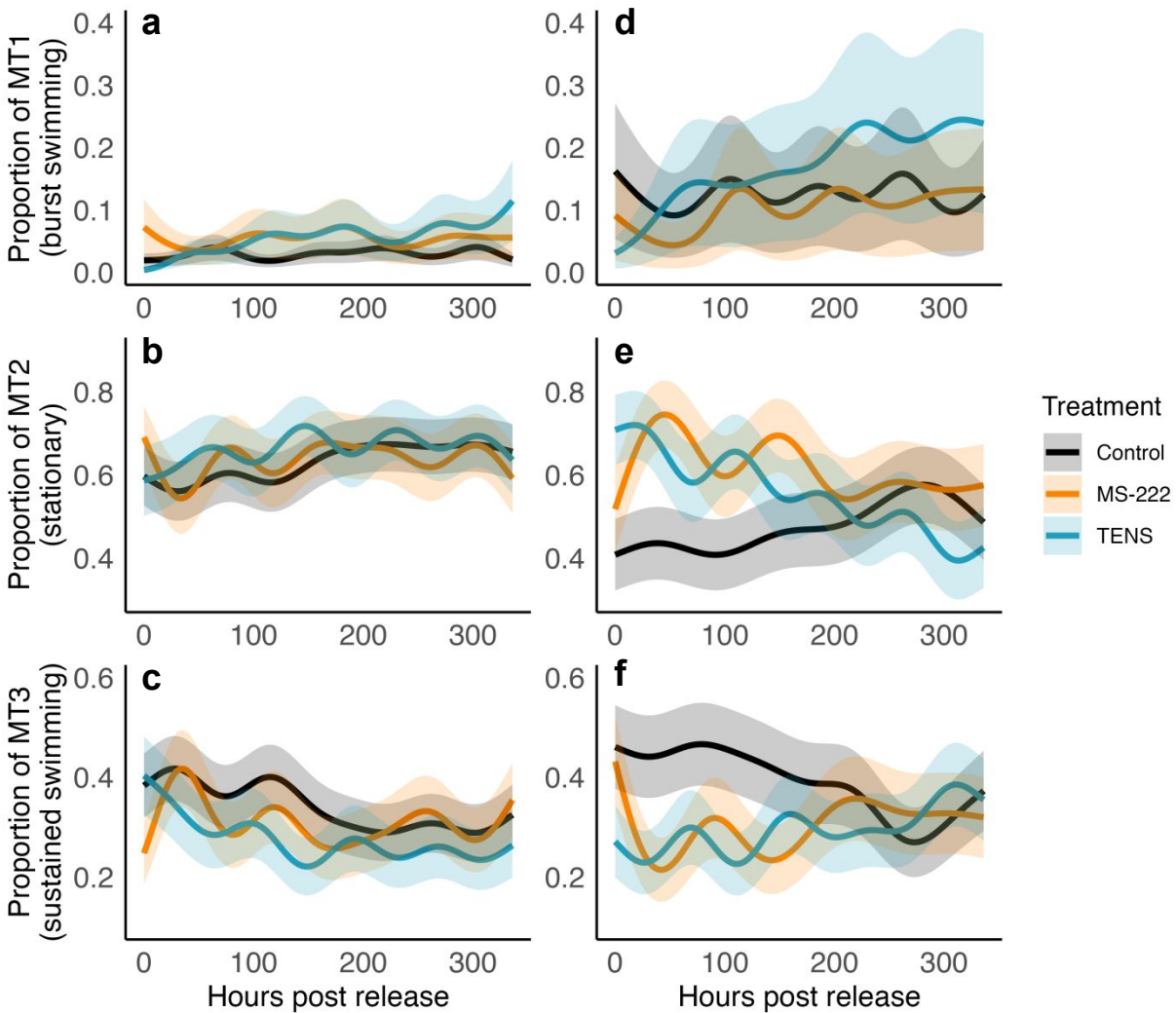


21 **Fig. 3:** Movement speed of a) northern pike (*Esox lucius*;  $n = 48$ ), and b) largemouth bass  
 22 (*Micropterus nigricans*;  $n = 54$ ) for 2 weeks after intracoelomic tagging via sedation with MS-  
 23 222 or electro-immobilization with TENS. Control fish were tagged ~6 months prior and were  
 24 assumed to have fully recovered from tagging. Overlaid means  $\pm$  95% confidence intervals  
 25 represent model fits from generalized additive mixed models (Table S1). Movement speeds  
 26 exceeding 0.15 m s<sup>-1</sup> (a) and 0.25 m s<sup>-1</sup> (b) were omitted from plotting to visualize model fits.

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31 **Fig. 4:** Movement types of (a–c) northern pike (*Esox lucius*;  $n = 48$ ), and (d–f) largemouth bass  
 32 (*Micropterus nigricans*;  $n = 54$ ) for 72 hours after intracoelomic tagging via sedation with MS-  
 33 222 or electro-immobilization with TENS. MT1 was classified by high movement speeds and  
 34 low–moderate linearity ratios, representing fish that were actively swimming. MT2 was  
 35 classified by very low movement speeds and linearity ratios, representing stationary fish. Finally,  
 36 MT3 was classified by low–moderate movement speeds and high linearity ratios, representing  
 37 fish that were cruising. Control fish were tagged ~6 months prior and were assumed to have fully  
 38 recovered from tagging. Overlaid means  $\pm$  95% confidence intervals represent model fits from  
 39 generalized additive mixed models (Tables S2, S3)



40 **Fig. 5:** Movement types of (a–c) northern pike (*Esox lucius*;  $n = 48$ ), and (d–f) largemouth bass  
 41 (*Micropterus nigricans*;  $n = 54$ ) for 72 hours after intracoelomic tagging via sedation with MS-  
 42 222 or electro-immobilization with TENS. MT1 was classified by high movement speeds and  
 43 low–moderate linearity ratios, representing fish that were actively swimming. MT2 was  
 44 classified by very low movement speeds and linearity ratios, representing stationary fish. Finally,  
 45 MT3 was classified by low–moderate movement speeds and high linearity ratios, representing  
 46 fish that were cruising. Control fish were tagged ~6 months prior and were assumed to have fully  
 47 recovered from tagging. Overlaid means  $\pm$  95% confidence intervals represent model fits from  
 48 generalized additive mixed models (Tables S4, S5).