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MANAGEMENT BRIEF

Evaluation of Tag Retention, Healing, Growth, and Behavior in Age-0 Muskellunge Following Acoustic Transmitter Implantation

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Abstract

The development of small acoustic transmitters has enabled researchers to monitor earlier life stages and smaller fish species than was previously possible. The underlying assumptions of any telemetry study are minimal tag loss and negligible effects on the behavior, survival, and growth of tagged individuals. To that end, tag retention, healing, survival, specific growth rates, and behavior were evaluated for 96 age-0 Muskellunge *Esox masquinongy* (TL [mean ± SD] = 205 ± 10 mm) from three treatment groups. Tagged fish were compared to untagged controls and sham fish (fish that had undergone anesthesia and laparotomy but not transmitter implantation). Thirty-two fish (tagged group) were implanted with one of the smallest commercially available acoustic transmitters (Juvenile Salmon Acoustic Telemetry tag; 12.0 × 5.3 × 3.7 mm, 0.217 g in air, >120-d tag life) and monitored in a 4-month, overwinter tank experiment. Tricaine methanesulfonate was used for anesthesia, incisions were closed with a synthetic absorbable monofilament, and all surgeries were conducted by a single trained researcher. All tags were retained throughout the experiment; surgical wounds healed within 30 d, 32% of sutures were retained at 120 d postsurgery, and survival did not differ between treatments. No biologically significant effects of tagging on mean relative growth rates (percent change in weight/d) were observed among the three groups (tagged, untagged, and sham fish) at 4 months postprocessing. The reaction of tagged fish to a moving object within 15 minutes after tagging was slower than the reaction at 7 d postsurgery, reiterating the importance of testing appropriate sedation methods prior to releasing fish in field studies. Results validate the utility of surgical implantation of small acoustic transmitters in juvenile Muskellunge for future studies, although immobilization methods for early life stages require further study.

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Telemetry has become a common tool for studying the spatial ecology and survival of fish (Donaldson et al. 2014; Hussey et al. 2015). Early telemetry studies focused on larger-bodied fish species and adult life stages, but technological enhancements (e.g., smaller tag size and longer battery life) have enabled tracking and collection of real-time movement data for smaller life stages and species. In telemetry studies, it is often presumed that the behavior, condition, and fate of tagged fish are not influenced by transmitter presence or the tagging process, suggesting that tagged fish are representative of untagged conspecifics (Skalski et al. 2001; Bridger and Booth 2003; Caputo et al. 2009). However, the extent to which that presumption is correct has been questioned, particularly when transmitters are implanted in the coelom (Brown et al. 2011). Therefore, evaluation of tagging procedures to identify methods that minimize impacts on the welfare of tagged fish is important (e.g., Walsh et al. 2000; Wagner and Cooke 2005). The ratio of tag size (volume, shape, and mass) to fish body size has been identified as being influential (e.g., on healing, retention, behavioral impairments, and survival; Jepsen et al. 2004). Nonetheless, other aspects, such as suture material, incision location, surgeon experience, and tag type (e.g., presence of an antenna for radio tags; tag coating), are also important considerations (reviewed by Bridger and Booth 2003; Cooke et al. 2011; Thorstad et al. 2013). Clearly, there is a large degree of interspecific variation in responses that is further mediated by the environment (Cooke et al. 2011).

Researchers use telemetry tools to explore and assess the spatial behavior of fish in their natural environment (Lucas and Baras 2000), often to develop a mechanistic understanding of spatial patterns (e.g., Cooke et al. 2008). Research efforts are expanding to study the spatiotemporal ecology and survival of juvenile fish, including Muskellunge *Esox masquinongy* (e.g., Hanson and Margenau 1992; Owensby et al. 2017), Northern Pike *E. lucius* (Hühn et al. 2014), and both species in a sympatric setting (Farrell et al. 2014). Validating how telemetry tools may influence the welfare of fish or may limit data interpretation is therefore imperative. For instance, implantation of small tags (e.g., PIT tags) was not found to impair the welfare of or produce sublethal effects on age-0 fish (e.g., Acolas et al. 2007; Richard et al. 2013; Tiffan et al. 2015), including age-0 Muskellunge (Wagner et al. 2007; Younk et al. 2010) and Northern Pike (Hühn et al. 2014). Radiotelemetry has been a popular tool with which to estimate mortality, dispersal, and habitat use of stocked age-0 Muskellunge (Hanson and Margenau 1992; Wagner and Wahl 2011; Owensby et al. 2017); however, acoustic telemetry permits researchers to collect short-term movement and habitat use data (i.e., hourly) in challenging conditions (i.e., winter) and does not require an external antenna, which can be burdensome for small fish. Deters et al. (2010) evaluated implantation and retention associated with a micro-acoustic transmitter (e.g., Juvenile Salmon Acoustic Telemetry [JSAT] tag, 0.3 g; McMichael et al. 2010) and found that neither tag expulsion nor mortality was associated with implanting tags in juvenile Chinook Salmon *Oncorhynchus tshawytscha* (FL range = 96–121 mm; tag burden = 2–6% of body weight). Given that acoustic telemetry has grown in popularity as a tool for research on juvenile life stages and small fish (Hussey et al. 2015), there is merit in studying the effects of the surgical implantation and presence of these new mini-acoustic tags on other species.

With research gaps related to the spatial ecology of juvenile Muskellunge (e.g., Crane et al. 2015) and no publications evaluating the influence of acoustic telemetry tools for this species, the present paper focuses on the influence of surgical procedures, tag presence, and anesthesia on captive-reared age-0 Muskellunge. To this end, survival, tag retention, tag burden, tag encapsulation, incision healing, and growth rates of fish implanted with JSAT transmitters were compared with those of untagged controls and sham fish (fish that underwent surgery but not transmitter implantation). In addition, individual flight response was assessed after recovery from the anesthetic and at 7 d postsurgery. Endpoints were measured over specified sampling intervals (0–30, 30–60, 60–90, and 90–120 d posttagging) across the 120-d study.

METHODS

Fish Source and Rearing

Muskellunge used in this study were reared at the Sir Sandford Fleming College Muskellunge Hatchery (Lindsay, Ontario) in 2.4- × 1.2- × 1.2-m (length × width × height) tanks with a normal operating volume of 3,000 L of recirculated water. Eggs were collected from a native, wild stock in Gloucester Pool, Lake Huron (Wilson et al. 2016), and were fertilized on April 27, 2017. Fish were fed a manufactured salmonid Skretting diet: Nutra ST 0.3-mm crumble feed (58% protein, 18% lipid) as fry, then Nutra RC NP 1.8-mm (50% protein, 20% lipid) and Europa 15 4.0-mm (55% protein, 15% lipid) food pellets as juveniles grew.

Individual Identification, Anesthetization, and Surgical Procedures

A total of 96 Muskellunge (183–236 mm TL) were selected haphazardly from their source tanks and were assigned to one of three treatment groups for a total of 32 fish per treatment group (e.g., Younk et al. 2010): tagged (subjected to anesthesia, laparotomy, and acoustic tag implantation), sham (anesthesia and laparotomy but no tag implantation), and control (no anesthesia, laparotomy,

or tag implantation; measurements only). Sham controls were used to isolate the effects of the surgical procedure from the effects of transmitter presence in accordance with Cooke et al. (2011).

All fish were handled with electroimmobilization gloves (Roscoe Medical TENS 3000 unit, DT3002, low-voltage setting; as per Ward et al. 2017) and were implanted with two visible implant elastomer (VIE) tags for treatment group and individual identification prior to anesthetization for sham and tagged fish. The VIE tags were administered with handheld, 0.3-mL tuberculin syringes (29-gauge needle) coupled with syringe holders. The elastomer and curing agent were mixed for several minutes prior to injection. Ink was kept on ice to reduce coagulation during injections, and tag codes were implanted in a systematic order. Rather than being injected into the lower jaw, VIE tags were injected in the transparent tissue where each fin (pectoral, pelvic, anal, caudal, and dorsal) met the body cavity (Younk et al. 2010). All fish were weighed (nearest 0.1 g) and measured (nearest 1 mm TL) after VIE implantation. Because natural body markings are known as an effective identification tool (e.g., Wilson et al. 2006; Brooks et al. 2010; Barriga et al. 2015), the dorsal, caudal, and pelvic fins were photographed as a form of secondary identification by spot pattern. Photographs were actively consulted throughout the study when VIE tags in the sham group (tagged first) were lost.

To mimic in situ field surgery conditions, fish were not fasted prior to surgery. Before laparotomy, sham and tagged fish were anesthetized (70-mg/L solution of tricaine methanesulfonate [MS-222] in hatchery water) until opercular rates slowed and the fish were unresponsive to touch (see Carter et al. 2011; Wagner et al. 2011, 2014). Fish were placed supine on a surgery table in recirculating water and received a maintenance anesthetic dose (70-mg/L MS-222) by placing a small-diameter (8-mm) silicone rubber tube from a pump (in the recirculating tank) inside the mouth so that water gently flowed over the gills. A lengthwise incision (~5 mm) was made with a number-21 scalpel between the pelvic and pectoral fins and was closed with one simple interrupted suture (PDS II, 3/0; Ethicon, Inc.) after tag insertion. Tagged fish received a sterilized (with Virkon) JSAT tag ($12.0 \times 5.3 \times 3.7$ mm, 0.20 g in air; Pacific Northwest National Laboratory) implanted into the coelom. Surgical tools were sterilized in a diluted solution of Virkon between each surgery.

Incision Healing Assessment

Macroscopic inflammation (redness) and wound closure (amount of open dermal tissue) were scored as a percentage based on five incremented categories from 0 to 1 (Table 1), as established by Schoonyan et al. (2017). Photographs taken of the incision site in sham and tagged fish at each sampling interval were viewed and scored by two researchers independently. Due to the large variation in wound closure scores between each researcher for the 90- and 120-d sampling intervals, when sutures caused additional dehiscence (rupture), the more conservative scores were selected for analysis.

Flight Initiation Response

The sedation level of each fish was established postsurgery by lightly touching the fish every 30 s to determine its response stage as it recovered in an aerated cooler. Fish were no longer considered sedated when they reached equilibrium (became upright and displayed regular opercular and fin movements; Wagner et al. 2014). Activity and

TABLE 1. Scoring criteria used to identify levels of wound openness and inflammation in age-0 Muskellunge in a hatchery experiment. Criteria are from Schoonyan et al. (2017; modified from the incision index of Wagner et al. 2000).

Score	Incision openness	Inflammation
0.00	Dermis completely healed	No inflammation present
0.05–0.025	Dermis mostly healed (<25% of incision was open)	Low levels of inflammation present (<25% of sutures and incision inflamed); slightly pink
0.30–0.50	Obvious dermal healing along < 50% of the length of the incision (between 30% and 50% open)	Low to moderate levels of inflammation present (between 30% and 50% around incision site and around sutured region); pink to red
0.55–0.75	Noticeable dermal healing along < 75% of the length of the incision (between 55% and 75% open)	Moderate to high levels of inflammation present around incision site and sutures (between 55% and 75% inflamed); reddened
0.80–0.95	Only trace evidence of dermal healing (80–95% open)	High levels of inflammation present throughout entire incision and sutures; very red
1.00	No evidence of healing (100% open)	Very high levels of inflammation; may have spread to dermal tissue outside of suture region

reaction time were recorded using a Hero 3 GoPro, mounted above a 40-L aquarium (51 × 25 × 30 cm). A Styrofoam sheet with 2.54- × 2.54-cm grid squares was placed under the aquarium to quantify movement. Fish were placed in the aquarium once they reached equilibrium, and they were permitted to acclimate for 5 min. Fish activity was recorded between the 5- and 10-min mark, when a moving object (76.5-cm, plastic, hollow, hexagonal cross section control rod from a set of horizontal mini-blinds, with a square, silicate aquarium air-stone attached by white electrical tape; total length of 79 cm) was placed in the fish's line of sight, on the opposite end of the aquarium from the fish, and was slowly moved toward the fish. Video recordings of activity responses after recovery from the anesthetic were randomly viewed and blindly scored to avoid observer bias associated with treatment. Active movement and response to a moving object, including flight initiation distance, were scored based on movement during the acclimation period (1 or 2; fish crossed <2 or ≥2 grid squares in 5 min), flight initiation reaction (1–4; fish did not react, reacted <2 or ≥2 grid squares from the object, or reacted before the object entered the tank), and postexposure response (1–4; fast: fish crossed the tank in 1 s, once the object was <2 grid squares away or the object had been in the water less than 5 s; moderate: fish swam to maintain a minimum distance of 2 grid squares; slow: fish swam slowly as the object pursued the fish [less than a 2-square distance]; none: fish exhibited no response through swimming).

Processing Considerations

For control fish to be processed (that is, held in anesthetic and recovery bins) in the same fashion as sham and tagged fish, the average time for which sham and tagged fish underwent anesthesia and recovered was calculated and used to time the processing of control fish. To achieve this, all fish from each treatment were processed at the same time (e.g., all sham, all tagged, or all control) rather than in rotational order (sham, tagged, control), which is often used to remove biases associated with processing fish. Muskellunge were tagged by the same researcher to reduce the effects of surgeon bias on survival (Cooke et al. 2003; Richard et al. 2013; Tiffan et al. 2015). To compare healing rates and document potential pressure necrosis in tagged fish, the ventral side of tagged and sham fish that received surgery was photographed. Any abnormalities associated with fish and deviations from the tagging process were noted. After surgery, fish were held in a recovery cooler with aerated water. Surgically processed fish (both tagged fish and sham fish) were kept under sedation (anesthesia and laparotomy or surgery) for 2–6 min and recovered in 2–3 min. Processed fish were systematically assigned to one of four tanks, so eight fish from each treatment group resided in each tank, with a total of 24 fish/tank (e.g., Tiffan et al. 2015).

Muskellunge were removed from their tanks at specific sampling intervals (7, 30, 60, 90, and 120 d; e.g., Wargo-Rub et al. 2011) to measure body size (nearest 1 mm TL; weight, nearest 0.1 g) and photograph spot pattern changes over time. Individual fish were rotated between tanks once processed to ensure that individuals were not sampled twice. At each sampling interval, all fish were first sampled (weighed, measured, and photographed) from tank 2, and temporarily housed in tank 1 once processed. Fish from tank 3 were then sampled and moved to tank 2. This process continued with tank 4 fish being moved to tank 3 (after processing) and tank 5 fish being moved to tank 4. Once all fish from tank 5 were moved, all fish stored in tank 1 were moved to tank 5, as tank 1 did not have the same dimensions as tanks 2–5.

Environmental Measurements and Fish Health

Tanks were visually scanned for expelled tags during daily, routine cleaning, and the lone drain for all hatchery effluent was covered by a small-mesh net to ensure that expelled tags would be located, if not observed upon initial inspection. Water temperature and dissolved oxygen levels were documented daily for each tank after processing. Average temperatures (based on sampling intervals) declined after 60 d (14.4°C in December; 11.3°C in January) and then increased to 16.9°C in February (90 d) and 18.1°C in March (120 d).

Fish health was monitored daily by hatchery staff to assess survival, and individuals that suffered mortality were kept frozen until a necropsy could identify the location of the transmitter, whether peritoneal infection (presence of viscous, pale-pink fluid in a sack around the transmitter or in the body cavity) was evident (Walsh et al. 2000), and whether the tag was encapsulated or free-floating.

Statistical Analyses

Survival, tag retention, tag burden, and growth rates.—Survival and tag retention were calculated as a percentage (the total number of fish that survived and/or retained their tags, respectively, per treatment group; Gries and Letcher 2002). Tag burden was calculated as 100 × (tag weight)/(individual fish weight at tagging; Wootton 1990), where tag weight was 0.2 g.

To quantify how tag burden may influence growth in body size, tag weight (0.2 g) was subtracted from the weight of tagged fish measured at each sampling interval. To determine whether final body size of fish after 120 d from each treatment group may have been biased by their initial size, the TLs of fish randomly picked for each group at the start of the study were compared using a one-way ANOVA. The potential effects of treatment group (sham, tagged, and control) and time under anesthesia on recovery time were evaluated using ANCOVA,

with treatment as the categorical variable and time as a continuous variable.

Relative growth rates for individual fish were calculated as percent weight change per day based on the exponential curve using the following formula: $\exp[(\ln w_2 - \ln w_1)/(t_2 - t_1)] - 1$, where w_2 and w_1 are fish weights at sampling intervals t_2 and t_1. The number of days between sampling intervals t_2 and t_1 was calculated by subtracting an interval value (i.e., 90 d) from the prior interval (i.e., 60 d). Collinearity between biological, temporal, and environmental factors (i.e., time, lnTL, ln*w*, and temperature) were compared using Pearson's product-moment correlation coefficients and variance inflation factors (VIFs; R package "car"; Fox and Weisberg 2011) prior to analysis. As anticipated, time and ln*w* had a VIF greater than 3, as time was collinear with temperature and ln*w* was collinear with lnTL. Growth rates were compared between each sampling interval with a repeated-measures analysis using linear mixed-effect type III ANOVA models as previously mentioned. Temperature, treatment, tank, and ln*w* were fixed effects; treatment × temperature and treatment × ln*w* were two (biologically relevant) interaction terms; individual fish (fish ID) was a random effect; and degrees of freedom were estimated using the Satterthwaite method. Eight models were compared using Akaike's information criterion corrected for small sample sizes (AIC_c) and the R package "MuMIn" (Barto 2018; Table 2). These eight models were fitted using the "corARMA" function (in the R package "nlme"; Pinheiro et al. 2018), which worked better than the corCAR1 and corAR1 functions. Temperature (fixed variable) was centralized to simplify interpretation and facilitate the comparison of relative importance (Schielzeth 2010). Settings were as follows: method = maximum likelihood; control list = "list (lmeControl (opt = 'optim'))"; maxIter = 10,000; and msMaxIter = 10,000 (maximum number of iterations for the nlm step). Multiple comparisons (due to significance found in more than one main term in the best-fitting model) were evaluated using Tukey's test ("glht" function in the R package "multcomp"; Hothorn et al. 2008).

Incision healing.—A linear mixed-effect model ("lmer" function in the R package "lmerTest"; Kuznetsova et al. 2017) was used to compare the number of Muskellunge scored for each wound type category (openness and inflammation; categorical response variable) per sampling period. Treatment and temperature were fixed effects, fish ID was a random factor, and degrees of freedom were estimated as previously described. The relationship between suture retention and time was assessed using a generalized linear mixed-effects model ("glmer" function). This type III ANOVA test included a binomial distribution, a log link function, a "bobyqa" (bound optimization by quadratic approximation) optimizer, maxfun = 100,000, and an nAGQ (adaptive Gauss–Hermite quadrature) value of 7 (e.g., Bolker et al. 2009). The nAGQ model is more accurate than Laplace estimations (Bolker et al. 2009) and increased the accuracy of the model's estimation (Pinheiro and Chao 2006). Fixed factors included time and treatment group, while fish ID was the random factor. Multiple comparisons (due to significance found in more than one main term) were performed using a Tukey's test.

Flight initiation response.—To assess the relationship between surgery and anesthetic exposure to flight behavior, a proportional odds ordinal logistic regression ("polr" function in the R package "MASS"; Venables and Ripley 2002) was used to analyze the ordinal dependent variable (reaction to a moving object after exposure). Time (repeated measurement) and treatment were fixed factors; activity (prior to flight response) and initial reaction were dependent, ordered factors; and fish ID was a random effect. The proportional odds assumption

TABLE 2. Nested linear mixed-effects models examined using Akaike's information criterion corrected for small sample sizes (AIC_c) to determine which model best describes the effect of fish size, temporal factors, and treatment group on Muskellunge relative growth rate (RGR) in a hatchery experiment after accounting for autocorrelation using the corARMA correlation function. All nested models included a random effect for individual fish, and parameters were standardized. The response variable is RGR; TR is the assigned treatment group of fish (tagged, sham, or control), TK is the tank a fish was sampled from (fish were rotated between tanks), TP is the centralized temperature averaged for all tanks over each sampling period (e.g., 7–30 d), and ln*w* is the centralized natural logarithm of weight. The best-fitting model is shown in bold italics.

Model	Formula	df	AIC_c
Full	RGR ~ TR + TP + TK + ln*w* + (TR × TP) + (TR × ln*w*)	16	−3,154.97
No TK	RGR ~ TR + TP + ln*w* + (TR × TP) + (TR × ln*w*)	13	−3,148.10
No ln*w*	RGR ~ TR + TP + TK + (TR × TP) + (TR × ln*w*)	16	−3,154.97
No TP	RGR ~ TR + TK + ln*w* + (TR × TP) + (TR × ln*w*)	16	−3,154.97
No TR × TP	***RGR ~ TR + TP + TK + lnw + (TR × lnw)***	***14***	***−3,155.81***
No TR × ln*w*	RGR ~ TR + TP + TK + ln*w* + (TR × TP)	14	−3,150.10
No interactions	RGR ~ TR + TP + TK + ln*w*	12	−3,153.48
Simple	RGR ~ 1	5	−3,097.05

was checked using the "sf" function. Variation between mean category levels was analyzed using least-squares means ("lsmeans" function in the R package "lsmeans"; Lenth 2016).

Processing considerations and missing data.— Neither TLs nor weights of fish processed at the start of the study were normally distributed based on a Shapiro–Wilk normality test ($W = 0.95985$, $P = 0.005$); therefore, both variables were natural log transformed, and the transformed values were used to calculate relative growth rates. Fish that were missing a measurement for one or more sampling intervals related to overall body size ($n = 2$), healing rate ($n = 3$), or activity response ($n = 17$) were omitted from their respective statistical analyses. Analyses were conducted in RStudio version 3.4.1 (RStudio Team 2016). Significance was identified if $P \leq 0.05$.

RESULTS

Size of Fish per Treatment Group

Fish selected for tagging had significantly larger body sizes than those selected for the control at the start of the study (Tukey's test: $z = 2.91$, $P = 0.01$) and at 120 d (Tukey's test: $z = 2.83$, $P = 0.01$), but the actual mean size difference was only 7 mm in terms of length (control [mean ± SD]: 201 ± 9.7 mm TL; sham: 206 ± 10.3 mm TL; tagged: 208 ± 10 mm TL) and 3 g in terms of body mass (control: 29 ± 5.4 g; sham: 32 ± 5 g; tagged: 32 ± 4.6 g). Average overall size of Muskellunge at 120 d varied little among treatment groups (control [mean ± SD]: 260 ± 14.6 mm TL; sham: 263 ± 17.4 mm TL; tagged: 263 ± 16.0 mm TL), as lengths ranged from 210 to 300 mm at 120 d postprocessing.

Survival Rates, Necropsy Results, Tag Retention, Tag Burden, and Surgery Times

Survival rates were 100% for sham fish, 100% for control fish, and 94% for tagged fish, as two tagged fish jumped to their deaths. After necropsy, no peritoneal infection was observed, although tags had begun to adhere to the serous membrane between the liver and stomach (Figure 1). Tag burden was low from the initial processing day (0.6%) to the end of the study (0.3%) at 120 d post-tagging (Table 3).

Although we aimed to process sham fish and tagged fish similarly, the amount of time under sedation differed significantly between treatment groups (sham [mean ± SD]: 368 ± 102 s; tagged: 280 ± 47 s; $F_{1,\ 62} = 10.93$, $P = 0.002$). Recovery time after anesthetic exposure was also significantly different between treatment groups (sham [mean ± SD]: 241 ± 93 s; tagged: 183 ± 44 s; $F_{1,\ 62} = 10.90$, $P = 0.0005$). All tagged fish retained their acoustic tags.

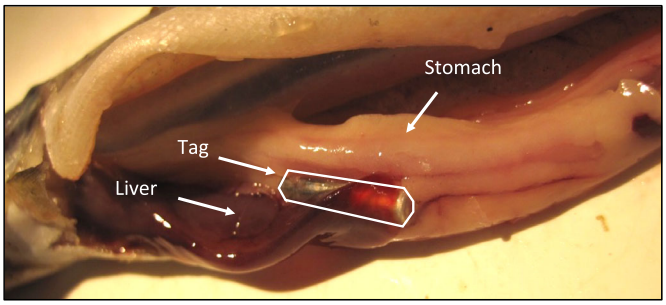

FIGURE 1. Photograph depicting a 0.2-g Juvenile Salmon Acoustic Telemetry tag (outlined in white), which adhered to the serous membranes of a 250-mm, 64.4-g juvenile Muskellunge at 90 d after tag implantation. [Color figure can be viewed at afsjournals.org.]

Growth Rates

High variation (mean = 0.007%; 95% confidence interval [CI] = 0.0003–0.01) was noted in mean relative growth rates for all treatment groups during the first 7 d. Despite no significant difference in mean growth rates between treatment groups ($\chi^2 = 2.75$, df = 2, $P = 0.25$) in the first week, tagged fish grew the slowest. Relative growth rates decreased in the first 60 d (30 d: mean = 0.007%, 95% CI = 0.005–0.009; 60 d: mean = 0.002%, 95% CI = 0.003–0.001), increased considerably to a mean of 0.01% (95% CI = 0.001–0.01) by 90 d, and then decreased to a mean of 0.008% (95% CI = 0.007–0.01) by 120 d (Table 3; Figure 2). Total length of all fish significantly increased over time ($\chi^2 = 55.18$, df = 22, $P < 0.001$), as anticipated; however, the growth rates of all fish in tank 5 were significantly lower than those of fish in tank 3 (Tukey's test: $z = -2.88$, $P < 0.02$).

The best model (lowest AIC_c) found a significant influence of temperature ($\chi^2 = 7.51$, df = 4, $P = 0.006$) and housing tank ($\chi^2 = 8.81$, df = 3, $P = 0.03$) on relative growth rates, as well as a significant treatment × ln$w$ interaction ($\chi^2 = 7.45$, df = 2, $P = 0.02$); a significant increase in growth of tagged fish ($t = 2.48$, $P < 0.01$) was noted over the 120-d study. Mean growth rates were significantly greater for all treatment groups when temperatures were above 14°C relative to the drop in temperature to 11.3°C at 30 d (14.4°C: $t = 5.12$, $P < 0.001$; 14.8°C: $t = 6.23$, $P < 0.001$; 16.9°C: $t = 2.96$, $P < 0.003$), but no difference was found between 11.3°C and 18.1°C ($t = -1.29$, $P = 0.19$). Pairwise comparisons further noted a significant reduction in growth at temperatures above 17.0°C (18.1°C versus 14.4°C, Tukey's test: $z = -4.92$, $P < 0.001$; 18.1°C versus 14.8°C, Tukey's test: $z = -5.31$, $P < 0.001$; 18.1°C versus 16.9°C, Tukey's test: $z = -4.80$, $P < 0.001$).

Incision Healing

Despite increased vertical (dorsal to ventral) tearing of dermal tissue in approximately 25% of tagged Muskellunge (induced by protracted suture retention) 60 d into

TABLE 3. Mean TL and weight (*w*) of juvenile Muskellunge studied at the Sir Sandford Fleming College Muskellunge Hatchery by treatment group (Group) over six sampling intervals (Time) between November 2017 and March 2018 (means are presented with SDs; RGR = relative growth rate). Weight for tagged fish excludes the 0.2-g tag. Number of Muskellunge per treatment group (*N*) changed from 32 to 30 fish at 90 and 120 d. Tag burden (%; weight of the tag [g] relative to fish body mass [g; without the tag]) decreased from 0.6% to 0.3% over the experiment. Significant values are highlighted in bold italics.

Group	*N*	Time (d)	TL (mm)	*w* (g)	Tag burden (%)	ln*w*	RGR (% change in weight/d)	Average temperature (°C)
Control	32	1	201 ± 9.7	29 ± 5.4		3.36 ± 0.17		
	32	7	203 ± 10.1	31 ± 6.0		3.41 ± 0.18	***0.008 ± 0.02***	
	32	30	212 ± 11.5	37 ± 6.8		3.59 ± 0.17	***0.008 ± 0.005***	14.4
	32	60	217 ± 10.6	38 ± 6.8		3.64 ± 0.16	***0.002 ± 0.002***	11.3
	32	90	242 ± 11.6	55 ± 10.4		4.00 ± 0.19	***0.01 ± 0.004***	16.9
	32	120	***261 ± 14.6***	71 ± 15.5		4.24 ± 0.23	***0.008 ± 0.003***	18.1
Sham	32	1	206 ± 10.4	32 ± 5.0		3.45 ± 0.15		
	32	7	207 ± 9.6	34 ± 5.6		3.51 ± 0.16	***0.01 ± 0.01***	
	32	30	215 ± 10.4	39 ± 7.1		3.65 ± 0.18	***0.006 ± 0.006***	14.4
	32	60	221 ± 10.5	42 ± 7.0		3.73 ± 0.16	***0.003 ± 0.004***	11.3
	32	90	245 ± 13.1	58 ± 11.2		4.04 ± 0.20	***0.01 ± 0.003***	16.9
	32	120	264 ± 17.4	75 ± 17.6		4.28 ± 0.26	***0.008 ± 0.003***	18.1
Tagged	32	1	208 ± 10.0	32 ± 4.6	0.6 ± 0.08	3.47 ± 0.14		
	32	7	211 ± 12.8	33 ± 5.6	0.6 ± 0.08	3.49 ± 0.17	***0.003 ± 0.02***	
	32	30	217 ± 10.2	39 ± 5.5	0.5 ± 0.07	3.66 ± 0.14	***0.008 ± 0.006***	14.4
	32	60	224 ± 9.6	42 ± 6.8	0.5 ± 0.06	3.71 ± 0.13	***0.002 ± 0.002***	11.3
	30	90	246 ± 11.3	56 ± 10.4	0.4 ± 0.07	4.00 ± 0.19	***0.009 ± 0.004***	16.9
	30	120	***264 ± 16.1***	72 ± 18.2	0.3 ± 0.08	4.24 ± 0.26	***0.008 ± 0.004***	18.1

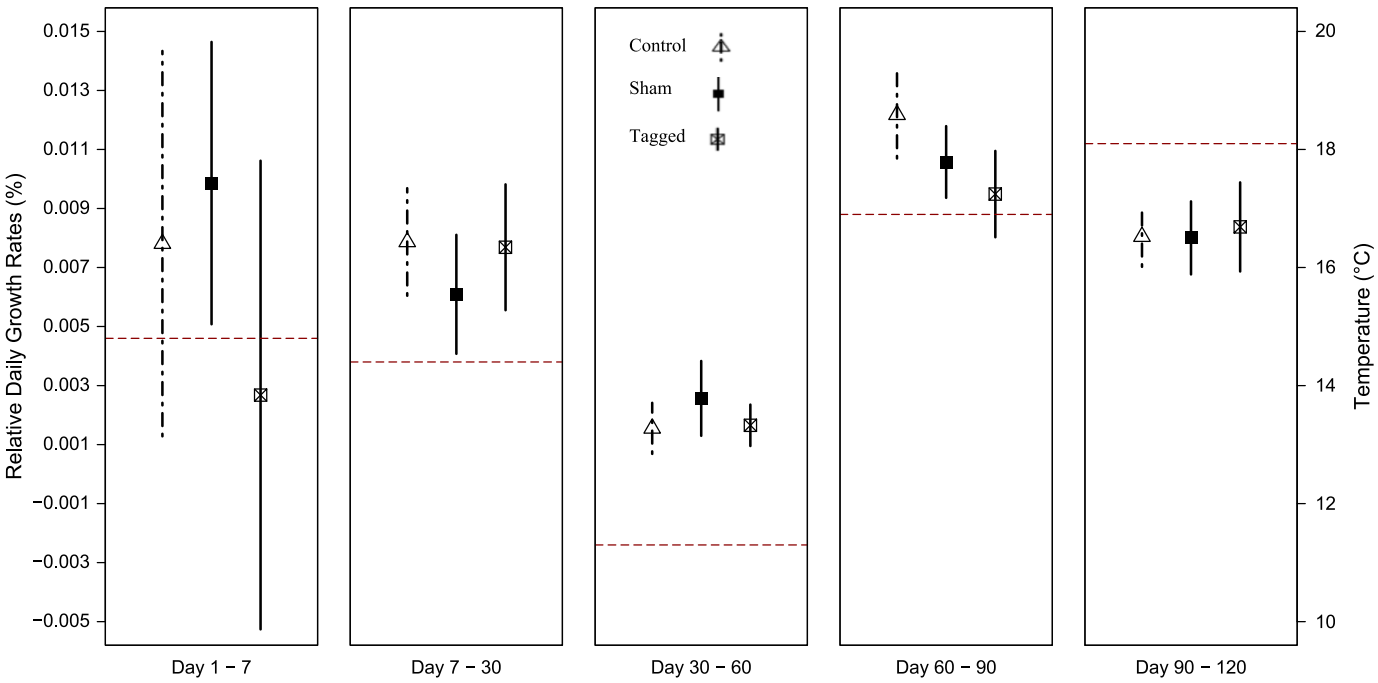

FIGURE 2. Relative growth rates (%/d) by weight of age-0 Muskellunge in three treatment groups (open triangles = control; solid squares = sham; squares with × = tagged) over five sampling intervals. Error bars represent the SEs of treatment-specific means. Average water temperature (secondary *y*-axis) for each sampling interval is represented by the horizontal dashed line.

the study, over 75% of incision wounds were fully healed (fully closed; no suture or erythema) within 120 d postsurgery for sham and tagged fish (e.g., Figure 3). Wound dehiscence (dermal tearing or rupture) scores decreased throughout the study for both sham and tagged fish. Sham fish exhibited low levels of dehiscence (0–0.3%), while dehiscence in tagged fish ranged from 0% to 0.15% (categories 1–3; Table 1; Figure 3).

Fish ID as a random factor was not found to influence healing, as an MuMIn::AIC_c comparison between a linear mixed effect model and a linear model found the linear model a better fit. The number of Muskellunge that were scored in each dehiscence (least regression [LR]: $\chi^2 = 40.46$, $P < 0.001$) and inflammation (LR: $\chi^2 = 31.05$, $P < 0.001$) category was not independent of sampling interval. Level of inflammation ranged from 0% to 0.45% (categories 1–4) for both treatment groups. Significantly fewer fish exhibited low levels of inflammation at the start of the study (as wounds had not yet begun to heal), and significantly fewer fish exhibited open wounds (90 d: $z = -4.20$, $P < 0.001$; 120 d: $z = -3.63$, $P < 0.001$) or inflammation later in the study (90 d: $z = -5.00$, $P < 0.001$; 120 d: $z = -5.23$, $P < 0.001$) as temperatures increased and healing was expedited. Relative to sham fish, significantly more tagged fish exhibited cases of dermal rupture after 60 d (when temperature dropped), and significantly more tagged fish exhibited inflammation after 120 d. Though few fish exhibited low levels of inflammation during the first 30 d, significantly more sham fish exhibited high levels of inflammation and dermal rupture during this period relative to tagged fish.

Incisions healed by 60 d; however, 32% ($n = 29$ fish) of sutures were retained by both groups (tagged and sham fish) at 120 d postsurgery. The rate at which sutures fell out significantly declined with each sampling interval (60 d: $z = -2.22$, $P = 0.02$; 90 d: $z = -3.08$, $P = 0.002$; 120 d: $z = -3.34$, $P = 0.001$).

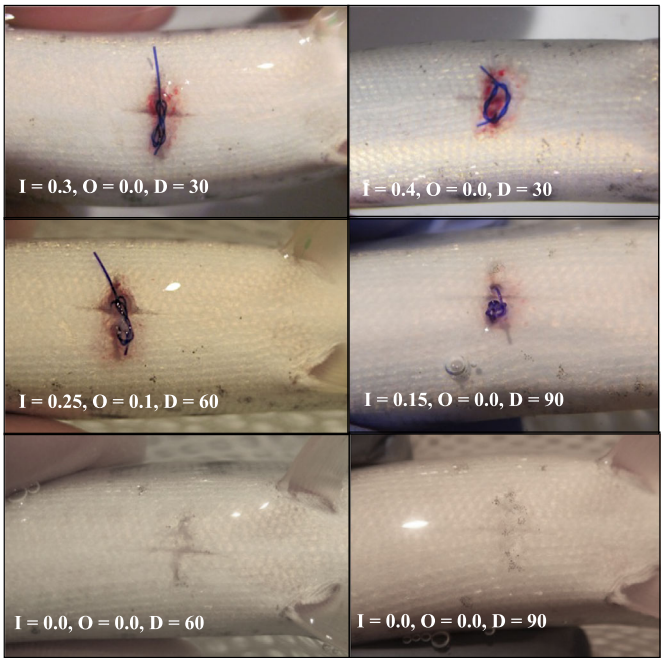

FIGURE 3. Representative photographs of six different juvenile Muskellunge, illustrating ranges of inflammation and wound openness. The key on each panel designates scores for inflammation (I) and wound openness (O) from 0 to 1 and the number of days postsurgery (D). Scores were based on six different categories (Table 1; see Schoonyan et al. 2017). [Color figure can be viewed at afsjournals.org.]

Flight Initiation Response

Nearly 25% of tagged and sham fish responded immediately to the moving object presented after sedation (Figure 4). No variation in behavior was noted during the acclimation period, as mean activity levels (category 1 or 2) postsedation (control: 1.39; sham: 1.25; tagged: 1.80) were not significantly different (95% CI = −0.23 to 0.72) from those observed at 7 d postrecovery (control: 1.29; sham: 1.40; tagged: 1.25). Mean response to object exposure (categories 1–4) postsedation (control: 2.65; sham: 2.85; tagged: 1.96) was significantly slower for tagged fish (LR: $\chi^2 = 7.8387$, df = 2, $P = 0.01985$) relative to the response at 7 d postrecovery (control: 3.0; sham: 2.50; tagged: 2.71). Moreover, mean initial reaction (categories 1–4) postsedation (control: 2.54; sham: 2.85; tagged: 2.18) and at 7 d postrecovery (control: 2.81; sham: 2.70; tagged: 2.54) significantly influenced the mean response to object exposure for all treatment groups—specifically, category 2 (the fish moved when the object came within < 2 grid squares upon exposure; 95% CI = 3.79–6.64) and category 4 (the fish reacted before the object entered the water; 95% CI = 0.04–1.37). The odds of exhibiting a fast or moderate postexposure response compared to a slow response were 183.28 times greater (95% CI = 49.39–907.08) if the flight initiation response occurred in close range (<2 grid squares). When the flight initiation response of a Muskellunge occurred before the object hit the water, the odds of exhibiting a faster postexposure response were 2.03 times greater (95% CI = 49.39–907.08). Mean initial flight reactions (categories 1–4) were significantly different ($LR_{3,7}$: $\chi^2 = 93.04$, $P < 0.001$) between sampling periods.

DISCUSSION

The present study suggests that mini-acoustic transmitter implantation did not impair short-term growth (120 d) of juvenile Muskellunge. Our finding is consistent with earlier studies in which growth was not impaired for free-ranging, multi-tagged (i.e., PIT, T-bar anchor, and streamer tags)

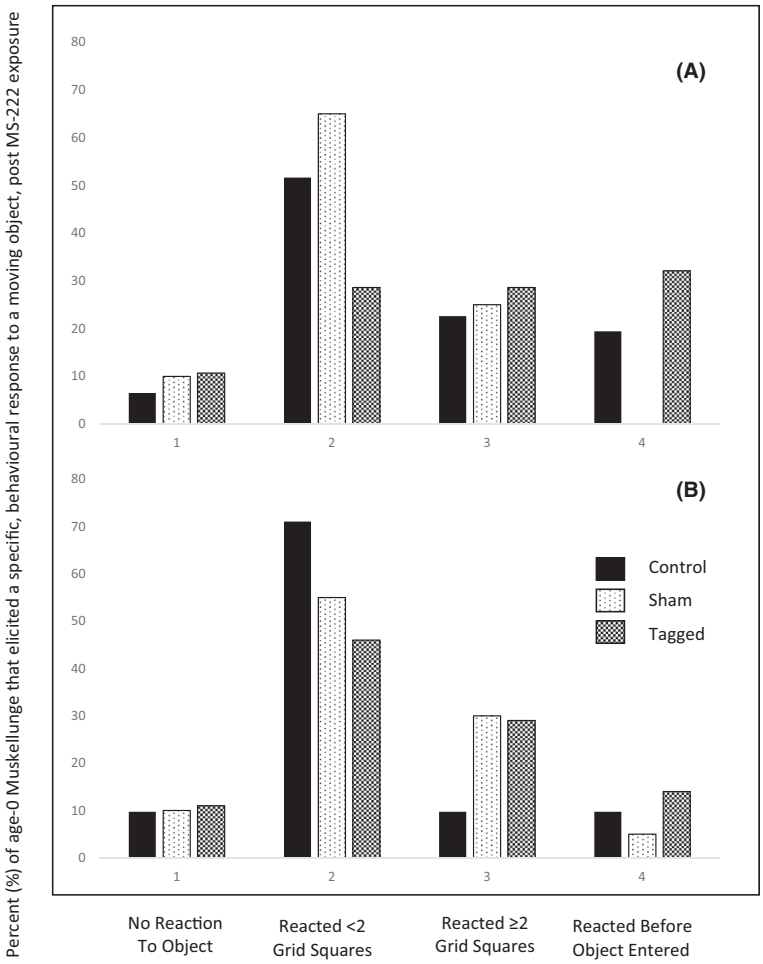

FIGURE 4. Percentage of juvenile Muskellunge from control ($n = 31$; black bars), sham ($n = 20$; dotted bars), and tagged ($n = 28$; checked bars) treatment groups that scored 1, 2, 3, and 4 in response to a moving object in the behavioral experiment. The reactions of fish were scored **(A)** after recovery from the anesthetic and **(B)** at 7 d after surgery. Fish that did not have a matching score (postrecovery or 7 d postsurgery) due to processing difficulties or due to a lack of video were eliminated from the analysis. No significant differences were found in the percentages of fish per reaction group over time.

age-0 Northern Pike smaller than 480 mm TL (Hühn et al. 2014), for Northern Pike larger than 480 mm TL implanted solely with radio transmitters (Hühn et al. 2014), or for Atlantic Salmon *Salmo salar* smolts implanted with dummy acoustic transmitters (Lacroix et al. 2004). Reduced size and growth rates of tagged age-0 Muskellunge (anticipated results of an invasive procedure) were not observed after 4 months (120 d), by which time growth was comparable to that of intensively reared age-0 Muskellunge in Chautauqua Fish Hatchery (New York) ponds after 50 d (Colesante and Bubnack 1992). The initial lag in growth of tagged fish during the first week may have persisted beyond 1 week but was negligible beyond the first month. Sham and tagged fish may have allocated more energy to continue wound closure than to somatic growth once temperatures increased beyond 14°C; however, the noted declines in growth for all treatment groups were likely attributable to water temperatures falling below preferred thresholds (≥12°C; Kerr and Lasenby 2001).

Low tag burden, high tag retention, and high survival suggest that micro-acoustic tags do not harm juvenile Muskellunge based on our methods and are appropriate for use in research. Tag burden was lower than observations from most micro-acoustic and PIT tag implantation studies (1–9%; e.g., Winter 1983; Panther et al. 2011; Tiffan et al. 2015), and high retention rates mimicked findings on PIT tag retention (>95%) previously reported for age-0 Muskellunge (Younk et al. 2010) and findings on JSAT tag retention (>99%) reported for juvenile Chinook Salmon (Wagner et al. 2014) and Bloater *Coregonus hoyi* (Klinard et al. 2018). Moreover, neither tag loss nor tagging-induced mortalities were found to occur for multi-tagged (PIT tag, fin clip, and external clip) age-0 Northern Pike up to 325 d posttagging (Hühn et al. 2014).

Though the present study cannot confirm whether expulsion occurred in age-0 Muskellunge beyond the 4-month poststocking study period, partial encapsulation at 3–4 months postimplantation and the healing of the cavity wall in two fish suggest that expulsion is unlikely. Detections from age-0 Muskellunge over 180 d after implantation of the JSAT tag used in the present study (e.g., S. E. Walton-Rabideau, unpublished data) reiterate a low likelihood of expulsion within the first 6 months of tagging; however, more research is required to determine when tag encapsulation occurs within juvenile Muskellunge. Tagging fish at cooler temperatures (i.e., 12–14°C) is known to result in higher tag and suture retention, lower incision openness, and less wound inflammation (i.e., in juvenile Chinook Salmon; Deters et al. 2010). To this end, future studies may wish to more closely examine wound dehiscence or levels of infection exacerbated by suture presence over the period of suture retention in tagged fish.

Results of the present study may be transferable to micro-radio transmitter implantation in age-0 Muskellunge in the Great Lakes basin. Our juvenile Muskellunge did not experience tag loss or mortality within the first 30 d in situ, and all incisions healed. In contrast, Owensby et al. (2017) documented low (<30%) ex-situ survival rates for stocked age-0 Muskellunge after 90 d in North Carolina rivers; those fish received implanted micro-radio transmitters (9 × 19 × 7 mm, 3.3 g in air, 200-mm trailing whip antenna; Advanced Telemetry Systems), and their low survival was due to predation (a common pattern for stocked fish in that region). When miniature radio transmitters (5 × 14 mm, 0.7 g in water; Advanced Telemetry Systems) were surgically implanted in the coelom of juvenile Chinook Salmon (114–159 mm FL), fish grew at rates comparable to those of our sham and control juvenile Muskellunge after 54 d, tags were not expelled, and low levels of inflammation were noted in less than 25% of fish (Adams et al. 1998).

We used MS-222 concentrations within acceptable limits (60–100 mg/L), and fish were induced and sedated within recommended timelines and manufacturer guidelines (see Wagner et al. 2011, 2014). Increased exposure to MS-222 (relative to tagged fish) did not appear to influence immediate recovery for sham fish; however, the reduced distance maintained by sham fish between themselves and the object at 7 d postsedation may have been a delayed reaction attributable to lingering effects of increased MS-222 exposure. In addition, the reduced reaction of tagged fish suggests that individuals may experience short-term behavioral impairments after transmitter implantation. Despite the fact that fish maintained some distance from the moving object once they exhibited a flight initiation response, tag implantation and MS-222 exposure may have short-term, sublethal implications (i.e., predator avoidance) after release. This short-term influence on the postsedation behavior of *Esox* sp. requires additional research.

In summary, low variation in growth rates between treatment groups at the end of this short-term study, as well as the negligible mortality, low tag burden, and 100% transmitter retention suggest that mini-acoustic transmitters can successfully be implanted intracoelomically into juvenile Muskellunge. However, sedation methods and level of exposure, confounded by laparotomy, could feasibly influence short-term growth and behavioral responses.

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