

METHODOLOGY

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# A comparison of survival and behavior of lake whitefish following transmitter implantation using electro- or chemical immobilization

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## Abstract

**Background** The number of telemetry studies focused on lake whitefish (*Coregonus clupeaformis*) in the Laurentian Great Lakes has steadily increased over the last decade, but field tests of immobilization methods used for tag implantation, which have the potential to affect survival and behavior of fish after release, are lacking. We compared post-tagging survival and behavior of lake whitefish that were immobilized for tag implantation using electroimmobilization via a transcutaneous electrical nerve stimulation (TENS) unit or by chemical immobilization via exposure to 10% eugenol.

**Results** Acoustic tags were implanted into 126 adult lake whitefish ( $N = 126$ ;  $N = 67$  TENS treatment group,  $N = 59$  eugenol treatment group) collected from the Fox River, Wisconsin, during the spawning period in November 2021. We found no significant differences between treatments in the number of days that lake whitefish spent in the Fox River following tagging (TENS mean = 13.4 days, eugenol mean = 14.7), and also found that the proportions of fish within each treatment group that returned to the Fox River during fall 2022 (51% from TENS treatment group, 49% from eugenol treatment group) did not differ from the proportions for all fish that were confirmed to be alive at that time. The best Cormack–Jolly–Seber model indicated no differences in survival between the two treatment groups (monthly survival = 0.980, 95% CI 0.970–0.987). Fish immobilized using TENS underwent almost immediate induction and recovery from surgeries, while fish immobilized using eugenol had induction times that ranged 167–487 s (mean = 347 s) and recovery times that ranged 51–2358 s (mean = 1242 s).

**Conclusions** Short- and long-term behavior (time to exit of Fox River, return to Fox River in the next spawning season) and monthly survival estimates of lake whitefish did not differ between the immobilization treatments. Either method may be suitable for immobilization during tag implantation, but the additional time needed for induction and recovery of fish when using eugenol may be a limiting factor in some field-based tagging situations.

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## Background

Telemetry studies have proven useful in informing fisheries management [1], particularly in large, multi-jurisdictional systems, such as the Laurentian Great Lakes [2–4]. Advances in technology, such as the miniaturization of transmitters and the development of passive receivers and deployment methods, have spurred the expansion of telemetry-based approaches for understanding fish movements, particularly since the early 2000s [5]. However, with increased interest in using telemetry to address management questions, it is important to make sure that tagging methods are optimized, especially for species that have not been the focus of many previous telemetry studies.

Telemetry often involves the surgical implantation of a transmitter (although external attachment is also common), which requires immobilization during the surgical process. While many immobilization options are available for use during surgery [6], specific guidance regarding the “best” method is often lacking, especially in understudied or new focal species or when tagging expands to include fish of different sizes and ages. Methods used in transmitter implantation surgeries need to be evaluated to ensure that the processes involved with capture, handling, and tagging have minimal effect on post-tagging behavior and survival [7].

Lake whitefish (*Coregonus clupeaformis*) is a species of economic and cultural importance in the Laurentian Great Lakes. The species supports Tribal, recreational, and commercial fisheries throughout the region, with the dockside value of commercial harvest exceeding \$9.5 million USD on an annual basis [8]. Lake whitefish stocks are managed using geographically defined management zones, making the movement of fish among zones an important management consideration [9–11]. Additionally, recent recruitment declines [12] have resulted in an increase in research focused on lake whitefish, including telemetry-based studies [13–16]. While earlier studies in smaller lakes have used tricaine methanesulfonate (MS-222) for immobilization of lake whitefish prior to acoustic transmitter surgery [17, 18], this chemical immobilization method is not an option for field-based studies in the Great Lakes region because it requires a 21-day withdrawal period before fish can be released if they could potentially be harvested for consumption [19]. Only one previous study evaluated the potential differences in short-term survival (48-h) of lake whitefish held in captivity following transmitter implantation using two immobilization methods approved for immediate release of tagged fish: electroimmobilization via transcutaneous electrical nerve stimulation (TENS) and chemical immobilization via exposure to 10% eugenol (synthetic clove-oil) [20]. These methods are commonly

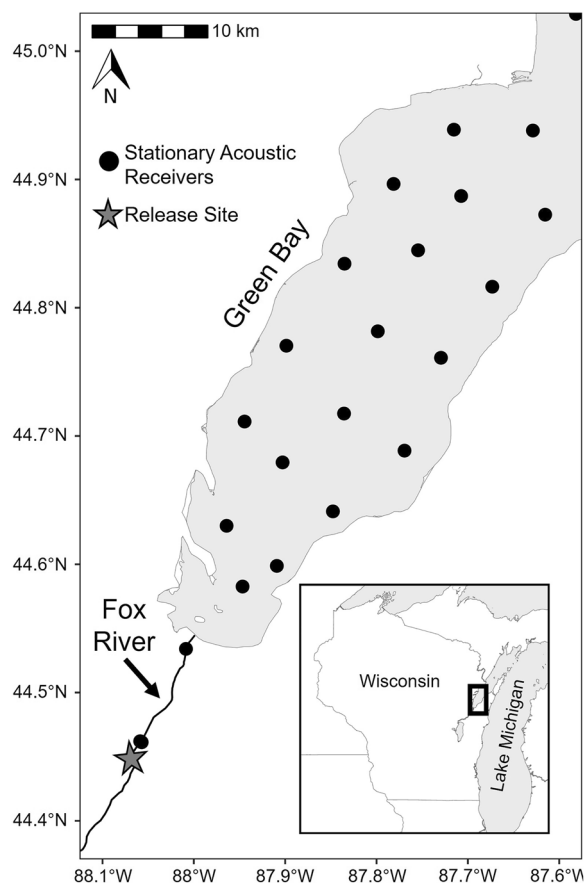
used to immobilize fish in the Great Lakes region during acoustic transmitter implantation [3, 21–26]. The TENS unit immobilizes fish using low-voltage electricity, which can elicit electrotetanus (muscle contraction) or electronarcosis (muscle relaxation or unconsciousness) in fishes [27–29]. Eugenol, in the form of Aqui-S 20E (Aqui-S New Zealand, Ltd. New Zealand), is an investigational new animal drug (INAD) that requires a special permit and monitoring process during use. The drug blocks the transmission of nervous signals through permeable membranes [19] to induce a sedative effect.

Both immobilization methods yielded high 48-h post-tagging survival of lake whitefish held in captivity [20]. Conversely, estimates of lake whitefish survival where fish were released into the Great Lakes following tag implantation where TENS was used for immobilization have been highly variable; for example, in Green Bay, the percentage of tags implanted in lake whitefish that had detections for more than 30 days ranged from 15 to 66% among five tagging groups [30]. These results prompted interest in determining whether use of chemical immobilization via exposure to eugenol might provide higher survival rates. Consequently, our objective was to determine if survival and post-tagging behavior of adult lake whitefish varied when immobilizing fish via TENS or through exposure to eugenol for implantation of acoustic transmitters.

## Methods

### Sampling and tagging

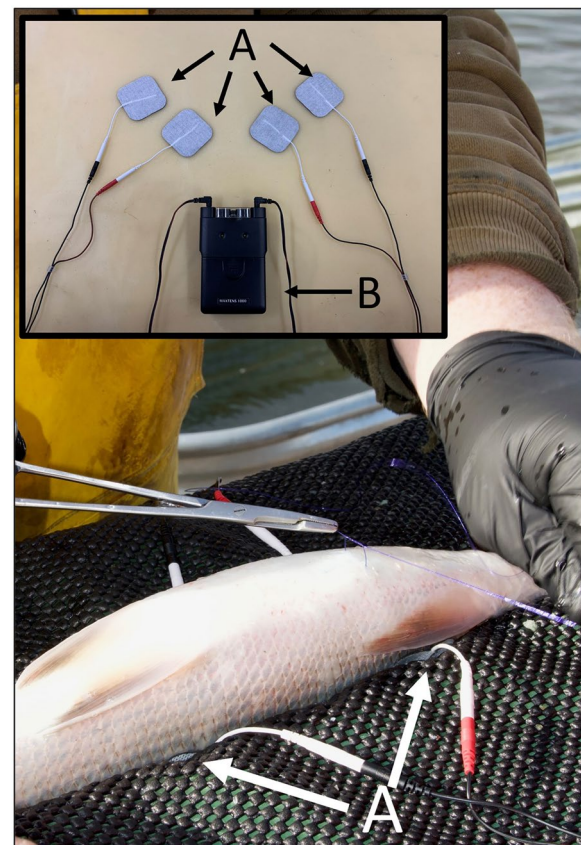
Adult lake whitefish were collected from the lower Fox River that flows into southern Green Bay (Fig. 1) over six sampling events during November 2021 (November 1, 2, 9, 10, 15, and 16) using daytime boat electrofishing (pulsed-DC; 3–5 A). Electrofishing occurred in the area < 1 km downstream of De Pere Dam in De Pere, Wisconsin, where lake whitefish spawning habitat is located. Following each electrofishing event, fish were transferred to two large cylindrical holding tanks (1,552 L) supplied with fresh, aerated water for a brief observation period (1–2 min) to assess general behavior and condition. Fish that were not in good condition for surgery (e.g., inability to maintain equilibrium, abnormal swimming behavior or opercular movement) were released back into the Fox River; no more than 12 fish were held in each tank at any one point in time. Holding tanks were equipped with submersible pumps to provide a moderate circular flow (i.e., resistance to aid fish in maintaining equilibrium). Water temperature (°C) and dissolved oxygen concentration (DO; mg/L) were monitored in holding tanks and in the ambient Fox River; tanks were re-filled periodically throughout each tagging event and ice blocks were periodically added so



**Fig. 1** Map of the study area, including Green Bay and the Fox River, Wisconsin. For visual clarity, the map includes only southern Green Bay, but the grid of acoustic receivers extends throughout all of Green Bay. Stationary acoustic receiver stations are indicated by black circles, and the grey star indicates the release site for lake whitefish (*Coregonus clupeaformis*) tagged with acoustic transmitters. The release site was located just downstream of the De Pere Dam in De Pere, Wisconsin

water conditions in holding tanks did not deviate from ambient conditions. Only fish  $\geq 406$  mm total length (TL; mm) that appeared to fully recover from the initial stressors of collection, handling, and transfer were selected for further processing and transmitter implantation. Fish were held for up to 20 min prior to tagging.

Once selected for further processing, lake whitefish were measured for TL, identified as male or female based on extrusion of gametes or visual inspection of gonads (when possible) through the incision, and a small caudal fin clip was removed for genetic analysis. Lock-on high-reward (\$100 USD) loop tags (Floy Model FT-4; Floy Mfg., Seattle, Washington) were attached to all lake whitefish prior to surgery by inserting the tag into a hollow stainless needle that was passed through the dorsal musculature posterior to the dorsal fin



**Fig. 2** Photographs (credit: D.J. Dembkowski) of transcutaneous electrical nerve stimulation (TENS) unit (MAXTENS 1000; BioProtech, Inc., Korea; *inset*) and how the unit was set up to immobilize lake whitefish (*Coregonus clupeaformis*) for surgical implantation of acoustic transmitters. **A** paired adhesive electrodes (shown placed on sides of fish in main panel); **B** battery-operated pulse generator

approximately 1 cm below the dorsal surface. External tags were affixed to aid in recovery of acoustic transmitters from fish caught by fishers.

Lake whitefish were haphazardly assigned to one of the two immobilization treatments, with approximately equal proportions of both sexes assigned to each treatment. For fish in the electroimmobilization treatment group, electroimmobilization was administered during tag implantation using a handheld battery-operated TENS unit (MAXTENS 1000; BioProtech, Inc., Korea) with adjustable power settings capable of producing a variable output of 0–80 mA pulsed direct current. When fish were placed in a slotted foam surgery platform, paired adhesive electrodes (i.e., positive–negative) were placed on either side of the fish near the pectoral fins and at a point that intersected the adipose and anal fins (Fig. 2). The power setting of the TENS unit was increased from zero until a point at which fish exhibited electrotetanus; power settings were adjusted for individual fish but

generally ranged 2–3 mA. Induction for lake whitefish using the TENS unit was instantaneous [20], so induction times were not recorded for the electroimmobilization treatment group. For each fish, the power setting used to induce electrotetanus was maintained throughout the duration of the surgery.

For the chemical immobilization treatment, fish were anesthetized in a bath of Aqui-S 20E (10% eugenol) at a concentration of 40 mg/L. A dosage of 25–40 mg/L of Aqui-S 20E is recommended for salmonids [31]; the specific concentration used herein (40 mg/L) was recommended to induce surgery-level anesthesia for adult lake whitefish by fish health specialists with the Wisconsin Department of Natural Resources responsible for administering the INAD permit (D. Godard and N. Nietlisbach, written communication, September 2021). An induction bath of Aqui-S 20E was prepared in an aerated 142-L tank and fish were induced one at a time; the induction bath was changed as necessary (1–2 times on each day of tagging) to maintain an appropriate concentration of the chemical. Induction time (s) for fish in this treatment group was measured from when fish were placed in the induction bath until a complete loss of equilibrium and cessation of reflex activity (Stage-4 anesthesia; [28]), at which point fish were transferred from the induction bath to undergo surgical implantation of an acoustic transmitter.

Surgical procedures for fish in both treatment groups were the same and generally followed Great Lakes Fishery Commission standard operating procedures for intra-coelomic implantation of acoustic transmitters [32]. Lake whitefish were placed ventral-side up in a slotted surgery platform with rubberized foam padding and fish in both treatment groups received a constant supply of fresh water during the surgical procedure. No maintenance dose of eugenol was used during acoustic transmitter surgery. Transmitter implantation involved making a 2–3 cm mid-ventral incision posterior to the pelvic girdle through which a sterilized acoustic transmitter (V13-1H; 13 mm × 36 mm; 11 g in air; 6 g in water; Innovasea, Boston, Massachusetts) was inserted into the body cavity. Acoustic transmitters and surgical tools were sterilized in a betadine solution (povidone-iodine; 7%) and rinsed with sterile saline solution between each surgery. Surgical incisions were closed with 2–3 simple interrupted sutures (2/0 PDO monofilament absorbable suture; 24-mm reverse cutting needle; Covetrus, Dublin, Ohio). Surgeries were performed by two experienced surgeons and surgery time (s) was recorded from when fish were first placed on the surgery platform to completion of the final suture. Following surgery, fish were immediately transferred to one of two large cylindrical holding tanks for a recovery and observation period prior to release.

Based on previous research [20], recovery times for lake whitefish immobilized using the TENS method were typically instantaneous or less than 30 s; thus, recovery times were not recorded for fish in this treatment group. Recovery time (s) for fish in the eugenol treatment group was measured from the point fish were placed in the recovery tank to when equilibrium, station-holding, swimming ability, and normal opercular movement were observed (Stage-0 anesthesia; [28]).

### Monitoring movements and behaviors

Movements of lake whitefish were monitored using an array of stationary acoustic receivers equipped with omnidirectional hydrophones (Innovasea VR2W or VR2AR) deployed as part of the Great Lakes Acoustic Telemetry Observation System (GLATOS) receiver network [2]. The receiver array in Green Bay included 78 open-water receivers positioned in an 8-km grid and 3 receivers in the Fox River (Fig. 1). Detection data were downloaded from receivers on an annual basis; receivers relevant to this study were downloaded during June–July 2022 and 2023.

Before analyses, acoustic detection data were filtered for false detections using the 'glatos' package in R [33]. The detection filter, which follows the protocols outlined by Pincock [34], removed 18,412 detections, which represented 1.31% of the total data set. Additionally, abacus plots that display the location of individual fish over time [33] were examined for the study period (November 2021 through November 2022) to look for evidence of presumed mortalities or tag shedding events near a receiver. These types of mortalities or tag shedding events in a highly mobile species like lake whitefish would have been expected to result in consistent detections in a single location for >1 month until the end of the study period [35]. For the purposes of our analyses, we assumed that mortality or shedding occurred when the fish first arrived at the last receiver site [35], but any detections before that timepoint were used in our analyses. Besides fish that met this criterion, all others were assumed to be alive if they were detected on any receiver during a given time period of interest.

### Data analysis

Two-sample *t*-tests were used to determine if TLs and surgery times differed between immobilization treatment groups. In order to look at potential effects of immobilization treatment on tagged lake whitefish, we examined (1) behavior after tagging, using time in days from tagging until entry to Green Bay (i.e., egress time); (2) survival after tagging, using Cormack–Jolly–Seber (CJS) models to investigate monthly survival; and (3) the proportion that returned to the Fox River the following year during



the spawning period. Statistical analyses for metrics (1) and (2) were conducted in R version 4.1.2 [36], and statistical analyses for metric (3) were performed using the SAS statistical program [37]. For all analyses, differences were considered significant at an  $\alpha$  level of 0.05.

### (1) Behavior after tagging

Egress time was chosen as a behavioral metric because tagged lake whitefish were in the Fox River for spawning, and the expected behavioral pattern would be for fish to exit the river after spawning. Fish needed to travel approximately 10 km from the release point to the mouth of the Fox River to enter Green Bay. Potential differences in egress time between immobilization techniques could imply that choice of technique differentially alters the behavior of lake whitefish following tagging. In previous years, telemetry indicated that some lake whitefish overwinter in the Fox River after spawning ( $N=5$  to 10/year). To avoid potential biases resulting from this behavior, we only examined egress times for lake whitefish that exited the river before January 1, 2022. Only 13 individuals remained in the Fox River overwinter, so we were not able to evaluate statistically if there was a difference in the prevalence of this behavior between treatment groups. For lake whitefish that exited the Fox River in November or December, entry time into Green Bay was calculated using the first detection on any receiver in the open waters of Green Bay proper. A two-sample  $t$ -test was used to determine if time of entry to Green Bay after tagging differed between the two immobilization treatments.

### (2) Survival after tagging

To estimate survival after tagging, we used acoustic detection data and CJS models to estimate monthly apparent survival ( $\phi$ ) and detection probability ( $p$ ) for each treatment group. The term apparent survival is used because the model cannot distinguish between mortality and emigration from the study area. However, previous research indicates that lake whitefish spawning in the Fox River rarely leave southern Green Bay [30], so we assumed that the  $\phi$  parameter represented a proxy for true survival probability of these tagged cohorts. Detection data were reduced into monthly capture histories indicating whether a fish was detected (1 for detected, 0 for not detected) on any receiver in the array (both in the Fox River and in Green Bay) during each month from December 2021 through October 2022. Since lake whitefish were tagged in November 2021, all fish received a 1 for that month, and the small number ( $N=5$ ) of tagged fish that were harvested during November 2021 were removed from survival analysis. Fish that were identified as mortalities or tag shredding events based on

abacus plots (see description above) received a 1 for the month they arrived at the final receiver, and then 0 s for the remaining months in the study. October 2022 was selected as the endpoint for survival analysis to avoid including potential mortality that would have occurred during the spawning period in late October/early November.

Survival analysis was implemented in R using the RMark analysis package [38]. The RMark package runs using program MARK [39], which allows for construction of potential CJS models and their comparison to select the “best fit” model for the data. Since the focus of this study was survival, and  $p$  could be considered a nuisance parameter, both time-varying and constant  $p$  models were initially tested to determine which provided the best fit for the  $p$  parameter. Preliminary results indicated that constant  $p$  was supported over time-varying  $p$ , and thus was used in all subsequent survival models. A total of four models were investigated for the  $\phi$  parameter: (1) constant  $\phi$ , (2) time-varying  $\phi$ , by month, (3)  $\phi$  varying by immobilization treatment, and (4)  $\phi$  varying by month and by immobilization treatment. Prior to model selection, program MARK was used to estimate the median  $\hat{c}$  as a measure of goodness-of-fit [40]. The global model used to assess goodness-of-fit included time-varying  $\phi$  and constant  $p$ , as well as an effect of treatment group on  $\phi$ . If overdispersion was identified, the median  $\hat{c}$  value was used to adjust Akaike's information criterion (AIC) scores to QAICc (quasi-AIC corrected for low sample size) scores for model comparison. Models with a  $\Delta$ QAICc score  $< 2$  were considered competitive [41].

### (3) Fox River returns

Lake whitefish were tagged during the spawning period, which involves a distinct migration from Green Bay proper into the Fox River, and we wanted to examine if fish behavior during the following spawning period may have been influenced by choice of immobilization method. We used a Chi-squared test to determine if the proportions of fish within each treatment that returned to the Fox River during fall 2022 differed from the proportions for all fish confirmed to be alive in October 2022. A difference in the treatment proportions of fish returning to the Fox River in 2022 would suggest differential behavior between treatments during the spawning run the year after tagging occurred.

## Results

A total of 126 adult lake whitefish (68 males, 52 females, and 6 fish of unknown sex) were implanted with acoustic transmitters during November 2021 (Table 1). Sixty-seven of the 126 fish (53%) were included in the TENS treatment group and 59 (47%) were included in

**Table 1** Lake Whitefish Tagging Summary

Treatment	N	Minimum	Mean	Maximum
Overall	126	430	494	570
Male	68	430	490	570
Female	52	431	502	547
Unknown	6	438	467	499
TENS	67	430	491	570
Male	36	430	485	570
Female	28	446	501	540
Unknown	3	460	477	499
Eugenol	59	431	496	563
Male	32	442	496	563
Female	24	431	502	547
Unknown	3	438	457	484

Number tagged (N) and minimum, mean, and maximum total lengths (mm) of lake whitefish (*Coregonus clupeaformis*) in each immobilization treatment group for acoustic tag surgery that took place in the Fox River, Wisconsin, during November 2021. TENS = transcutaneous electrical nerve stimulation; Eugenol = Aquis 20E (10% eugenol) at a concentration of 40 mg/L

the eugenol treatment group. Similar proportions of male, female, and unknown-sex lake whitefish were included in each treatment group (Table 1). Total length of lake whitefish ranged from 430 to 570 mm TL (mean = 494 mm) and did not differ significantly between treatment groups ( $t=0.92$ ;  $P=0.36$ ). For fish in the eugenol treatment group, induction times ranged from 167 to 487 s (mean = 347 s) and recovery times ranged from 51 to 2358 s (mean = 1242 s). Surgery time ranged from 115 to 282 s (mean = 163 s) and did not differ significantly between treatment groups (TENS mean = 162 s, eugenol mean = 164 s;  $t=0.29$ ;  $P=0.77$ ). Across tagging dates, ambient water temperature ranged from 5.9 to 10.0 °C and DO was between 9.7 and 12.5 mg/L; temperature and DO conditions in holding and recovery tanks were maintained within  $\pm 1$  °C and  $\pm 1$  mg/L of ambient conditions, respectively.

### (1) Behavior after tagging

A total of 104 lake whitefish (58 TENS treatment, 46 eugenol treatment) left the Fox River in November or December of 2021 and were used to assess potential differences in egress time between immobilization treatments. In the TENS treatment, lake whitefish spent on average 13.4 days after tagging in the Fox River before entering Green Bay (range: 2.1–42.1 days), while lake whitefish in the eugenol treatment spent 14.7 days on average in the Fox River after tagging before entering Green Bay (range: 2.4–45.9 days). There was no significant difference between egress times for lake whitefish in the two immobilization treatment groups ( $t=0.604$ ;  $P=0.55$ ; Fig. 3a). The 22 lake whitefish that did not leave

the Fox River in November or December were either harvested in November ( $N=5$ ), harvested in February and March ( $N=2$ ), left the river in March through May ( $N=13$ ), or never left the river during the study period and their fate was unknown ( $N=2$ , one from the TENS treatment group and one from the eugenol treatment group).

### (2) Survival after tagging

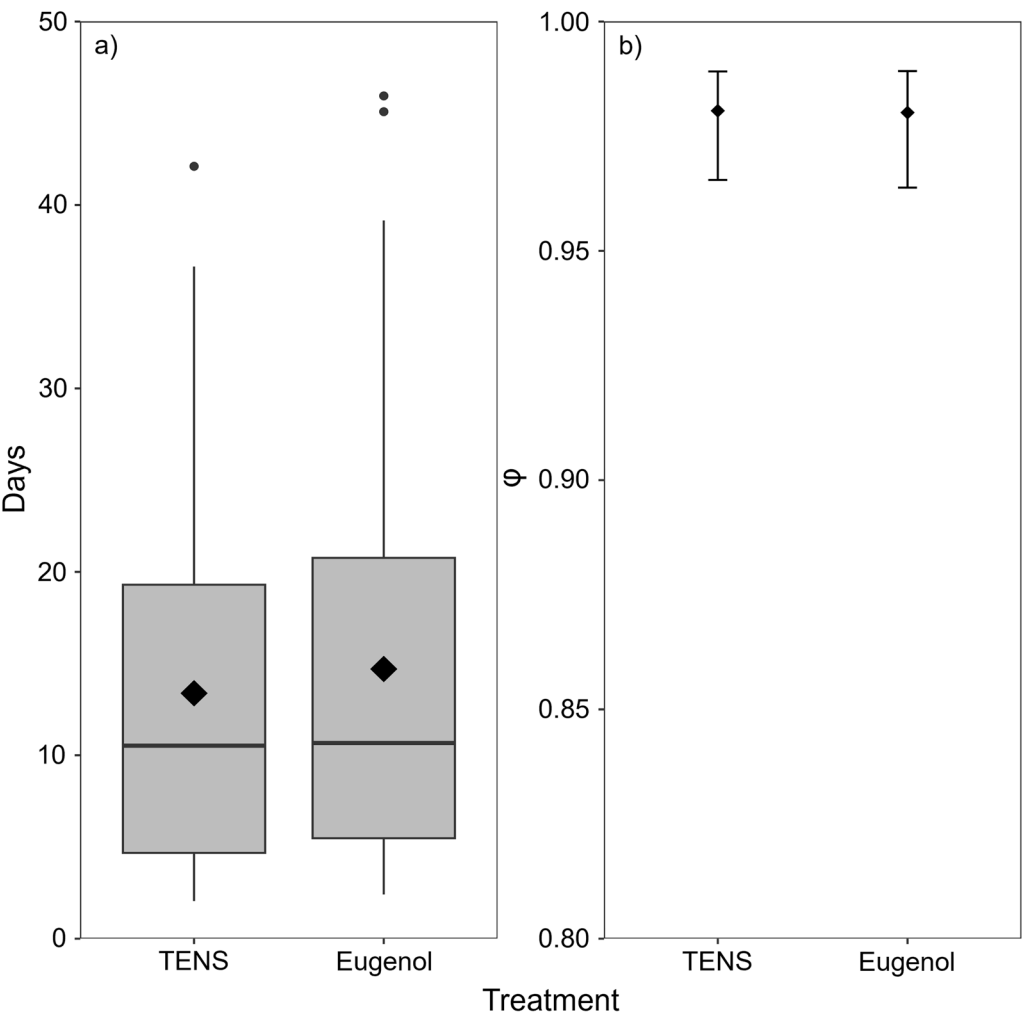
A total of 121 lake whitefish were used in survival analysis (TENS = 63 individuals, eugenol = 58 individuals). A median  $\hat{c}$  value of 2.06 (SE = 0.01) was estimated by the goodness-of-fit test and was subsequently used to calculate QAICc values for the four models tested. The best supported model included constant  $\phi$  over the study period ( $\phi=0.980$ , 95% CI 0.970–0.987), while the next best model ( $\Delta\text{QAICc}=2.01$ ) included the effect of immobilization treatment on  $\phi$  (Table 2). However, the model that included an effect of immobilization treatment on  $\phi$  showed no differences in the monthly survival estimates (TENS:  $\phi=0.981$ , 95% CI 0.965–0.989; eugenol:  $\phi=0.980$ , 95% CI 0.964–0.989; Fig. 3b). Parameter estimates for the top two models are reported in Table 3.

### (3) Fox River returns

Detection data indicated that 84 lake whitefish (TENS = 45 individuals, eugenol = 39 individuals) were still assumed alive and available to return to the Fox River in October 2022. Of those, 74 lake whitefish returned to the Fox River (return rate = 88%); 38 (51%) were from the TENS treatment group and 36 (49%) were from the eugenol treatment group. The proportion of fish in each treatment group detected in the Fox River during the spawning period in 2022 did not differ significantly from the proportion of fish in each treatment group that were at large and available to return ( $\chi^2=0.21$ ;  $P=0.65$ ).

## Discussion

Based on multiple metrics, our study suggests that short- (1–2 month) and long-term (spawning run the following year) behavior and monthly survival of adult lake whitefish in the year after tagging did not differ when using eugenol or TENS to immobilize fish for surgery. While an increasing number of telemetry studies focusing on lake whitefish have occurred in the last ten years [13–16], to our knowledge this is only the second study to evaluate the effects of part of the acoustic tagging process on this species. The results of our field-based study mirror those of the short-term trials conducted by Dembowski et al. [20], which found high (100%) survival of lake whitefish up to 48 h following surgical tagging procedures with no differences noted between eugenol and TENS immobilization treatments.



**Fig. 3** Comparison of post-tagging egress times from the Fox River **(a)** and survival estimates **(b)** for two immobilization treatments (TENS = transcutaneous electrical nerve stimulation; Eugenol = Aqui-S 20E (10% eugenol) at a concentration of 40 mg/L) tested on lake whitefish (*Coregonus clupeaformis*) for acoustic tagging in the Fox River, Wisconsin, in November 2021. Boxplot in panel a includes a horizontal black line representing the median, a black diamond representing the mean, boxes showing the interquartile range, and whiskers showing the extremes (1.5 × the interquartile range). The y-axis represents the number of days from tagging until fish were detected on any acoustic receiver in Green Bay (see Fig. 1 for details of acoustic receiver array). Plot in panel b shows monthly survival ( $\phi$ ) estimated using the Cormack–Jolly–Seber model (see Tables 2, 3) that included the effect of immobilization method on survival, with error bars representing 95% confidence intervals. No differences were detected between the two immobilization treatments based on these metrics

**Table 2** QAICc Table, CJS Models of Lake Whitefish Survival

Model	Parameters	QAICc	$\Delta$ QAICc	$w_i$	QDeviance
$\phi(\text{constant}), p(\text{constant})$	2	271.31	0.00	0.73	78.52
$\phi(\text{immobilization}), p(\text{constant})$	3	273.32	2.01	0.27	78.51
$\phi(\text{time}), p(\text{constant})$	12	283.40	12.09	0.00	70.34
$\phi(\text{time} + \text{immobilization}), p(\text{constant})$	13	245.95	11.15	0.00	70.34

Quasi-Akaike information criterion corrected for low sample size (QAICc) table showing the results of Cormack–Jolly–Seber models used to test if immobilization treatment had an effect on monthly survival of lake whitefish (*Coregonus clupeaformis*) after acoustic tag surgery in the Fox River, Wisconsin, in November 2021, including QAICc scores, the difference in QAICc score between the specified model and the top model ( $\Delta$ QAICc), and the Akaike weights ( $w_i$ ). Values were corrected for overdispersion based on a median  $\hat{c}$  value of 2.06

**Table 3** CJS Model Summaries

Model	Parameter	Description	Estimate	95% CI
(1)	$\phi(\text{constant}), p(\text{constant})$			
	Monthly survival ( $\phi$ )	All	0.980	0.970–0.987
(2)	Detection probability ( $p$ )	All	0.966	0.953–0.975
	$\phi(\text{immobilization}), p(\text{constant})$			
	Monthly survival ( $\phi$ )	TENS	0.981	0.965–0.989
		Eugenol	0.980	0.964–0.989
	Detection probability ( $p$ )	All	0.966	0.953–0.975

Cormack–Jolly–Seber model estimates and 95% confidence intervals (CI) for monthly survival ( $\phi$ ) and detection probability ( $p$ ) of lake whitefish (*Coregonus clupeaformis*) acoustically tagged in the Fox River, Wisconsin, for the top two models based on Quasi-Akaike information criterion corrected for low sample size (QAICc) scores (see Table 2 for further information on model selection). TENS = transcutaneous electrical nerve stimulation; Eugenol = AQUI-S 20E (10% eugenol) at a concentration of 40 mg/L

While we did not detect any differences between immobilization treatments based on the behavior and survival metrics that we examined, there may have been other effects from using either TENS or eugenol for immobilization. For example, there may have been immediate post-release differences in behavior that we could not detect in our study design, but these potential differences did not appear to influence when fish left the Fox River or estimated survival. Additionally, we did not collect data on reproductive status prior to tagging, which could have influenced egress times from the Fox River. Lastly, we could not use reference fish to test if behavior or survival of tagged fish differed in any way from those that had not undergone acoustic tag implantation. Use of telemetry assumes that implantation of transmitters does not affect fish in ways that may alter our ability to draw accurate conclusions from the data collected [7]. Previous work showed that lake whitefish that underwent acoustic tagging surgery showed no differences in mortality compared to reference fish that only received a loop tag [20], so we assume that the surgery itself, including immobilization, did not have a significant effect on survival of lake whitefish in our study.

We noted long recovery times (> 20 min) for lake whitefish that were immobilized using eugenol. Recovery times for multiple chemical anesthetic agents, including eugenol, have been observed to be temperature-dependent, with recovery times decreasing as water temperature increase [42–44]. Tagging for our study took place during the lake whitefish spawning season in the fall, when water temperatures were  $\leq 10$  °C. It is possible that recovery times would be reduced under warmer water conditions (i.e., outside of the spawning season). It is also possible that changing the dose could shorten the recovery time while still providing adequate sedation for acoustic tag

surgeries. The dose chosen (AQUI-S 20E, 10% eugenol at a concentration of 40 mg/L) was recommended by fish health specialists with the Wisconsin Department of Natural Resources (D. Godard and N. Nietlisbach, personal communication), and it was outside the scope of our study to investigate multiple dosing options. In other species, including warm-, cool-, and cold-water fishes, previous work has noted that higher doses of eugenol often lead to shorter induction times but may increase recovery times [43, 44]. Future research that aims to use eugenol would benefit from controlled studies examining the relationships between dose, water temperature, and recovery and induction times to find the optimal combination for field-based tagging efforts.

Because our results demonstrate that either immobilization method is suitable for transmitter implantation, the decision of which method to use may ultimately depend on the location and equipment available for tagging operations. Some potential downsides to the use of eugenol for immobilization include the increased time needed for induction and recovery, additional time for changing the induction bath during sampling, the need for an increased number of fish holding facilities (separate tanks for induction and recovery), appropriate disposal of the chemical anesthetic bath, and extra monitoring of water quality, induction, and recovery times under the INAD permit process that is currently required to use the anesthetic in immediate-release situations. Some of these issues may not be of concern, particularly for shore-based tagging operations with additional space for tanks and personnel. However, more field-based tagging operations are occurring on boats (both research and commercial fishing vessels) to minimize fish displacement and transit time before tagging. In these cases, it may not be feasible to use eugenol for immobilization and TENS may be the preferred option.

In part, this study was spurred by previous lake whitefish tagging projects that indicated higher-than-expected assumed mortality (< 20 lake whitefish with available data out of 101 tagged; [30]). Survival was high in groups using both eugenol and TENS as immobilizing agents in the current study, so in the previous study that used TENS only it is unlikely that immobilization method was the primary cause of the high mortality. One potential reason for the differences in mortality between the current study and previous tagging efforts that was not investigated explicitly was the impact of pre-surgical holding on lake whitefish survival following transmitter implantation. For the previous field-based study, lake whitefish were held in 640 L oval cattle tanks prior to tagging. For the current study, fish were held in 1,552 L circular tanks prior to tagging. Additionally, a pump was used to create constant flow for fish to orient to and densities of fish



in the tank were low (<12 fish at a time). While crowding and holding during aquaculture-related activities has been shown to increase stress in fishes [45], and surgical best practices for telemetry studies note the importance of reducing stress during capture and handling [6, 46], studies focusing on telemetry that specifically aim to disentangle the impact of pre-surgical capture and handling and the tagging procedure itself are lacking [47, 48]. Future research that focuses on investigating and improving appropriate handling methods for fish species before and after tagging surgery may be beneficial to improving survival, and therefore inferences that can be drawn from acoustic telemetry projects.

## Conclusions

Our results demonstrate that lake whitefish immobilized using TENS or eugenol show no differences in survival and behavior based on multiple metrics. Therefore, either method may be suitable for immobilization during tag implantation, but the additional time needed for induction and recovery of fish when using eugenol may be a limiting factor in some field-based tagging situations. The data we have presented provide important information for those planning acoustic telemetry studies of lake whitefish (or related species) regarding choice of most appropriate immobilization treatment. For lake whitefish as well as other fish species, considering the impacts of different components of the surgical process, including pre- and post-surgical handling in both the laboratory and the field, may be important to optimizing the use of telemetry to study fishes.

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## Author contributions

This study was designed by DAI, DJD, TRB, and CSV. Fish tagging was conducted by DAI and DJD. SPH coordinated Wisconsin Department of Natural Resources sampling to provide lake whitefish for tagging for this study. Data

analysis, interpretation, and the initial draft of the manuscript were completed by LKI and DJD. All authors read, edited, and approved the final manuscript.

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## Availability of data and materials

At the time of publication, data were not publicly available through GLATOS. The datasets used and/or analyzed during the current study are available from the corresponding author on request. Data inquiries can be directed to L.K. Izzo (lizzo1@utk.edu).

## Declarations

### Competing interests

The authors declare no competing interests.

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