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SPECIAL SECTION: ANGLING FOR DINOSAURS

Intracoelomic Implantation of Transmitters in Longnose Gar

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Abstract

Fish in the family Lepisosteidae (hereafter, gars) have unique ganoid scales that pose inherent challenges to implanting electronic tags in their coeloms for telemetry studies. In this paper we outline a unique approach to conducting laparotomic surgery in gars, with a focus on the Longnose Gar *Lepisosteus osseus*. An electric rotary tool with a circular cutting blade was used to cut through the scales, and the same tool, with a drill bit, was used to create holes through which to run the suture material. The final incision into the body cavity was made with a scalpel, and the incision was expanded using surgical scissors. Using a passive acoustic telemetry array, the survival of 12 of 15 tagged Longnose Gars was confirmed over a 123-d period based on their detection at receivers outside of their areas of capture and release. Two individuals were recaptured 17–19 weeks postsurgery and showed complete healing of the wounds with limited evidence of scarring. This approach will allow for the long-term tagging and tracking of gars to help elucidate their ecology.

Fish in the family Lepisosteidae (hereafter, gars) are part of an ancient lineage that diverged from Teleostei approximately 250 million years ago (Schultze 2016). These fishes are facultative air breathers that can tolerate warmwater conditions with low dissolved oxygen. While they have long been considered nuisance species in direct competition with more economically important recreational and commercial fishes (e.g., Largemouth Bass *Micropterus salmoides* and Northern Pike *Esox lucius*; Scarnecchia 1992), recently there has been interest in developing a better understanding of gar ecology. This is particularly true in areas where gars have been identified as species at risk (e.g., Spotted Gar *Lepisosteus oculatus* in Canada; COSEWIC 2005).

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Biotelemetry is frequently used to track fish and provide insights into their basic ecology (Lucas and Baras 2008; Cooke et al. 2013, 2016). The utility of this approach is predicated on the assumption that the attachment of a transmitter does not significantly affect a fish's welfare, behavior, life history, or survival (Brown et al. 2011). While several transmitter attachment techniques exist (i.e., gastric, external), intracoelomic transmitter implantation has been used successfully in a wide variety of fishes and is considered the best attachment technique for long-term tracking, as the transmitters stay in the fish longer than gastric transmitters and are less energetically costly than externally attached transmitters (reviewed in Bridger and Booth 2003; Cooke et al. 2011). Standardized techniques for intracoelomic transmitter implantation have been developed (see Cooke et al. 2011; Wagner et al. 2011), but most of the literature on which those methods are based stems from studies of teleost fishes. Their sister infraclass in the subclass Neopterygii, the Holosteii, pose a unique challenge, and while intracoelomic implantation has been successfully applied to fish in the order Amia (Traslavina 2010; Midwood et al. 2018), to our knowledge no species in the gar family have been internally tagged. This is likely due to the challenges associated with breaching the ganoid scales of gars. These scales are composed of multiple layers with a thick osseous plate at the base, overlapping collagenous layers in the middle, and ganoine (an enamel-like substance) on the surface (Elliott 2011a). The result is a series of rigid plates that are connected by peg-and-socket joints, which collectively provide rigidity to the body wall, support their swimming activity (Long et al. 1996), provide protection from predation (Yang et al. 2013), and pose a unique challenge for intracoelomic implantation using traditional scalpel-based surgical approaches.

Despite the physical challenges related to tagging gars, external attachments of radio and acoustic transmitters to their dorsal fins and T-bar and anchor tags have been used to evaluate habitat use (Spotted Gar: Sneddon et al. 1999; Glass et al. 2012; Alligator Gar *Atractosteus spatula*: Buckmeier et al. 2013), migration and movement patterns (Longnose Gar *Lepisosteus osseus*: Johnson and Noltie 1996; McGrath et al. 2012; Alligator Gar: Solomon et al. 2013), and tag retention (multiple Lepisosteidae: Buckmeier and Reeves 2012). While the application of these techniques has contributed greatly to our understanding of gar ecology, external attachment of transmitters can have negative long-term consequences (Jepsen et al. 2015). Furthermore, external transmitters can become biofouled, which is particularly true for species such as gars that spend a considerable amount of time in shallow, vegetated waters (Bridger and Booth 2003). This type of biofouling on external tags can lead to infection or tissue necrosis (Atlantic Salmon *Salmo salar*: Thorstad et al. 2000) and may affect the swimming performance of tagged individuals (Atlantic Salmon: McCleave and Stred 1975; White Sturgeon *Acipenser transmontanus*: Counihan and Frost 1999). Therefore, developing techniques to surgically implant transmitters into gars would be desirable.

Using the Longnose Gar as a model species, our objectives in the present study were to (1) outline an intracoelomic tagging approach for gars, (2) provide a preliminary evaluation of posttagging survival, and (3) where possible, assess the extent of wound closure.

METHODS AND RESULTS

On June 29 and 30, 2016, 15 Longnose Gars were captured using boat electrofishing (Smith-Root SR 21EH work boat, 7.5 kW Generator Powered Pulsator) in the Ottawa Street Slip, which is situated along the south shore of Hamilton Harbour at the western tip of Lake Ontario (Figure 1). Water temperatures in this slip are generally higher and less variable than those in other areas of the harbor due to the effluent from an adjacent steel plant. For the duration of this study, water temperatures ranged from a high of 30°C in August to a low of 21°C in October (C. McGinley, ArcelorMittal, personal communication). The length of captured Longnose Gars ranged from 671 to 978 mm, with a wet mass of 1.2 ± 0.5 kg (mean $\pm$ SD; Table 1). Individuals were held in a 341-L live well with constant water circulating in from the slip. Animal care approval for this study was provided by the Carleton University Animal Care Committee under permit 102935. This permit was provided provisionally to support the current pilot study to develop an intracoelomic tagging technique for Longnose Gars and required immediate reporting of any adverse outcomes to the Carleton University veterinarian.

Anesthesia.— We explored two options for anesthesia prior to surgery. The first was electro-anesthesia using the boat electrofisher to stun the Longnose Gars with a setting of 9.8 A for 6 s. Similar approaches have been used successfully with other fish species (i.e., Bluegill *Lepomis macrochirus*; Rous et al. 2015); however, given the extended length of the surgery for the Longnose Gars (outlined below), this approach did not provide adequate induction. Furthermore, electro-anesthesia has been found to increase mortality in some fishes, particularly those with elongated bodies (i.e., Northern Pike; Peat et al. 2016). This concern, paired with the limited induction time, likely makes electro-anesthesia inappropriate for gar surgeries, which require deeper anesthesia; however, this approach may still be valuable for applications where less complete induction is required (i.e., external tagging or to facilitate handling).

The second approach used a solution of clove oil emulsified in ethanol (with eugenol as the active ingredient) in a 1:10 ratio at a dose of 0.065 mL/L. This dosage is frequently used with other fishes that are not easily induced

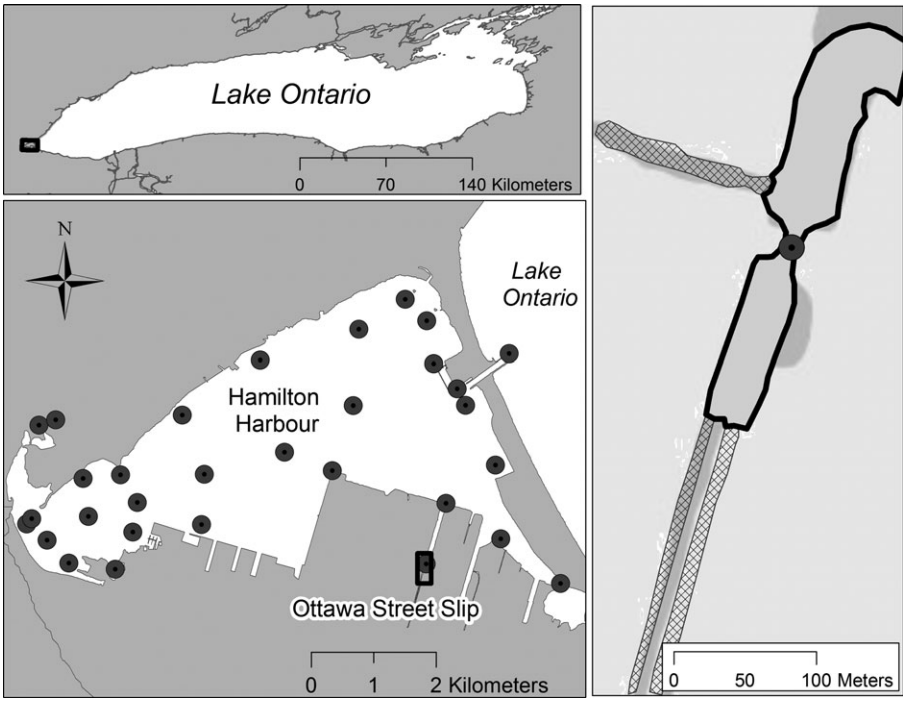

FIGURE 1. Map of the Ottawa Street Slip in Hamilton Harbour, Lake Ontario. The locations of the acoustic telemetry receivers are indicated by gray circles with black dots in the middle. The zone where Longnose Gars were captured and released is outlined by a thick black line in the panel on the right, and areas within the slip that fall outside of this receiver's detection zone are indicated by hatch marks.

TABLE 1. Date of tagging and basic physical characteristics of the 15 Longnose Gars used in this study. Individuals are listed in the order in which they were tagged. Details on the duration of induction in a 0.065 mL/L solution of clove oil and surgery (plus any additional immersions in the clove oil bath) are provided. A fish's relative activity, which was used to confirm survival, is summarized based on the number of acoustic receivers at which it was detected each month between tagging and October 31, 2016. Several individuals were detected leaving the array, and these are identified as being "Out" in a given month.

ID	Date tagged[a]	Total length (mm)	Wet mass (g)	Initial induction time (min)	Surgery + additional induction time (min)	Number of additional immersions	Number of stations where detected				Confirmed survival
							Jul	Aug	Sep	Oct	
LNG15217[b]	Jun 29	680	680	4.75	6.80		1	1	2	12	Yes
LNG15214	Jun 29	834	1,290	5.50	4.60		5	2	21	23	Yes
LNG15210	Jun 29	902	1,330	4.62	7.67	2	17	Out	Out	Out	Yes
LNG15211	Jun 29	781	1,110	5.43	8.50	2	12	2	16	17	Yes
LNG15212	Jun 29	858	1,650	5.15	4.00		22	14	Out	23	Yes
LNG15213	Jun 29	752	970	8.85	5.83	2	11	5	5	11	Yes
LNG15206	Jun 29	704	790	9.87	4.27		1	1	1	1	No
LNG15207[b]	Jun 29	728	1,000	9.38	3.83		2	2	2	20	Yes
LNG15208	Jun 29	727	910	6.73	4.17		2	2	2	2	Yes
LNG15209	Jun 29	978	2,370	8.93	3.98		21	22	1	1	Yes
LNG15205	Jun 30	920	1,750	5.83	9.92	1	2	2	4	11	Yes
LNG15204	Jun 30	916	1,750	12.43	3.62		1	0	0	0	No
LNG15203	Jun 30	732	790	7.65	10.23	1	17	1	1	14	Yes
LNG15202	Jun 30	823	1,230	7.55	4.47		24	20	Out	Out	Yes
LNG15200	Jun 30	671	710	5.88	3.85		1	0	0	0	No

[a]All dates in 2016.
[b]Denotes individuals that were recaptured during the fall surveys.

using electro-anesthesia or when this technique cannot be practically applied based on field conditions (e.g., a remote location or lack of electricity; Bridger and Booth 2003; Javahery et al. 2012). Induction times with the clove oil varied among the Longnose Gars, with a mean of 7.23 min (SD, 2.18 min; Table 1). In several instances, additional shorter immersions in the clove oil bath were required to ensure complete induction, and these were completed prior to the start of surgery. Following the first induction period, the length and wet mass of each Longnose Gar were determined. If the individual had not reached stage IV induction (i.e., loss of equilibrium and slow but regular movements), it was returned to the clove oil bath until it no longer responded to external stimuli (e.g., grasping the caudal peduncle). The surgery timer was started when an individual was measured; therefore, the total surgery time is longer for fish that received additional immersions. Once an individual had reached stage IV induction, it was placed supine in a V-shaped trough with fresh slip water covering its head and most of its gills. Freshwater was added to the trough throughout the surgery; however, it may be prudent in future surgeries to pass a circulating maintenance dose of clove oil over the gills to prolong induction.

Surgery.— The incision was made along the ventral midline between the pectoral and pelvic girdles. The first step in the surgical procedure involved breaching the ganoid scales. This was accomplished using an 18-mm cutting disk on a rotary tool (12-V Mastercraft, Vonore, Tennessee); the initial incision was the same length as the diameter of the cutting disk but intentionally did not penetrate into the body cavity to avoid excessive damage to the peritoneum (Figure 2; see also Video S.1 in the Supplement available in the online version of this article). A scalpel (no. 21) was then used to breach the body cavity, with the assistance of forceps to prevent damage to internal tissue. Once the body cavity had been breached, surgical scissors were used to increase the length of the incision to approximately 40 mm (Figure 2) to allow for the insertion of an acoustic transmitter (V13P, 69 kHz, length = 45 mm, mass in water = 6 g, mean delay = 200 s, estimated tag life = 1,388 d, tag burden range = 0.3–0.9%; Vemco, Halifax, Nova Scotia). This multistep approach to creating the incision was used because the cutting disk was quite powerful and did not allow an incision as precise as the surgical scissors. Also, just using a scalpel, as is common with teleost fishes, was not an option since it could not penetrate the ganoid scales to start the incision or elongate it.

Once the incision was complete, the transmitter was inserted into the body cavity and four holes were drilled using a 1.5-mm drill bit in the rotary tool (Figure 2; Video S.1) to allow suture material to be passed through the ganoid scales. The handle portion of a stainless steel forceps was first inserted into the incision under the location

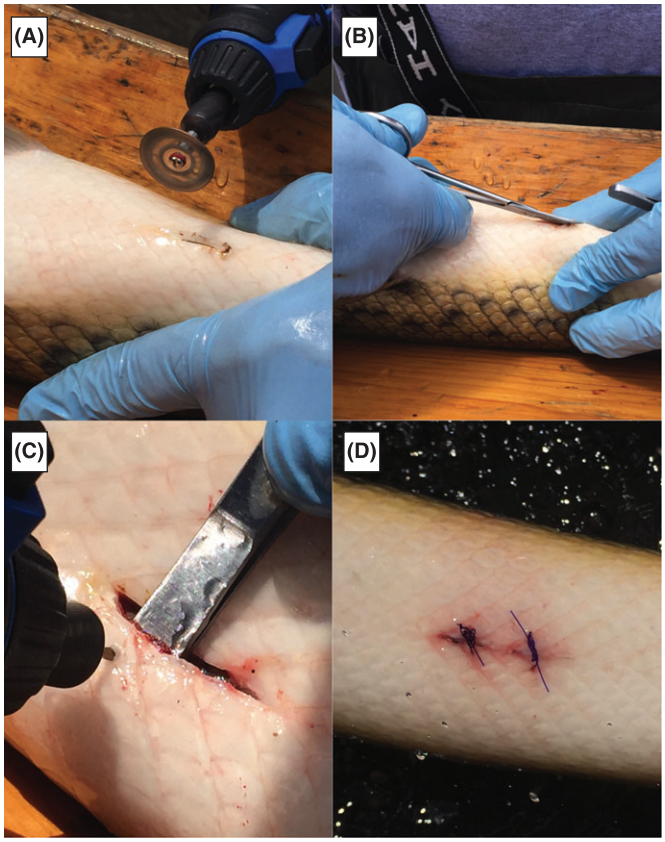

FIGURE 2. Photographs from Longnose Gar surgery showing **(A)** making the initial incision with a cutting disk, **(B)** opening the incision with scissors, **(C)** using a drill to create suture holes and shielding internal organs with the handle of a pair of forceps, and **(D)** the closed surgical incision. Photo: Dave Reddick. [Color figure can be viewed at afsjournals.org.]

of the hole to stop the drill bit from penetrating too deeply into the body cavity, thus protecting internal organs.

To begin closure of the incision, two sutures (3–0 monofilament with a reverse cutting needle) were held in Olsen-Hegar needle holders. Two pairs of needle holders were used (in contrast to more typical surgeries, which just use one) because the rigidity of the body wall in Longnose Gars made it challenging to thread the second suture through the suture holes after one had already been closed. Thus, each suture was first threaded through the suture holes with the aid of blunt curved forceps and then the sutures were tied using a surgeon's knot (2 × 3 throws followed by 1 × 2 throws; Figure 2; Video S.1). Again, due to the rigidity of the body wall, we were careful to ensure that the opposing body walls on either side of the incision were properly aligned prior to completing the suture knots. Proper apposition of the tissue on either side of the incision is well established as a critical component of proper healing for all forms of surgery (Wagner et al. 1999; Ream et al. 2003). With the exception of the cutting disk, all surgical equipment was disinfected between its

use on successive individuals using povidone iodine before being rinsed with distilled water.

The surgeon who performed the implants had extensive experience with similar implantation techniques in teleost fishes, having completed more than 100 surgeries on 12 different species. Surgery times were quite variable (3.62–10.23 min); however, much of this variation was driven by additional immersions in the clove oil bath for some individuals (Table 1). For those individuals that did not require additional immersion ($N = 10$), surgery times were less variable (4.37 ± 0.87 min) and generally decreased as the surgeon became more proficient with the required techniques (Table 1). Longer surgery times were also partially driven by bleeding at the incision site and/or the suture holes. This bleeding occurred during all surgeries and was followed by rapid clotting. For some of the Longnose Gars, blood pooled around the incision site, which made it challenging for the surgeon to thread the suture material through the holes, thus extending the overall length of the surgery (Table 1). In such cases a clean paper towel was used to remove excess blood.

Recovery.— Following surgery, the fish were returned to the live well, into which fresh lake water was continually pumped. Their recovery was monitored and once they exhibited signs of recovery (i.e., were right side up and responding to caudal tail grabs), which typically occurred within 10–20 min, they were returned to the water and held alongside the boat until they swam away volitionally. All Longnose Gars appeared to recover from the anesthesia and were active and responsive at the time of release.

Posttagging tracking.— Following their release, the fish were passively tracked on an acoustic array with 34 acoustic receivers (VR2W, 69 kHz; Vemco; Figure 1). One receiver was situated directly in the Ottawa Street Slip within the capture and release zone (Figure 1). Longnose Gar positions were tracked from their release on June 29 and 30, 2016, until October 31, 2016, when the receivers were recovered and the data were downloaded. Posttagging survival was assessed based on the level of activity (detections on multiple receivers) of each fish. In addition, electrofishing surveys were conducted in the Ottawa Street Slip on October 31 and November 15, 2016, to recapture the fish and visually evaluate incision healing. During these surveys, an active acoustic tracking unit (VR100; Vemco) was also deployed in an effort to detect Longnose Gars that were still present in the slip.

For 12 of the 15 Longnose Gars that were tagged, detections were recorded outside of the Ottawa Street Slip at 2–24 receiver stations (depending on the individual), suggesting that they had survived the surgical procedure and were still actively moving within the harbor. For the three remaining individuals, two were only detected a handful of times (LNG15200: $N = 9$; LNG15204: $N = 1$), and all detections were on the same day that they were tagged. Based on this, we assumed that these individuals either did not survive (which is more likely) or shed their transmitters in a location outside of the range of receivers (Figure 1). The final individual (LNG15206) was continuously detected at the station within its tagging zone and was found to be in the same position on both active tracking dates. This individual was therefore assumed either not to have survived the procedure or to have dropped its transmitter shortly after surgery.

All of the Longnose Gars observed during electrofishing were captured and held in a live well with circulating slip water. The hydrophone of the VR100 active tracker was placed inside the live well to determine whether any of these individuals had been tagged. When a tag was detected, individual fish were removed and thoroughly inspected for signs of tagging (often suture material is still present several months posttagging or there is some residual inflammation or scarring; Jepsen et al. 2000; Caputo et al. 2009). Regardless of whether or not they were recaptured fish, the length and wet mass of all individuals were measured. The transmitter IDs of recaptured fish were recorded, and photographs of their incisions were taken. In each of the recapture surveys a tagged Longnose Gar was recaptured. Fish LNG15207, captured on October 31, 2016, had no signs of inflammation and only faint scarring (all suture material was gone). In fish LNG15217, captured on November 15, 2016, part of the anterior suture was still present but the incision itself had healed completely, with inflammation localized around the suture material (Figure 3).

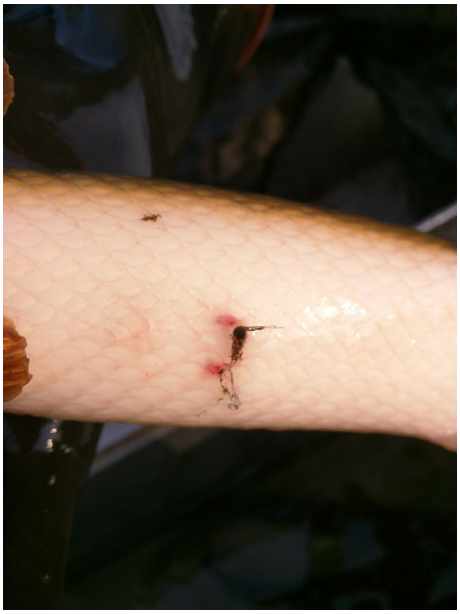

FIGURE 3. Photograph showing inflammation from the suture material in fish LNG15217, which was recaptured on November 15, 2016. The surgical incision has healed completely, with no apparent signs of scarring. Photo: Andrew Fernley. [Color figure can be viewed at afsjournals.org.]

DISCUSSION

In this article we have presented a relatively simple modification to standard fish surgical procedures for intracoelomic tag implantation that allows fish with ganoid scales to be implanted with electronic tags. Confirmed survival from the tagging procedure was 80%, with some evidence of complete closure of the incision with minimal scarring. For the three individuals for which survival could not be confirmed, two had long induction times in the clove oil bath. As rapid induction is a desirable feature of fish anesthetics (CCAC 2005), prolonged exposure to the anesthetic may have caused excess stress on these two individuals and affected their posttagging survival. There was no apparent difference in the surgical procedure for the final fish (LNG15200) and, indeed, all three of the individuals appeared to recover well from the surgery and were active at the time of release. Since the fate of these three individuals cannot be verified, the survival rate presented here represents a minimum estimate. Long-term monitoring on the receiver array paired with more in-depth active tracking in the Ottawa Street Slip may help to determine the ultimate fate of these individuals. An important shortcoming of the current study is the lack of information for each individual on the duration of its recovery postsurgery. Several Longnose Gars were recovered in the live well at the same time, so individual recovery durations are not available; however, future studies should explore the relationships among induction time, recovery time, and survival.

For the remaining Longnose Gars, it is clear that they were active in the system and, for the two that were recaptured, complete closure of the incision occurred within at least 18 weeks. Several factors likely contributed to the effective healing of the incision. First, the epidermal layers in fish are all metabolically active, so that epithelial cells immediately begin migrating to the site of an injury to cover and protect the wound (Elliott 2011a, 2011b). The rate of migration of these cells is heavily influenced by the surrounding water temperature (Anderson and Roberts 1975; Ream et al. 2003; Elliott 2011b), with warmer temperatures resulting in faster migration (which peaks between 25°C and 35°C) and ultimately to more rapid healing rates (Ream et al. 2003). Water temperatures within the Ottawa Street Slip during the present study fell within this optimal range (typically above 26°C), and this likely contributed to effective healing of the surgical incisions. Water temperature also plays an important role in the dissolution of absorbable sutures, with over 50% loss of suture material in Striped Bass *Morone saxatilis* after 30 d of exposure to water between 22°C and 29°C (Walsh et al. 2000). Finally, the ganoid-type scales of Longnose Gars and other Lepisosteidae possess a unique vascular system that penetrates the boney scales, supplying blood to external tissue layers. Lost and damaged scales can therefore be replaced if the integumental damage is not severe (Elliott 2011a). Combined, these three factors likely contributed to the successful closure of the surgical incisions and the dissolution of most of the suture material in a comparatively short time. A more detailed and controlled evaluation of wound healing under different water temperatures and conditions is likely warranted given the unique physiology and anatomy of gars.

We should note one caveat about this surgical approach: for many of the surgeries, we observed more bleeding than is typical for surgeries on teleost fishes. As stated above, the ganoid scales of gars are highly vascularized, such that the repeated penetration of the body wall that is necessary to complete the surgery will invariably disrupt vascular tissue. As the blood clotted rapidly, however, we do not believe that blood loss alone led to the apparent reductions in posttagging survival. Rather, the presence of clotted blood in the area of the incisions made for a more challenging surgery due to limited visibility. Repeated flushing with water and blotting with paper towels did help resolve all of these challenges, though removal of some of this material using suction may help further.

In their review of surgical techniques for implanting transmitters in fishes, Cooke et al. (2011) emphasized the importance of conducting studies on the efficacy of tagging techniques on a wider diversity of taxa. Here, we have documented a unique but effective approach for intracoelomic implantation of telemetry transmitters into a unique group of fishes, the Lepisosteidae. Hopefully, the methodology presented here will contribute to long-term studies of the spatial ecology, habitat use, and migratory behavior of these unique and ancient fishes. Similar approaches may work on other "armoured" fish species as well.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.