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Evaluation of optimal surgical techniques for intracoelomic transmitter implantation in age-0 lake sturgeon

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ABSTRACT

We evaluated the effects of incision placement (midline vs. lateral), closure method (absorbable monofilament suture vs. n-butyl-ester cyanoacrylate adhesive, Vetbond®, 3 M), and tag burden (PIT-tag only vs. PIT-tag and acoustic transmitter) on survival, post-operative complications (i.e., viscera expulsion, necrosis), incision dehiscence, incision apposition, transmitter retention, incision healing, inflammation, and growth for intracoelomic transmitter implantation in age-0 lake sturgeon (*Acipenser fulvescens*). The risk of death was 5.17 times higher and the risk of viscera expulsion was 6.21 times higher for sturgeon that had midline incisions closed with Vetbond compared to all other treatments. Incision dehiscence probabilities were low for all treatments, except for midline incisions closed with Vetbond. Time to complete incision apposition occurred most quickly in the lateral suture treatment followed closely by the midline suture and lateral vetbond treatment groups. Tissue strength was notably weaker in the midline region. Transmitter retention was 100% for all treatments except for midline incisions closed with Vetbond. Inflammation was low and slightly decreased over time for incisions closed with Vetbond, while incisions closed with suture exhibited significant increases in inflammation levels over time. Incisions closed with suture achieved better healing outcomes initially, but the healing process was 2–3 times more likely to relapse because of severe inflammation compared to lateral incisions closed with Vetbond. Sturgeon with midline incisions closed with Vetbond gained less weight compared to the other treatments, while sturgeon with lateral incisions closed with Vetbond gained similar amounts of weight relative to both suture treatments and the control group. Collectively, results suggest that Vetbond can be effectively used to close small lateral incisions (≤ 8 mm), with a lower risk of severe inflammation compared with sutures. For transmitter implantation, we recommend using a lateral incision through the hypaxial musculature and either closing the incision with suture or Vetbond.

1. Introduction

In telemetry and long-term monitoring studies the surgical implantation of acoustic transmitters, radio transmitters, and passive integrated transponder (PIT) tags is vital to fisheries research projects that seek to evaluate habitat use, spatial movements, and passage survival at hydroelectric projects. Intrinsic to these projects is the assumption that transmitters are not lost and that surgically tagged fish behave and survive similarly compared to un-tagged conspecifics. However, surgical incisions disrupt homeostasis and elicit a whole series of interconnected immune responses (Broughton et al., 2006; Fontenot and Neiffer, 2004). The process through which incisions are healed is intricate and dynamic, and it involves four stages in vertebrates: hemostasis, inflammation, proliferation, and remodeling (Guo and DiPietro, 2010). Surgical incisions involve the cutting of connective tissue, muscle tissue, and associated nerves and blood vessels. In addition, the surgically implanted transmitters themselves can add significant weight and can put pressure on internal organs and the incision site (Brown et al., 2010, 1999). This represents a significant physiological impairment that can affect growth, behavior, and survival, which can confound research results (Benson et al., 2005; Panther et al., 2011; Wagner et al., 2011). Thus, significant clinical evidence is required to validate and optimize surgical procedures.

There are many factors that can delay incision healing, but the primary factors usually are dehiscence, poor apposition, and inflammation (Boyd et al., 2011; Guo and DiPietro, 2010; Miller et al., 2014; Petering and Johnson, 1991). Dehiscence and poor apposition cause incisions to heal through a longer process termed secondary

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intention instead of through primary intention (Barbul, 2005; Panther et al., 2011). In secondary intention the gap between the incised tissues must be filled with fibrous granulation tissue. This granulation tissue is then able to contract and close the incision opening via myofibroblasts (Barbul, 2005; Panther et al., 2011). Inflammation is a natural part of the incision healing process, but prolonged inflammation can cause the healing process to relapse (Guo and DiPietro, 2010; Miller et al., 2014). Inflammation may be prolonged because of the inefficient removal of microbes and decontamination of the wound site, which leads to elevated levels of pro-inflammatory cytokines (Guo and DiPietro, 2010; Koh and DiPietro, 2011). In telemetry studies inflammation can also be prolonged by suture material, which elicits a foreign body immune response. Unless the suture material is promptly removed the inflammation can persist for long periods of time, as most suture material takes longer than 90 days to be completely absorbed (Dunn, 2007). In addition, prolonged inflammation results in higher levels of metalloproteases at the incision site, and these proteases degrade the newly forming extracellular matrix of the wound, reversing healing progress (Guo and DiPietro, 2010). Over time this can lead to the complete dehiscence of the incision, thereby, completely reversing the healing process (Miller et al., 2014). Many researchers are not able to hold their tagged fish for post-operative evaluations and suture removal. Consequently, researchers may be unaware that their study fish are exhibiting severe inflammation that is harming the healing process and potentially affecting health and behavior.

There are two main incision locations that have been used for intracoelomic transmitter implantation in fishes. Incisions can be made along the midline linea alba, which is composed of less perfused connective tissue, in both salmonids (Panther et al., 2011; Wagner et al., 2011) and sturgeon (Murray, 2002; Smith and King, 2005). In humans this is the preferred incision location because it is associated with quicker healing, stronger closure, and less muscle and nerve damage (Rath et al., 1996; Tera and Aberg, 1977). Alternatively, incisions can be made parallel and lateral to the midline through the hypaxial musculature in both salmonids (Wagner et al., 2011) and sturgeon (Crossman et al., 2013; Liss et al., 2018; Miller et al., 2014). Lateral incisions can cause both muscle and nerve damage, which can take longer to heal (Burger et al., 2002; Tera and Aberg, 1977; Wagner et al., 2011). One commonly cited benefit of lateral incisions in salmonids is that the incision is located in an anatomic region that is less vulnerable to contact with bottom substrate and debris; however, in dorsal-ventrally flattened fish, like lake sturgeon (*Acipenser fulvescens*), there is no such benefit (CCAC, 2005; Wagner et al., 2011). While studies have compared optimal incision site location in salmonids (Panther et al., 2011; Wagner and Stevens, 2000), to our knowledge only one study has evaluated the subject matter on age-0 white sturgeon (Liss et al., 2018) and no comparable studies have examined optimal incision placement in age-0 lake sturgeon.

Incisions can be closed with a variety of methods. Absorbable monofilament suture material is commonly used for closing incisions in salmonids (Deters et al., 2010; Wagner et al., 2011) and sturgeon (Boone et al., 2013; Hondorp et al., 2015; Liss et al., 2018). The simple interrupted closure pattern is also routinely used to close incisions in salmonids (Deters et al., 2012; Wagner et al., 2011) and sturgeon (Hondorp et al., 2015; Liss et al., 2018), as it provides high closure strength and requires less suture material than mattress patterns, which are often associated with more inflammation (Deters et al., 2012; Wagner et al., 2011). Transmitters have continued to decrease in size and this has subsequently led to a reduction in incision length. Traditionally at least two sutures have been used to close incisions, but the reduction in incision length now makes single suture closure and even self-closure (i.e., no suture or closure method) possible (Deters et al., 2012; Liss et al., 2018; Wagner et al., 2011). Using less suture material is advantageous because less foreign material in tissues results in less problematic inflammation and better incision healing (Deters et al., 2012; Liss et al., 2018; Miller et al., 2014).

Surgical grade adhesives (i.e., n-Butyl cyanoacrylate and octyl cyanoacrylate) are another option for closing increasingly smaller incisions, and surgical grade adhesives are generally bactericidal, dissolve rapidly, and do not illicit a strong inflammatory response (Bhagat and Becker, 2017; Bruns and Worthington, 2000; Elmasalme et al., 1995; Romero et al., 2009). To our knowledge there are only two studies that have evaluated the efficacy of a surgical grade adhesive for incision closure in fish (Jepsen et al., 2017; Lowartz et al., 1999). The few other studies available on the subject have examined household superglue, ethyl 2-cyanoacrylate (Baras and Jeandrain, 1998; Kaseloo et al., 1992; Nemetz and Macmillan, 1988; Petering and Johnson, 1991). Some studies examining adhesives have found that it is associated with poor closure and tissue inflammation, while other studies have observed strong closure and minimal inflammation. Household superglue was once used in medical procedures, but it is now considered medically contraindicated because it is severely cytotoxic (Sohn et al., 2016; Toriumi et al., 1990). No studies that we are aware of have evaluated the efficacy of adhesives for closing incisions on sturgeon.

Over the past two decades there has been increased research interest and regulatory concern regarding juvenile lake sturgeon passage behavior and survival at hydroelectric dams (Coscarelli et al., 2011; Jager et al., 2016). In regulatory survival studies it is imperative that surgical procedures do not affect survival or behavior. The significant lack of information on optimal surgical incision placement and closure methods for age-0 lake sturgeon led to the development of this project. In this study our objectives were to evaluate (1) optimal incision placement (midline vs. lateral), (2) the suitability of single suture incision closure, (3) the efficacy of surgical adhesive for closing incisions, (4) the influence of tag burden (PIT-tag only vs. PIT-tag and acoustic transmitter), and (5) the importance of genetic effects in relation to incision dehiscence characteristics, incision apposition quality, inflammation, incision healing, transmitter retention, and mortality.

2. Methods

2.1. Surgical treatments

Four surgical treatments were evaluated, along with a control group. All surgical procedures and animal husbandry practices were reviewed and approved by Michigan State University's animal care program (IACUC#: 03/14-041-00). Two different incision locations and two different closure methods were assessed. Surgical incisions were made ventrally either at the midline along the linea alba or lateral and parallel to the midline in the hypaxial musculature anterior to the pelvic girdle. Incisions were either closed with a single interrupted absorbable monofilament suture or with n-butyl-ester cyanoacrylate surgical adhesive (Vetbond®, 3 M). A total of 32 lake sturgeon were evaluated for each surgical treatment, and a total of 16 lake sturgeon were evaluated in the control group. Half of the lake sturgeon in each surgical treatment were surgically implanted with only PIT-tags, while the remaining half were implanted with PIT-tags and acoustic transmitters to assess the influence of tag burden and design. The PIT-tags used were cylindrical in shape, 23 mm long, 3.65 mm in diameter, and weighed 0.6 gs (Oregon RFID, Portland, Oregon; Fig. 1b). The acoustic transmitters used were developed by the U.S. Army Corps of Engineers for the Juvenile Salmonid Acoustic Telemetry System (JSATS). These transmitters are irregularly shaped with a length of 11.1 mm, a width of 5.5 mm, a height of 3.7 mm, and they weighed 0.32 gs (Lotek Wireless Inc., Newmarket, Ontario, Canada; model: L-AMT-1.421; Fig. 1a).

The different treatments have been abbreviated to facilitate discussion. The MS (midline-suture) treatment consisted of a midline incision closed with suture; the LS (lateral-suture) treatment consisted of a lateral incision closed with suture; the MV (midline-Vetbond) treatment consisted of a midline incision closed with Vetbond; the LV (lateral-Vetbond) treatment consisted of a lateral incision closed with Vetbond; and the CA (control anesthetic only) or control group

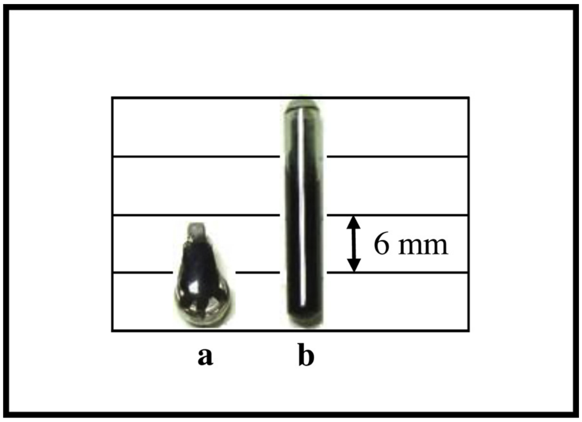

Fig. 1. Picture displaying the 0.32 g JSATS L-AMT-1.421 acoustic transmitter with dimensions of 11.1 × 5.5 x 3.7 mm manufactured by Lotek Wireless (a) and the 0.6 g PIT-tag with dimensions of 3.65 x 23 mm manufactured by Oregon RFID (b).

treatment consisted of the group of control fish that did not receive an incision, but did undergo general anesthesia and were placed on the operating table for five minutes as though they were having a surgery.

2.2. Experimental design

A total of sixteen 26.2 L tanks were utilized to house all lake sturgeon used in this experiment. Three different families were also used in the experiment to examine family genetic effects. The first family consisted of 36 fish, the second family consisted of 54 fish, and the third family consisted of 54 fish. Two fish from each surgical treatment category were placed in each tank. One fish from each treatment category was surgically implanted with only a PIT-tag, while the other fish from the treatment was surgically implanted with a PIT-tag and an acoustic transmitter. In addition, one control fish was placed in each tank, bringing the total number of fish in each tank to nine. The order in which the tanks were filled was randomized, as was the order in which the families were selected to fill a tank. A given tank was filled with nine fish from the same family. Furthermore, the order in which the surgical treatments were carried out for each tank was also randomized in order to reduce bias. Because the treatment types were all mixed together in each tank, each treatment was proportionately influenced by individual tank conditions, making the design robust to confounding tank effects. In the event that a mortality occurred in a given tank, a replacement fish, which did not undergo surgery, was substituted for the deceased fish in order to maintain equal fish densities in each tank throughout the experiment.

2.3. Fish husbandry

The 16 experimental tanks were housed at the Black River Sturgeon Rearing Facility along the Black River in Cheboygan County, Michigan. The Black River Sturgeon Rearing Facility is a streamside hatchery that uses continuously pumped river water from Kleber Reservoir (~680 L/min). Water entered each tank from above at a uniform flow rate and no water was recirculated. The lake sturgeon in each tank were fed approximately 10% of their body weight in bloodworms per day over three daily feeding times (Aloisi et al., 2006). Tanks were cleaned and maintained on a daily basis, and uneaten food was removed to ensure a healthy environment. Water temperature was recorded every hour with the use of an Onset Hobo temperature logger (Bourne, Massachusetts), and the average water temperature over the study was 20.47 °C (SD = 1.809, Range: 16.13–24.31 °C). All lake sturgeon were kept and monitored within the tanks for a total of 21 days.

2.4. Surgical procedures

Surgical procedures were carried out in 2016 between September 1st and September 6th by one experienced surgeon (Jonathan Hegna) in order to avoid the confounding effects of using multiple surgeons. All of the lake sturgeon used had been raised in the stream-side hatchery and were previously acclimated to the water conditions. Before surgery, sturgeon were anesthetized for approximately 5 min in an anesthetic bath with 125 mg/l of tricaine methanesulfonate (MS222). Once a sturgeon became fully sedated based on visually observing a loss of reflexes, slow heart rate, and slow opercular movements, wet weight and total length measurements were taken. On average the age-0 lake sturgeon weighed 30.23 g (SD = 4.308, Range: 20.3–43.6) and were 205.47 mm in total length (SD = 10.451, Range: 168–231). Average tag burden as a percent of total body weight was 1.99% (SD = 0.290, Range: 1.38–2.67%) for sturgeon implanted with only PIT-tags and 3.15% (SD = 0.450, Range: 2.44–4.53%) for sturgeon implanted with a PIT-tag and an acoustic transmitter. The sturgeon was then placed ventrally on the operating table and an oxygenated maintenance dose of 100 mg/l of MS222 was irrigated across the gills to ensure proper sedation throughout the entire operation. The anesthetic maintenance dose was not recirculated to ensure the efficacy of the anesthetic and to prevent infection. Protective padding was used on the operating table to prevent abrasions and injury. The randomized surgical treatment type was then carried out with the appropriate incision location and closure method. Sturgeon in the control group underwent the anesthetic bath and were put on the operating table with the maintenance dose for a total of five minutes, but they did not receive a surgical incision or transmitter. However, sturgeon in the control group did receive a small dorsal fin clip for identification.

Incision sites were disinfected with a 10% betadine solution prior to making the incision. Surgical incisions were either made ventrally at the midline along the linea alba or lateral to the midline anterior to the pelvic girdle with a size 15 surgical blade. Surgical incisions were measured to 8 mm in length with the use of a caliper. Incisions were either closed with one simple interrupted absorbable monofilament suture or with Vetbond. The suture was pre-sterilized with ethylene oxide and consisted of absorbable, monofilament polyglycolide-cocaprolactone 5-0 suture material (PGCL Unify®, AD-Surgical, Sunnyvale, California; comparable to Monocryl, Ethicon®) with pre-attached 3/8ths curvature, reverse-cutting suture needles. Olsen-hegar needle holders and small soft tissue forceps were also used during the operation. In order to apply the Vetbond, the two edges of the incision were firmly apposed by hand. Then one drop of Vetbond was applied and allowed to air dry for approximately 15 s, forming a thin coating over the incision. A second drop was subsequently added to the incision forming an additional adhesive coat that was allowed to air dry for an additional 20–30 seconds. Only a thin coating of the adhesive is recommended by the manufacturer (3M Health Care, 2016), and attention was given to avoid pooling of the adhesive on the skin. After the incision was securely closed, the sturgeon was then placed in its experimental tank and monitored until motor control was regained. We recorded the total time of the surgery starting from when the sturgeon were placed on and removed from the operating table. On average the MS treatment took 3.55 min (SD = 0.645, Range: 2.38–5.08 min.), the LS treatment took 4.00 min (SD = 1.009, Range: 2.13–7.67 min.), the MV treatment took 3.46 min (SD = 0.948, Range: 2.23–6.41 min), and the LV treatment took 3.35 min (SD = 0.743, Range: 2.32–5.08 min.). Between surgeries the surgical table and maintenance dose tubing was disinfected with a 10% betadine solution. All surgical instruments were disinfected with 95% ethanol for a minimum of ten minutes.

2.5. Post-operative evaluations

Post-operative evaluations were conducted every seven days for three weeks. Sturgeon were placed in an anesthetic bath for five

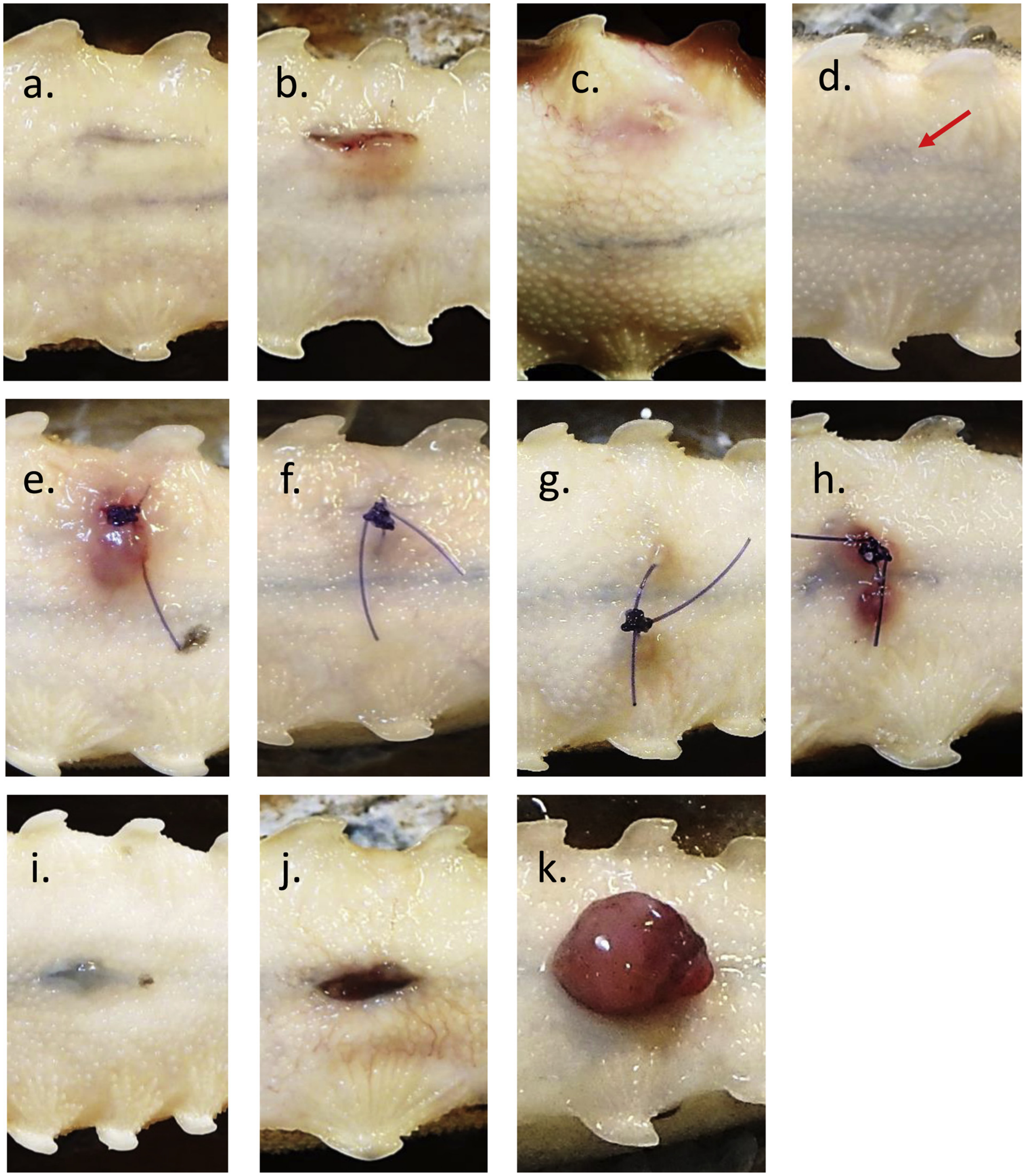

Fig. 2. Surgical pictures representing key incision healing characteristics and surgical complications for lateral and midline incisions closed with absorbable monofilament suture and Vetbond on lake sturgeon. Well approximated lateral incision closed with Vetbond; incision closure is secure, but relatively weak with no inflammation evident at 7 days post-surgery (a). Lateral incision closed with Vetbond with significant inflammation evident at 7 days post-surgery (b). Lateral incision closed with Vetbond exhibiting inflammation because remnants of the adhesive were still adhered to the skin at 21 days post-surgery (c). Lateral incision (location of arrow) closed with Vetbond showing reepithelialization and the relative completion of the healing process at 21 days post-surgery; approximation is excellent, incision closure is very strong, and no inflammation is evident (d). Lateral incision closed with suture exhibiting comprehensive severe inflammation and fibrosis at 21 days post-surgery (e). Lateral incision closed with suture displaying no inflammation at suture sites at 7 days post-surgery (f). Midline incision closed with suture displaying moderate inflammation at suture sites at 14 days post-surgery (g). Midline incision closed with suture displaying severe inflammation at suture sites at 21 days post-surgery (h). Midline incision closed with Vetbond; incision edges are not completely approximated, as the incision edges are separated by blue-colored fibrous scar tissue that has sealed the incision opening at 14 days post-surgery (i). Midline incision closed with Vetbond with complete dehiscence of the surgical incision; moderate inflammation is evident around the margins of the incision at 7 days post-surgery (*j*). Midline incision closed with Vetbond displaying severe viscera expulsion through the incision site at 14 days post-surgery (k) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

minutes until they were completely sedated prior to evaluation. At each evaluation we noted surgical complications and irregularities with the healing process. Surgical complications mainly included viscera expulsion and tissue necrosis (Fig. 2k). We also recorded how long Vetbond remained at the incision site and whether it caused severe inflammation or delayed healing. Each tank was also monitored multiple times each day for mortalities.

A number of different measurements were also taken during each evaluation, including open incision width and length (i.e., dehiscence characteristics; Fig. 2j). We also measured un-apposed incision width and length (Fig. 2i). Open incision width and un-apposed incision width were measured at the location where the width was greatest. Incision apposition refers to how closely aligned the two edges of the incision are. Some closure methods are not as effective at apposition and may result in scar tissue forming between the two incision edges, which ultimately closes the incision through secondary intention. In other cases, extremely poor apposition leads to dehiscence of the incision resulting in open incision width and length. Each tank was monitored multiple times each day for transmitters that may have fallen out of open incisions.

We also scored inflammation characteristics using a point-based rating system adapted from Wagner and Stevens (2000). Inflammation was rated from 1 to 4 along the margin of the incision, with a higher number indicating more inflammation along the incision. A rating of 1 indicated that no inflammation was present, a rating of 2 indicated that there was little inflammation along the incision site (i.e., less than 10% of incision site inflamed), a rating of 3 indicated that there was little to moderate inflammation along the incision site (i.e., 10–50% of incision site inflamed), and a rating of 4 indicated that there was moderate to high inflammation (i.e., up to 100% of incision site inflamed; Fig. 2**a,b,c,e**). Suture entrance and exit sites were rated on a binary basis regarding whether they exhibited inflammation or not. Furthermore, each suture site was also rated on a binary basis regarding whether it exhibited severe inflammation or not **(**Fig. 2**f,g,h)**. The rating scores for incision inflammation, suture site inflammation, and severe suture site inflammation were then summed together to determine the total level of inflammation.

In addition, we scored the incision healing process using a rating index adapted from Wagner and Stevens (2000) that ranged from 1 to 8 (Table 1; Fig. 2). The rating scale takes into account incision dehiscence, incision apposition, scar tissue formation, and inflammation. A rating of 1 indicates that the incision has healed, is completely secure, and that there is little to no inflammation, while a rating of 8 indicates that the incision is completely open. On the final post-operative evaluation on day 21 the sturgeon were weighed and their total length was measured again. This allowed total weight gain (g) and total length increase (mm) for the three week experimental time period to be determined.

2.6. Statistical analyses

Restricted maximum likelihood (REML) based mixed effects models were used to evaluate initial weight and length differences among treatments, along with incision healing scores and inflammation scores by treatment and week. In all relevant analyses fixed factors included surgical treatment group, tag burden (PIT-tag only vs. PIT-tag and acoustic tag), and family. Covariates included initial length, initial weight, average water temperature, and surgery time. The REML mixed model approach was used because it is robust to correlated variables, unequal variances, and can handle both balanced and unbalanced designs. Covariance structure in repeated-measures mixed model analyses was determined through comparing corrected Akaike information criterion (AICc) values for different structures. Multivariate analysis of variance (MANOVA) was used to evaluate weight gain and length increase over the course of the experiment. Firth generalized binomial regression was used to evaluate the probability of acoustic transmitter loss, probability of PIT-tag loss, probability of incision healing relapse, probability of mortality, probability of tissue necrosis, probability of viscera expulsion, probability of complete healing, and probability of incision dehiscence. For the probability analyses we were usually only able to evaluate the main surgical treatment effects, as the low incidence rate of the phenomena made more complex models with interactions infeasible. Parametric Weibull time-to-event analysis was used to evaluate time to complete apposition. All statistical analyses were carried out with the JMP Pro version 14 statistics software package (SAS Institute, Cary, NC, USA).

3. Results

3.1. Mortality rates and surgical complications

No mortalities were observed in fish from the MS, LS, LV, and CA treatments over the 21 day observation period. In the MV treatment a total of three mortalities (9.4%) were documented. Two deaths occurred on day 7 and one death occurred on day 10. The Firth generalized binomial regression model showed differences in mortality risk between treatments (LR-Chi square: 8.28, DF: 1, $p = 0.004$). Fish in the MV treatment were 5.17 times more likely (95% CI: 1.58–60.23, $p = 0.004$) to die compared to the other treatments. Tag burden (PIT-tag only vs. PIT-tag and acoustic transmitter) was not predictive of mortality (LR-Chi square: 0.00, DF: 2, $p = 1.000$).

Viscera expulsion was not observed in fish from the MS, LS, and LV treatments. In the MV treatment, at total of five fish (15.6%) exhibited some level of viscera expulsion (Fig. 2k**)**. Three of these fish presented with the complication at day 7, one at day 14, and one at day 21. The Firth generalized binomial regression model found that the risk of viscera expulsion for fish in the MV treatment was 6.21 times higher (LR-Chi-square: 13.51, DF: 1, $p < 0.001$; 95% CI: 2.04–71.52, $p < 0.001$) compared to the MS, LS, and LV treatments. Tag burden was not predictive of viscera expulsion (LR-Chi-square: 0.072, DF: 1, $p = 0.788$). Additionally, three fish developed tissue necrosis over the course of the experiment. Two fish (6.2%) exhibiting tissue necrosis were in the MS treatment group and the other fish (3.1%) was in the MV treatment group.

Vetbond was associated with a low incidence rate of surgical complications. At 21 days post-operatively, we observed that the Vetbond

Table 1
Description of the incision healing scoring system used to assess the healing process, adapted and modified from Wagner and Stevens (2000).

Incision Healing Score	Rating Description
1	Incision is closed and completely secure. There is little to no inflammation and healing is essentially complete.
2	Incision is closed and completely secure, but inflammation is evident and is impeding the healing process.
3	Incision is closed and relatively secure, although the closure is not overtly strong and healing is not complete. Inflammation may or may not be evident.
4	Incision is closed, but only with thin fragile tissue or a fragile scab. Inflammation may or may not be evident.
5	Incision is held in proximity, but not completely closed, as edges still slide. Inflammation may or may not be evident.
6	Incision is partially open at one end or in the middle (< 50% open). Inflammation may or may not be evident.
7	More than 50% of incision is open. Inflammation may or may not be evident.
8	Incision is completely open. Inflammation may or may not be evident.

only caused inflammation that delayed the healing process in two fish (3.3%) treated with the adhesive (groups pooled together; inflammation score ≥ 4). Both affected fish were in the LV treatment, accounting for 6.2% of the treatment group. The inflammation associated with the Vetbond was caused by remnants of the surgical adhesive, which managed to stay adhered to the incision area eliciting a foreign body immune response. At the conclusion of the experiment, the Vetbond that was causing inflammation in the two fish was easily excised with a scalpel and forceps.

3.2. Incision dehiscence probability by week

The probability of an incision being open or dehisced varied significantly by treatment at 7 days post-surgery based on the Firth generalized binomial regression model (L–R Chi-Square: 33.27, DF: 3, $p < 0.001$). By day 7, three fish from the MS treatment (9.4%), one fish from the LS treatment (3.1%), fifteen fish from the MV treatment (46.9%), and zero fish from the LV treatment had an incision that was dehisced. Fish in the MV treatment were 9.44 times more likely than fish in the MS treatment (95% CI: 3.90–36.38, $p < 0.001$), 9.45 times more likely than fish in the LS treatment (95% CI: 3.89–36.34, $p < 0.001$), and 9.49 times more likely than fish in the LV treatment (95% CI: 3.89–36.23, $p < 0.001$) to have a dehisced incision at 7 days post-surgery. Average open incision length at 7 days post-surgery was 1.82 mm (SE = 0.279) for the MV treatment, 0.19 mm (SE = 0.275) for the MS treatment, 0.05 mm (SE = 0.272) for the LS treatment, and open incision length was non-existent for the LV treatment. At 14 and 21 days post-surgery, only four fish (12.5%) had dehisced incisions documented in the MV treatment group, while no fish had dehisced incisions documented in the other treatment groups. Fish in the MV treatment were 5.73 times more likely (95% CI: 1.55–32.79, $p = 0.003$) than fish in the other treatments to have a dehisced incision. Fish in the MV treatment still had on average 0.20 mm (SE = 0.244) of open incision length by day 21

3.3. Time to complete incision apposition

Time until complete apposition varied significantly by treatment according to the parametric Weibull time-to-event model (Wald Chi-Square: 193.80, DF: 3, $p < 0.001$; Fig. 3). Fish in the MV treatment took 1.72 times longer than fish in the MS treatment (95% CI = 1.51–1.93, $p < 0.001$), 2.17 times longer than fish in the LS treatment (95% CI = 1.90–2.48, $p < 0.001$), and 1.64 times longer than fish in the LV treatment (95% CI = 1.41–1.90, $p < 0.001$) for complete and sustained incision apposition to occur. Fish in the MS treatment took 1.24 times longer (95% CI = 1.13–1.35, $p < 0.001$) and fish in the LV treatment took 1.35 times longer (95% CI = 1.22–1.48, $p < 0.001$) for complete apposition to occur compared to fish in the LS treatment group.

We also found evidence that there was a significant interaction between treatment and tag burden with regards to time to complete apposition (Wald Chi-Square: 10.77, DF: 3, $p = 0.013$), indicating that within the LV treatment group those fish implanted with PIT-tags and acoustic transmitters took 1.15 times longer (95% CI = 1.01–1.31, $p = 0.0331$) to reach complete incision apposition compared to those fish implanted with only PIT-tags. Greater initial total length prior to surgery was also associated with a marginal decrease in time to complete apposition (ETR = 0.98, 95% CI = 0.97 – 0.99, $p = 0.014$), while the other covariates were not significant ($p > 0.200$).

3.4. Transmitter retention

We did not observe transmitter losses in fish from the MS, LS, and LV treatments over the 21 day experiment. In the MV treatment group a total of five PIT-tags (15.6%) and one acoustic transmitter (6.2%) were lost. One PIT-tag was lost on day 2, one on day 5, two on day 6, and one on day 7 of the experiment. The one acoustic transmitter was lost on day 5. The Firth generalized binomial regression model showed that fish in the MV treatment were 6.21 times more likely (LR-Chi-square: 13.51, DF: 1, $p < 0.001$; 95% CI: 2.04–71.52, $p < 0.001$) to lose a PIT-tag compared to fish in the MS, LS, and LV treatments. Fish in the MV treatment were also marginally 3.06 times more likely (LR-Chi-square: 1.87, DF: 1, $p = 0.172$; 95% CI: 0.69–37.34, $p = 0.172$) to lose an acoustic transmitter compared to fish in the MS, LS, and LV treatments. Tag burden was not predictive of PIT-tag loss (LR-Chi-square: 0.000, DF: 1, $p = 1.000$).

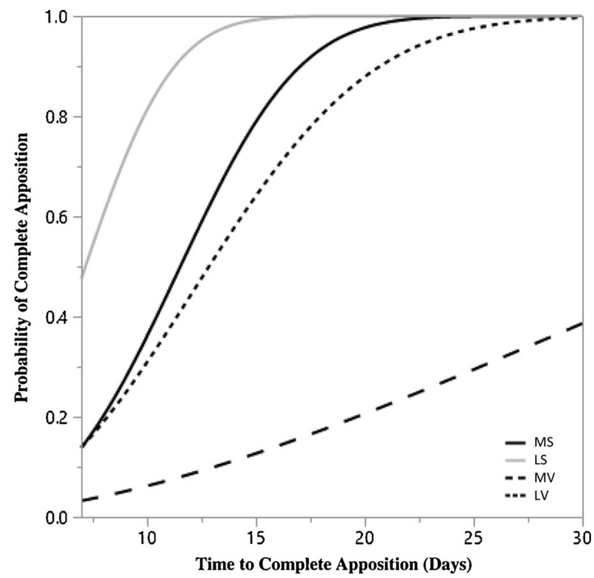

Fig. 3. Parametric Weibull time-to-event analysis showing observed and projected probabilities for time until complete incision apposition for each surgical treatment group of lake sturgeon. Treatment groups: MS = midline incision closed with suture, LS = lateral incision closed with suture, MV = midline incision closed with Vetbond, LV = lateral incision closed with Vetbond.

3.5. Incision inflammation by week

Inflammation by week was found to vary significantly by treatment (F-Ratio: 17.52, DF: 6, $p < 0.001$; Fig. 4). The interaction between treatment, family, and week was marginally significant (F-Ratio: 1.63, DF: 12, $p = 0.087$), while the interaction between treatment and family was significant (F-Ratio: 3.81, DF: 6, $p = 0.002$). However, the interaction between tag burden and treatment was not significant (R-Ratio:

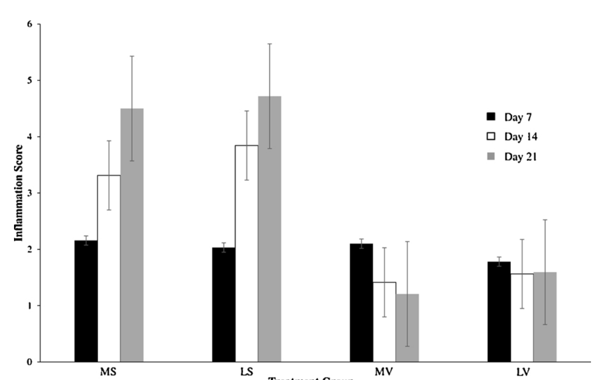

Fig. 4. Average inflammation score by week for each surgical treatment group of lake sturgeon. Means are shown (± SE). Treatment groups: MS = midline incision closed with suture, LS = lateral incision closed with suture, MV = midline incision closed with Vetbond, LV = lateral incision closed with Vetbond.

0.66, DF: 3, p = 0.577). All covariates were not significant (p > 0.340), except for average temperature (R-Ratio: 48.49, DF: 1, p < 0.001). At 7 days post-operatively, the level of inflammation experienced by fish was relatively uniform across the MS (mean = 2.81, SE = 0.154), LS (mean = 2.52, SE = 156), MV (mean = 2.63, SE = 157), and LV (mean = 2.26, SE = 0.155; Tukey HSD, p > 0.800) treatment groups. By day 14 the level of inflammation that fish in the MS (mean = 3.39, SE = 0.220) and LS (mean = 3.83, SE = 0.221) treatment groups exhibited had greatly increased and was substantially higher than the level of inflammation observed in the MV (mean = 1.42, SE = 0.227) and LV (mean = 1.58, SE = 0.221) treatment groups (Tukey HSD, p < 0.001).

At 21 days post-operatively, inflammation continued to increase among fish in the MS (mean = 4.13, SE = 0.2216) and LS (mean = 4.18, SE = 0.2229) treatment groups causing inflammation levels to become significantly higher compared to fish in the MV (mean = 0.78, SE = 0.2296) and LV (mean = 1.14, SE = 0.2221; Tukey HSD, p < 0.0001) treatment groups. For comparison, at 21 days post-operatively 65.6% of the MS treatment group and 68.7% of the LS treatment group had significant inflammation that was delaying the healing process (inflammation score ≥ 4), while no fish from the MV treatment group and only 6.3% of the LV treatment group displayed a similar level of detrimental inflammation. Fish in the MS treatment were 10.07 times more likely (95% CI: 4.09–35.87, p < 0.0001) and fish in the LS treatment were 11.47 times more likely (95% CI: 4.66–41.26, p < 0.0001) to experience severe inflammation compared to fish in the LV treatment group. Fish in the MS and LS treatment groups exhibited a marked increase in inflammation over time between day 7 and day 21 (Tukey HSD, p < 0.0001), while fish in the MV and LV treatment groups exhibited a slight decrease in inflammation over time between day 7 and day 21 (Tukey HSD, p ≤ 0.0014).

3.6. Incision healing

3.6.1. Incision healing scores by week

The mixed model analysis revealed significant differences in healing scores (Table 1**)** among the surgical treatments (F-Ratio: 34.64, DF: 3, p < 0.001; Fig. 5). There was also a significant interaction between treatment and examination week (F-Ratio: 8.46, DF: 6, p < 0.001) and between family and treatment (F-Ratio: 3.37, DF: 6, p = 0.004) with regards to incision healing. Seven days post-surgery we observed significant differences in healing between fish in the MV treatment group (mean = 4.85, SE = 0.330) and the MS (mean = 3.61, SE = 0.325), LS (mean = 2.65, SE = 0.320), and LV (mean = 2.94, SE = 0.329) treatment groups (Tukey HSD, p < 0.001). Fish in the MV treatment group exhibited poor healing characteristics. The level of healing experienced by fish in the LV treatment group was intermediate to that experienced

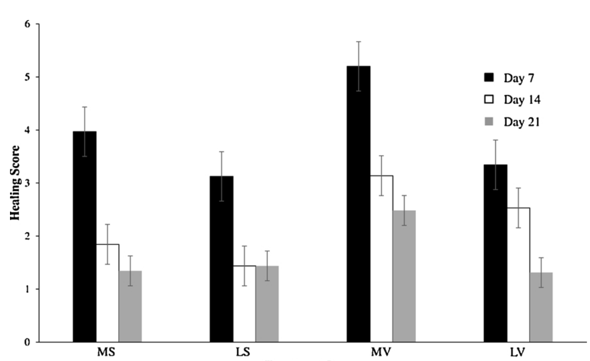

Fig. 5. Average healing score by week for each surgical treatment group of lake sturgeon. Means are shown (± SE). Treatment groups: MS = midline incision closed with suture, LS = lateral incision closed with suture, MV = midline incision closed with Vetbond, LV = lateral incision closed with Vetbond.

by the MS (Tukey HSD, T-Ratio: 2.90, p = 0.149) and LS (Tukey HSD, T-Ratio: -1.23, p = 0.986) treatment groups.

Incision healing by day 14 improved drastically in all treatments compared to measurements taken on day 7 (Tukey HSD, p < 0.001). Fish in the MV treatment group (mean = 3.28, SE = 0.330) continued to experience the poorest level of healing, especially compared to the LS (mean = 2.06, SE = 0.179; Tukey HSD, T-Ratio: p < 0.001) and MS (mean = 1.49, SE = 0.184; Tukey HSD, p < 0.001) treatment groups. The observed improvement in the MV treatment group was largely due to three fish within the treatment dying and, thus, measurements were no longer taken. Fish in the LS treatment group experienced somewhat better healing than fish in the LV treatment group at 14 days post-operatively (Tukey HSD, T-Ratio: -5.14, p < 0.001).

Incision healing by day 21 improved significantly for fish in the LV treatment group (Tukey HSD, T-Ratio = 4.10, p < 0.003), marginally for fish in the MS (Tukey HSD, T-Ratio: 1.18, p = 0.990) and MV (Tukey HSD, T-Ratio: 1.70, p = 0.866) treatments, and no major improvement was observed for fish in LS treatment group (Tukey HSD, T-Ratio: -0.92, p = 0.999) relative to measurements taken on day 14. Fish in the MV treatment (mean = 2.86, SE = 0.285) displayed a significantly poorer level of healing compared to fish in the MS (mean = 1.77, SE = 0.281), LS (mean = 1.71, SE = 0.288), and LV (mean = 1.72, SE = 0.278; Tukey HSD, p < 0.001) treatments. The fish in the MS, LS, and LV treatments comparatively all displayed similar levels of healing by day 21 (Tukey HSD, p = 1.000). However, fish in the MS treatment were 2.58 times (15.6% incidence rate) more likely (95% CI: 1.09–10.31, p < 0.001), and fish in the LS treatment were 3.16 times (18.8% incidence rate) more likely (95% CI: 1.17–12.51, p < 0.001) to experience a healing relapse event compared to fish in the LV treatment group between day 14 and day 21. The healing relapse events observed in the MS and LS treatment groups largely arose because of severe inflammation that developed between day 14 and day 21 from the prolonged immune response to suture material. Fish in the MV (3.1% incidence rate) and LV (0.0% incidence rate) treatments had similar low to non-existent rates of healing relapse (p = 1.000).

3.6.2. Probability of complete healing by day 21

The Firth generalized binomial regression model showed that there were significant differences in complete healing probabilities at the end of the experiment (LR-Chi square: 21.55, DF: 3, p < 0.001). At 21 days post-operatively, fish that had undergone the MS treatment were 5.62 times (95% CI 1.92–16.45, p = 0.002), those that underwent the LS treatment were 4.20 times (95% CI 1.48–11.94, p = 0.007), and those that underwent the LV treatment were 7.86 times (95% CI 2.56–24.15, p < 0.001) more likely to be completely healed compared to fish in the MV treatment group. Probabilities of complete healing at 21 days post-operatively were similar among fish from the MS, LS, and LV treatment groups (p > 0.260). There was not a significant interaction between treatment and tag burden (LR-Chi square: 1.40, DF: 3, p = 0.706), but there was a significant interaction with family (LR-Chi square: 35.52, DF: 6, p < 0.001). All covariates were not significant (p > 0.16).

3.7. Growth

3.7.1. Weight gain

There were no significant differences in weight among the treatment groups at the beginning of the study (F-Ratio: 0.61, DF: 4, p = 0.656). The MANOVA analysis showed differences among treatment groups with respect to weight gain and total length increase at the end of the study (Pillai's Trace: 0.18, F-Ratio: 3.86, DF: 6, p = 0.001; Fig. 6). There was also no significant interaction between treatment and tag burden (Pillai's Trace: 0.07, F-Ratio: 1.36, DF: 6, p = 0.231) or with family (Pillai's Trace: 0.06, F-Ratio: 0.428, DF: 16, p = 0.974). All covariates were not significant (p > 0.110), except for initial total length (Pillai's Trace: 0.06, F-Ratio: 3.475, DF: 2, p = 0.034). Fish in

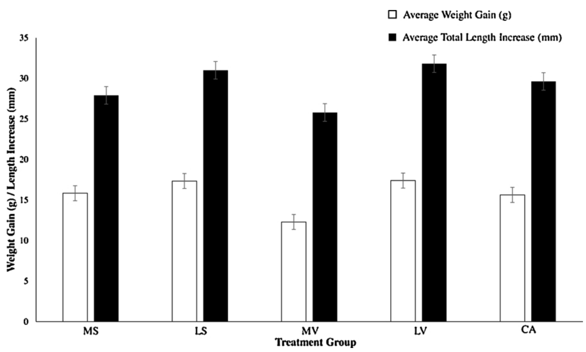

Fig. 6. Average weight gain (grams; white bars) and total length increase (mm; black bars) over the 21 day experimental time period for each surgical treatment group of lake sturgeon. Means are shown (± SE). Treatment groups: MS = midline incision closed with suture, LS = lateral incision closed with suture, MV = midline incision closed with Vetbond, LV = lateral incision closed with Vetbond, CA = control group treated with anesthetic only.

the MS (mean weight gain = 15.84 g, SE = 0.837), LS (mean weight gain = 17.34 g, SE = 0.837), LV (mean weight gain = 17.40 g, SE = 0.837), and control (mean weight gain = 15.63 g, SE = 1.184) treatment groups gained similar amounts of weight over the course of the 21 day experiment (Tukey HSD, $p > 0.600$). The fish in the MV treatment group gained significantly less weight than the fish in the MS (Tukey HSD, T-Ratio: 3.00, $p = 0.026$), LS (Tukey HSD, T-Ratio: 4.27, $p < 0.001$), and LV (Tukey HSD, T-Ratio: -4.32, $p < 0.001$) treatment groups. However, the fish in the MV treatment group gained a comparable amount of weight relative to the control group (Tukey HSD, T-Ratio: -2.30, $p = 0.150$).

3.7.2. Total length increase

There were no significant differences in total length among the treatment groups at the beginning of the study (F-Ratio: 0.59, DF: 4, $p = 0.669$). Fish in the LS (mean length increase = 31.00 mm, SE = 0.986) and LV (mean length increase = 31.81 mm, SE = 0.986) treatment groups grew in length similarly over the course of the 21 day experiment (Tukey HSD, T-Ratio: -0.58, $p > 0.977$), as did fish in the MS (mean length increase = 27.91 mm, SE = 0.986) and LS (Tukey HSD, T-Ratio: -2.22, $p = 0.179$) treatment groups (Fig. 6). However, fish in the MS treatment group grew marginally less in length compared to fish in the LV treatment group (Tukey HSD, T-Ratio: -2.80, $p = 0.045$). Fish in the MV treatment group (mean length increase = 25.79 mm, SE = 1.036) grew significantly less in length than fish in the LS (Tukey HSD, T-Ratio: 3.64, $p = 0.003$) and LV (Tukey HSD, T-Ratio: -4.21, $p < 0.001$) treatments, but grew comparably relative to the control group (mean length increase = 29.62 mm, SE = 1.395; Tukey HSD, T-Ratio: -2.21, $p = 0.184$) and the MS treatment group (Tukey HSD, T-Ratio: 1.48, $p = 0.579$).

4. Discussion

4.1. Mortality and surgical complications

We examined optimal incision placement and the efficacy of using Vetbond surgical adhesive for closing incisions in age-0 lake sturgeon. Mortality was only associated with sturgeon in the MV treatment group. The three mortalities that occurred in the experiment were associated with viscera expulsion, which was the most likely cause of death. During viscera expulsion internal organs may be damaged by the external environment, osmoregulation capability is severely degraded, the risk of infection greatly increases, and the incision is generally not able to close (Boyd et al., 2011; Panther et al., 2011). Boyd et al. (2011) noted that viscera expulsion was more common in smaller fish and for midline incisions closed with a single suture than for incisions closed with two sutures when Chinook salmon smolts were exposed to simulated turbine passage.

The occurrence of tissue necrosis was very low, only occurring along midline incisions of sturgeon in the MS (6.25%) and MV (3.1%) treatment groups. The tissue necrosis observed in the sturgeon in the MS treatment group likely resulted from the suture material causing the tissue to become ischemic (Whipple and Elliott, 1938). The tissue necrosis observed in the sturgeon in the MV treatment group was associated with dehiscence of the incision. The lower perfusion of the tissues along the midline linea alba (Panther et al., 2011) makes tissue necrosis more of a concern in this region compared to lateral incisions made along the hypaxial musculature. However, lateral incisions can damage vascular muscle tissue (Tera and Aberg, 1977) and nerve axons (Burger et al., 2002), which can result in greater blood loss (Nygaard, 1996) and longer healing times (Anderson and Roberts, 1975; Wagner et al., 2011).

4.2. Incision dehiscence and apposition

Our experimental results clearly demonstrate that Vetbond was not able to adequately close midline incisions, while it was efficient at closing lateral incisions. The LS and LV surgical treatments were the most robust against incision dehiscence, and they provided the highest level of closure. Midline incisions closed with sutures experienced lower apposition quality and a higher rate of dehiscence compared to lateral incisions closed with suture. Tissue in the midline region was also more fragile, and the incisions tended to close with delicate scar tissue. The higher level of dehiscence and poorer apposition quality in the MV and MS treatment groups would cause incisions to heal through the much longer process of secondary intention. This ultimately delays the re-epithelialization process, increases susceptibility to infection, reduces osmoregulatory performance, and requires more energy (Guo and DiPietro, 2010).

The lower apposition quality and higher incidence rate of dehiscence observed in the midline region can likely be explained by several factors. The midline region is the connection point for the abdominal muscles used during locomotion, which makes the midline region more vulnerable to higher levels of lateral tension that could lead to poor apposition and dehiscence (Boyd et al., 2011; Panther et al., 2011). Transmitters are also more likely to rest on and apply pressure to the midline incision area. Furthermore, the midline linea alba region is poorly vascularized, which may result in weaker fibrin clot formation and slower healing compared with lateral incisions made through the hypaxial musculature (Panther et al., 2011). Histological results reported by Panther et al. (2011) showed that midline incisions for Chinook salmon smolts healed poorly with less fibrotic tissue at the incision site, the edges of the incision curled inward, and the incision was only connected by a thin layer of epithelial cells compared to lateral incisions.

4.3. Transmitter retention

Incision closure and apposition characteristics are directly linked to transmitter retention. PIT-tag loss was only observed by sturgeon in the MV treatment group (15.6%). Sturgeon in the MV treatment group were 6.21 times more likely to lose a PIT-tag compared to fish in the other treatments. Acoustic transmitter loss was rare, with only one transmitter lost in the MV treatment group (3.1%). The irregular shape of the JSATS transmitter may have contributed to the low level of loss observed. LS, MS, and LV surgical treatments were all robust to transmitter loss, indicating that comparable tag retention can be achieved when Vetbond is used to close lateral incisions. This also indicates that only one suture is necessary to ensure high transmitter retention in lateral or midline incisions. Similar research on juvenile Chinook salmon found that only one simple interrupted suture was required for closing midline incisions, maintaining apposition, and achieving high transmitter retention (Deters et al., 2012).

Studies on juvenile sturgeon transmitter retention have generally focused on larger juveniles than the size range we evaluated (mean weight: 30 g). Miller et al. (2014) reported 93.75% transmitter retention for lateral incisions closed with suture in juvenile green sturgeon (mean weight: 347 g; *A. smedirostris*), although the sample size was low (N = 16). Conversely, Crossman et al. (2013) found that transmitter retention of relatively large transmitters (length: 2.7 cm, diameter: 0.9 cm) inserted through lateral incisions was only 25% for non-anchored transmitters and 88% for transmitters anchored to the peritoneum in juvenile shortnose sturgeon (mean weight: 318 g; *A. brevirostrum*). Smith and King (2005) also experienced poor transmitter retention with midline incisions in a telemetry study, reporting 40% transmitter loss with yearling lake sturgeon (mean weight: 207 g). More recently, Liss et al. (2018) observed 100% transmitter retention for ventral-lateral and dorsal-flank incisions in age-0 white sturgeon (weight: ˜ 69 g). Similarly, Ashton et al. (2017) noted 100% transmitter retention in age-0 white sturgeon for un-sutured dorsal-flank incisions for a variety of weight classes (weight: 26–126 g).

4.4. Incision healing and inflammation

While lake sturgeon in the MS and LS treatment groups generally attained complete healing first, their healing process began to relapse and deteriorate significantly by day 21 compared with the LV treatment group because of severe inflammation generated by the immune response to the suture material. In contrast, inflammation associated with the Vetbond surgical adhesive was very low, and healing was robust in the LV treatment group. However, lake sturgeon in the MV treatment group exhibited poor healing throughout the entire experiment.

To avoid the detrimental effects of inflammation, absorbable monofilament suture material should be promptly removed once incisions have become securely closed, usually 7–14 days post-surgery. Sutures cease to be beneficial after incisions are securely closed and will quickly become harmful to the healing process (Deters et al., 2012; Dunn, 2007; Liss et al., 2018). Miller et al. (2014) reported that severe inflammation in response to suture material caused lateral incisions to reopen by the third week in green sturgeon, and they recommended using the least amount of suture material possible. Liss et al. (2018) also found that suture material impaired the healing process in age-0 white sturgeon, and they recommended not using sutures to close small 7–9 mm incisions. In mammals Moncryl absorbable monofilament suture (Ethicon, Somerville, New Jersey) has been shown to lose all of its tensile strength at 28 days post-surgery, but the suture material itself is not fully absorbed until 91–119 days post-surgery (Dunn, 2007). This is a fact that is commonly overlooked in studies, as inflammation will continue to worsen until either the suture material is removed or it is completely absorbed. The inflammation can potentially affect the health and behavior of the fish under study, which can confound the results of important research (Bridger and Booth, 2003; Deters et al., 2012; Guo and DiPietro, 2010).

4.5. Growth characteristics

Lake sturgeon in the MV treatment group gained significantly less weight than sturgeon in the other treatment groups. Sturgeon in the MV treatment group also grew less in length compared to sturgeon in the LS and LV treatments. In contrast, sturgeon in the LV treatment grew similarly compared to sturgeon in the LS treatment and control group. This provides additional evidence that Vetbond can be safely used to close lateral incisions, without causing adverse health impacts. It seems likely that the poor incision healing and incision dehiscence experienced by the MV treatment group resulted in poorer growth compared to the other treatments (Boyd et al., 2011; Guo and DiPietro, 2010).

Transmitter burden was not linked to differences in growth characteristics likely because the level of tag burden tested relative to body weight was very low (mean: 1.99% vs. 3.15%). Some researchers have found that fish can be robust against the effects of tag burden (Ammann et al., 2013; Brown et al., 1999; Miller et al., 2014), while others have found clear deleterious effects on growth (Brown et al., 2010; Chittenden et al., 2009; Robertson et al., 2003). Sutton and Benson (2003) found that larger external radio transmitter burdens (Range: 1.25%–6%) on juvenile lake sturgeon caused significantly lower growth, and they recommended that transmitter weight should not exceed 1.25% of body weight. Ashton et al. (2017) observed that transmitter burden (0.6–2.6%) only had a small effect on the growth of age-0 white sturgeon, and the sturgeon were able to regain their growth potential by 28 days post-implantation.

4.6. Prior research on cyanoacrylate adhesives for incision closure in fishes

To our knowledge, only two studies have examined the efficacy of a surgical grade adhesive (e.g., Vetbond) for intracoelomic transmitter implantation. Jepsen et al. (2017) found that 10–15 mm incisions on brown trout (*Salmo* trutta) closed with histo-glue (chemically identical to Vetbond) presented with the best healing characteristics (i.e., limited inflammation and necrosis), but 30% of the transmitters were lost in the treatment group. Lowartz et al. (1999) observed that 5 mm incisions on larval sea lamprey closed with Vetbond were associated with more inflammation and higher mortality compared with suture treatments.

The other studies on the subject matter have all examined the efficacy of household superglue. Petering and Johnson (1991) examined the efficacy of a gel based superglue for closing 20–25 mm incisions on black crappies (*Pomoxis nigromaculatus*), and they found that incisions closed with superglue healed slower, there was a much higher frequency of incision dehiscence, and at 21 days post-operatively 70% of the transmitters had been expelled. The researchers also observed that the superglue caused less inflammation than sutures, took 38% less time to apply compared to sutures, and at 3 days post-operatively the superglue began to fall off. Other researchers have similarly noted poor closure characteristics with household superglue (Kaseloo et al., 1992; Raoult et al., 2012). In contrast, Baras and Jeandrain (1998) noted good closure performance, better survival, and considerably less inflammation than sutures when a biological bandage was used in conjunction with superglue to close 20 mm incisions on yellow eels (*Anguilla Anguilla*), and Nemetz and Macmillan (1988) similarly noted limited inflammation and good closure quality with minor mesentery adhesions when superglue was used to close 15 mm incisions on channel catfish (*Ictalurus punctatus*).

In the present study we found that Vetbond surgical adhesive was effective at closing lateral incisions, but was not effective at closing midline incisions. We also did not observe severe tissue inflammation or necrosis associated with the adhesive. The differences observed among the studies presented in this section are likely the result of multiple factors, including incision length, incision location, surgical technique, surgical experience, structural tissue differences, immune response differences, surgical site infection, and the amount of tension that the incision is exposed to. The characteristics of the adhesive itself are also a vital factor. Surgical grade adhesives have limited cytotoxicity, while household superglue is severely cytotoxic and medically contraindicated (Sohn et al., 2016; Toriumi et al., 1990). In addition, most of the studies that evaluated the efficacy of household superglue applied a thick superglue gel to the incision cites, which is not recommended, as you should only apply a very thin coating of surgical adhesive that is in liquid format (usually only 1–2 drops), making sure to avoid the pooling of the adhesive on the skin (3M Health Care, 2016).

4.7. Surgical recommendations

We found that midline and lateral incisions 8 mm in length on age-0 lake sturgeon can be safely closed with a single suture, while achieving high survival and transmitter retention. However, the midline linea alba tissue is more delicate and our results showed poorer apposition and closure characteristics in this area. Therefore, we recommend using a minimum of two simple interrupted sutures to close midline incisions. We also found that Vetbond was not able to safely close midline incisions, while it was effective at closing lateral incisions. Consequently, small lateral incisions can be safely and effectively closed with either a single interrupted suture or with Vetbond surgical adhesive. If the sutures are not able to be removed within 7–14 days post-surgery, then we recommend that Vetbond be used because it is associated with significantly less inflammation and better incision healing in the long-term. Our research also showed that PIT-tags can be safely inserted into the body cavity of age-0 lake sturgeon with high retention and fewer problems than what has been reported for the traditional dorsal musculature insertion region (Damon-Randall et al., 2010; Hamel et al., 2012; Moser et al., 2000). In order to minimize potential family genetic effects on incision healing, care should also be taken to ensure adequate diversity (Blankenhorn et al., 2009; Liaw et al., 1998; McBrearty et al., 1998). Future simulated turbine passage research is needed to evaluate the reliability of these closure methods and incision locations for lake sturgeon that may rapidly out-migrate through hydroelectric infrastructure (Boyd et al., 2011).

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