High Mortality of Largemouth Bass Implanted with Transmitters at Colder Water Temperatures

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Abstract: Biotelemetry via surgical implantation of an electronic tag is a common way to examine fish behavior and movement. Previous studies suggest higher post-operative survival should be expected when implanting tags at colder water temperatures. However, during the initial part of our study, all 26 adult largemouth bass (*Micropterus salmoides*) we implanted with transmitters at water temperatures from 14 to 17 C at Toledo Bend Reservoir, Texas, died within 4 wk post-surgery. To further investigate this phenomenon, we conducted two tagging trials at 13 C, observing post-operative mortality of 100% ($n = 5$) and 58% ($n = 12$); all fish that died developed external fungal infections (i.e., saprolegniasis). Post-operative survival was 100% in a third trial at 24 C ($n = 6$) and no fungal infections were observed. Subsequently, tagging mortality was ≤ 20% when 81 largemouth bass were tagged at water temperatures from 22 to 30 C at Toledo Bend and Lake Fork reservoirs. Our results suggest that immunosuppression of largemouth bass coupled with greater fungal virulence in colder water likely caused the complete mortality of 31 largemouth bass initially implanted with transmitters at Toledo Bend Reservoir and in our first trial. Investigators conducting surgeries during similar conditions should be aware that high post-surgical mortality could result if fungal virulence is high during tagging.

Key words: surgery, immunosuppression, fungus, *Saprolegnia*, acclimation

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Biotelemetry is commonly used to monitor fish movement and habitat use (Baras 1991, Cooke et al. 2012, Hockersmith and Beeman 2012), and many biotelemetry studies have focused on largemouth bass (*Micropterus salmoides*) (e.g., Warden and Lorio 1975, Mesing and Wicker 1986, Colle et al. 1989, Sammons et al. 2003, Hunter and Maceina 2008, Goclowski et al. 2013) due to their importance as sport fish (Heidinger 1976, Pullis and Laughland 1999, USFWS and USCB 2018). Surgical implantation of an electronic tag into the coelom is common in long-term biotelemetry studies (Bridger and Booth 2003, Cooke et al. 2011). As such, the invasive nature of the approach requires consideration of post-operative effects because reduced health can affect fish behavior and habitat use, and mortality can reduce sample size.

Water temperature is considered the controlling variable of fish biology and can influence the surgical process in numerous ways, including magnitude of capture and handling stress, effectiveness of anesthesia, rate of incision healing and recovery, and suture performance (Cooke et al. 2011). However, of the 108 surgical or tagging-effects studies reviewed by Cooke et al. (2011), only three examined water temperature. Bunnell and Isely (1999) found that water temperature (either 10 or 20 C) during surgery had no effect on mortality of rainbow trout (*Oncorhynchus mykiss*), but transmitter expulsion rates were higher at warmer water temperatures. Knights and Lasee (1996) and Walsh et al. (2000) found post-operative mortality was only observed at warmer water temperatures for bluegill (*Lepomis macrochirus*) (20 C) and hybrid striped bass (*Morone saxatilis x M. chrysops*) (22–29 C). No mortalities were observed in either study at lower temperatures (6 C and 12–18 C, respectively). Although warmer water temperatures typically increase wound-healing rates (Anderson and Roberts 1975), Knights and Lasee (1996) and Walsh et al. (2000) recommended implantation of transmitters at colder temperatures due to slower fish metabolism, decreased tissue inflammation and infection rate, and reduced mortality.

Consistent with these procedural recommendations, we implanted radio transmitters into adult largemouth bass at Toledo Bend and Lake Fork reservoirs, Texas, to investigate temporal movement and habitat use. We conducted our initial surgeries during fall when water temperatures were cooler and declining to minimize infection rate, handling stress, and mortality. The objectives of this paper were to 1) document the high surgery-related mortality we experienced under these conditions, which was unexpected and not described in previous research, and 2) examine causes of mortality via surgical trials and additional tag implantations at both reservoirs.

Methods

Toledo Bend Reservoir Radio Tag Study

During November 2019, 26 largemouth bass (358–545 mm TL) were collected for radio-tag implantation from Toledo Bend Reservoir, Texas-Louisiana, with boat-mounted, pulsed-60 DC electro-

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fishing gear (Model GPP 5.0, Smith-Root Inc., Vancouver, Washington) operated at 500 V and 4 amps. Surface water temperatures ranged 14–17 C and had consistently declined from 21 C during the 14 days prior to fish collection. Prior to surgery, instruments and radio transmitters (model F1835B, Advanced Telemetry Systems [ATS], Isanti, Minnesota; 16 g) were sterilized overnight in a 10% bleach/distilled water solution (Stoskopf 1993, Noga 2000) and Betadine® (10% povidone-iodine solution) (Bowker and Trushenski 2019), respectively. Fish were held for 10 to 30 min in a 379-L holding tank with reservoir water at ambient temperatures and dissolved oxygen at saturation levels, before being placed on the surgical table mounted inside the electrofishing boat and immobilized using electroanesthesia as described by Jennings and Looney (1998). Gills were irrigated with a continuous flow of oxygen-saturated water throughout the surgical process. The transmitter was washed with distilled water to remove Betadine®, inserted into the body cavity through a 2-cm incision posterior to the pelvic girdle and above the ventral midline, then pushed over the pelvic girdle to minimize risk of expulsion. The external antenna was passed through the body cavity wall approximately 2-cm posterior to the incision using the shielded needle technique described by Ross and Kleiner (1982). Incisions were closed using size one nylon monofilament non-absorbable suture material, with three simple interrupted knots, and three total sutures. Two sets of surgical equipment were alternated for a minimum of 10 min between surgeries (one used, one sterilized with 10% bleach/distilled water solution). Fish were externally tagged (Floy T-Bar model FD-68BC, Floy Tag and Mfg., Inc., Seattle, Washington) as described by Guy et al. (1996) for identification if caught by anglers. The duration of each procedure took 3.5 to 5.0 min. Prior to release, fish were immersed in a 3% non-iodized salt solution for 30 sec (Carmichael et al. 1984, Noga 2000), then held approximately 15 min for observation in the holding tank described above. All fish appeared healthy following surgery and were released within 100 m of their capture site to prevent displacement effects.

Fish were initially tracked 2 wk following surgery by boat using an ATS R2000 receiver and a directional yagi antenna. Each transmitter was equipped with a mortality sensor, such that signal rate doubled if there was no movement for 24 h. Due to high mortality observed during the initial tracking event, fish were tracked three times during the following 2 wk. During each of these tracking events, signals were obtained from all transmitters to confirm existing mortality signals and monitor surviving fish. The implantation of transmitters at Lake Fork Reservoir initially scheduled for December 2019 was delayed due to mortality observed at Toledo Bend Reservoir.

Experimental Trials of Tagging Mortality

Three surgical trials were conducted to examine post-operative mortality using identical collection, electroanesthesia, and surgical equipment and methods described above. The first two trials were conducted at ambient water temperatures. In the first trial, five implanted fish and five controls (i.e., untagged) 381–445 mm TL were collected from Lake Athens, Texas, in December 2019 at 13 C. In the second trial, 12 implanted fish and 2 controls (375–544 mm TL) were collected from Sam Rayburn Reservoir, Texas, in January 2020 at 13 C. Reservoir water temperatures were relatively stable for 3 and 6 wk prior to fish collection at Lake Athens and Sam Rayburn Reservoir, respectively. During the second trial, we investigated incision sealing by increasing suture number to either four or five sutures for three fish each. A tissue adhesive (3M Vetbond®, St. Paul, Minnesota) was also applied to the incision of six fish (two fish each with three, four, and five sutures). For both of these trials, surgeries were conducted during the day of collection, but fish were held 1–4 h prior to surgeries.

The third trial was conducted using six implanted fish and two controls (390–505 mm TL); fish were collected from Sam Rayburn Reservoir in February 2020 at an ambient water temperature of 13 C. Fish were transferred to hatchery raceways at 13 C, then water temperature was gradually heated to 24 C over a period of 7 days. Surgeries were conducted after an additional 7 days of acclimation with water temperature maintained at 24 C. After surgeries in all three trials, fish were held at 24 C for 5 wk in hatchery raceways for observation. All raceways were aerated (>7.5 ppm dissolved oxygen) via continuous water flow from the reservoirs in which fish were collected and juvenile koi (*Cyprinus carpio*) abundance was maintained to provide on-demand forage.

Subsequent Field Studies

During May to July of 2020 and May to June of 2021, a total of 43 (356–606 mm TL) and 38 largemouth bass (362–615 mm TL) were implanted with transmitters at Toledo Bend and Lake Fork reservoirs, respectively. Ambient water temperatures during surgeries ranged from 22–31 C. Fish collection and surgical procedures were identical to methods previously described, except that 13 fish from Toledo Bend Reservoir and 6 fish from Lake Fork Reservoir were collected via angling. Based on the findings from the 2019 Toledo Bend Reservoir study and our surgical trials described below (i.e., 37 of 38 fish died within 21 days post-surgery and the remaining fish died within 28 days), we conservatively defined surgery-related mortality as deaths occurring less than 28 days after surgeries.

Results

During the initial tracking event at Toledo Bend Reservoir 2 wk after surgeries, signals were obtained from all 26 transmitters and 23 were indicating mortality. During the following 2 wk, mortality signals were received from all 26 transmitters and no movement was observed, resulting in 100% mortality of our surgically implanted fish (Table 1; Figure 1).

Post-operative mortality from our two surgical trials at 13 C were also high. In our first trial in December 2019 at Lake Athens, all five of our implanted fish died, three within 2 wk post-surgery and two during the third week (Table 2; Figure 1). Conversely, all five control fish were alive and released at the conclusion of the trial. In our second trial in January 2020 at Sam Rayburn Reservoir, 7 of 12 implanted fish died, four within 1 wk post-surgery, and three during the second week (Table 2; Figure 1). Our additional measures to ensure incision sealing (i.e., added sutures and adhesive) did not reduce mortality. At the conclusion of the trial, five surgery fish and both control fish were alive and visibly healthy (i.e., no infections and healed incisions), and were released. All 12 fish that died from both trials became lethargic a few days prior to death, suspended high in the water column, and developed external infections that initially emanated from the incision site and then expanded over the body (Figure 2). These infections were likely *Saprolegnia* spp. or other fungi/water molds, although no necropsies were conducted.

During the third Sam Rayburn trial at 24 C, we observed no fungal infections or mortality of six implanted fish or two control fish over the 5-wk observational period (Table 2; Figure 1). Similarly, post-operative mortality from our subsequent field studies in

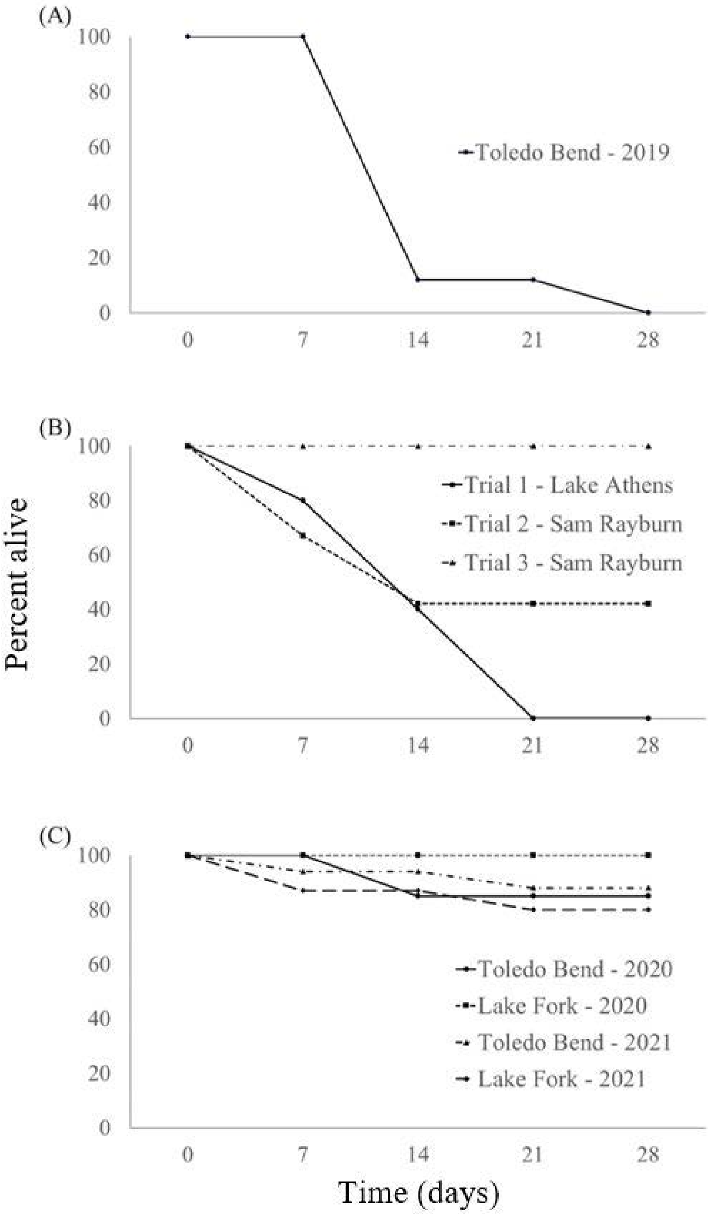

Figure 1. Percent of tagged fish found alive (1-wk intervals) during the initial study on Toledo Bend Reservoir, Texas (A), in three trials using fish from two Texas reservoirs (B), and during subsequent field studies on Toledo Bend and Lake Fork reservoirs, Texas (C).

Table 1. Post-operative mortality (defined as deaths occurring < 28 days after surgeries) of adult largemouth bass surgically implanted with radio transmitters and released at Toledo Bend and Lake Fork reservoirs, Texas. For implanted fish, mortalities are in parentheses.

Reservoir	Water temperature (C)	Date	Implanted fish	Mortality rate (%)
Toledo Bend	17	20 Nov 2019	15 (15)	
	14	21 Nov 2019	11 (11)	
		Total	26 (26)	100
Toledo Bend	23	5 May 2020	4 (0)	
	25	22 May 2020	7 (1)	
	27	2 June 2020	4 (0)	
	29	1 July 2020	7 (1)	
	30	29 July 2020	4 (2)	
		Total	26 (4)	15
Lake Fork	25	22 May 2020	11 (0)	
	25	28 May 2020	12 (0)	
		Total	23 (0)	0
Toledo Bend	22	24 May 2021	7 (1)	
	25	9 June 2021	8 (0)	
	29	24 June 2021	2 (1)	
		Total	17 (2)	12
Lake Fork	26	10 June 2021	2 (0)	
	31	15 June 2021	7 (1)	
	28	29 June 2021	6 (2)	
		Total	15 (3)	20

2020 and 2021 (22 to 30 C) was relatively low for both years (15% and 12% at Toledo Bend and 0% and 20% at Lake Fork; Table 1; Figure 1). Angled fish experienced 23% post-operative mortality at Toledo Bend (3 of 13 fish) and 17% at Lake Fork (1 of 6 fish). Mortality of fish collected from electrofishing was only 8% at Toledo Bend in 2020 (1 of 13 fish) but was 22% at Lake Fork in 2021 (2 of 9 fish).

Table 2. Results from three surgical trials examining post-operative mortality (defined as deaths occurring < 28 days after surgeries) of adult largemouth bass collected from two Texas reservoirs and implanted with radio transmitters, then held 5 wk in hatchery raceways for observation. For trials 1 and 2, surgeries were conducted at ambient water temperatures. For trial 3, fish were collected at an ambient water temperature of 13 C, then raceway water was heated to 24 C over a 7-day period. Surgeries were conducted after an additional 7 days of acclimation at 24 C. For implanted and control fish, mortalities are in parentheses.

Surgery trial	Water temperature (C)	Date	Implanted fish	Mortality rate (%)	Control fish
Trial 1 – Lake Athens	13	11 Dec 2019			
Three sutures			5 (5)	100	5 (0)
Trial 2 – Sam Rayburn	13	6–7 Jan 2020			
Three sutures			4 (0)		
Three sutures, adhesive			2 (2)		
Four sutures			1 (1)		
Four sutures, adhesive			2 (1)		
Five sutures			1 (1)		
Five sutures, adhesive			2 (2)		
		Total	12 (7)	58	2 (0)
Trial 3 – Sam Rayburn	24	4 Feb 2020			
Three sutures			6 (0)	0	2 (0)

Figure 2. Fungal infection emanating from the incision site on a largemouth bass that died from surgical implantation of a radio transmitter at a water temperature of 13 C.

Discussion

During preparation for largemouth bass telemetry research at Toledo Bend and Lake Fork reservoirs, our literature review found only three studies that examined transmitter implantation effects on largemouth bass; two on juvenile fish (Cooke et al. 2003, Thompson et al. 2014) and one on adult fish (Crumpton 1982). Crumpton (1982) found that surgically implanted dummy transmitters had no effects on swimming, feeding, spawning, or catchability of adult largemouth bass and no surgery-related mortality was reported. We found no previous research that had examined effects of water temperature on post-operative health and mortality of largemouth bass, but investigations with bluegill (Knights and Lasee 1996) and hybrid striped bass (Walsh et al. 2000) suggest that surgeries at colder temperatures (6 C and 12–18 C, respectively) minimize tagging mortality. Although prior studies examining impacts of water temperature on adult largemouth bass mortality are tournament-related, the relationship is exponential with approximately 10% mortality at 15 C and 24% at 25 C (Wilde 1998). Therefore, we expected low mortality associated with our first set of surgeries at Toledo Bend Reservoir when water temperatures ranged from 14–17 C. However, within 4 wk post-surgery, all 26 of the surgically implanted fish had died.

All fish that died during our two trials at 13 C acquired fungal infections (i.e., saprolegniasis), which have been previously observed in fish surgically implanted with transmitters. Mellas and Haynes (1985) attributed mortality of white perch (*Morone americana*) to saprolegniasis with surgeries at stable, warmer temperatures (20–23 C). Knights and Lasee (1996) observed post-operative saprolegniasis in bluegill at colder temperatures (6 C) but documented no mortality. However, immunosuppression of channel catfish (*Ictalurus punctatus*) and mortality from *Saprolegnia* spp. has been documented during colder and declining water temperatures. Bly and Clem (1991) found that a rapid water temperature decline (23 to 11 C in 24 h) produced immunosuppression in channel catfish, and fish required up to 6 wk of acclimation for immune responses to recover. Bly et al. (1992) determined that *Saprolegnia* spp. zoospore abundance and potential virulence was 2–5-fold higher at water temperatures of 10 C than 20 C. Further, a temperature shock from 22 to 10 C in 24 h resulted in immunosuppression of channel catfish, an infection rate of 92%, and a mortality rate of 67% within 21 days. The researchers identified *Saprolegnia* spp. as the primary pathogen and causative agent of mortality. However, when fish were acclimated at 10 C for 8 wk or were held at ambient 22 C prior to exposure to *Saprolegnia* spp. (i.e., immunocompetent in both cases), no mortalities were observed. A similar study found that temperature shock and skin abrasions were needed to induce high *Saprolegnia* spp.-related mortality in channel catfish (Howe et al. 1998).

We suggest that decreasing, colder water temperatures in November (range = 13–17 C and declined from 21 C in 14 days) resulted in immunosuppression of largemouth bass at Toledo Bend Reservoir concomitant with high virulence conditions for saprolegniosis (*sensu* Bly and Clem 1991). Coupled with stressors and incisions from surgeries, these factors may have been key contributors of the 100% mortality observed for the 26 largemouth bass tagged in Toledo Bend Reservoir. A similar set of conditions were present during our first Lake Athens trial, which occurred following only 3 wk of relatively stable water temperatures. This immunosuppression/saprolegniosis/acclimation hypothesis during colder and declining temperatures is supported by considerably lower post-operative mortalities observed from our second cold-water trial, and our third trial and field work at Toledo Bend and Lake Fork reservoirs at warmer water temperatures. The reduced mortality we experienced from the second trial at 13 C (58%) may have resulted from 6 wk of relatively stable water temperatures prior to the trial that allowed recovery of immune response by the fish. During the third trial at 24 C, we observed no saprolegniosis or mortality of six implanted fish or two control fish within 5 wk post-surgery. Similarly, post-operative mortality from our field work in 2020 and 2021 at 22–30 C was relatively low for both years. Angling imposes additional stress on largemouth bass (Gustaveson et al. 1991, Brownscombe et al. 2014, Dinken et al. 2022), which may have increased post-operative mortality at Toledo Bend Reservoir in 2020; however, mortality was similar between collection methods at Lake Fork Reservoir in 2021.

We could find no other research that implanted adult largemouth bass with transmitters during fall when water temperatures were below 20 C and declining that resulted in 100% post-operative mortality. However, although many largemouth bass telemetry studies have been conducted, only a few reported post-operative mortalities and details regarding water temperatures were often lacking. Two previous studies surgically implanted largemouth bass during colder winter months (December, Goclowski et al. 2013; February, Sammons et al. 2003). Although no specific water temperatures were reported, no surgery-related mortalities were observed, suggesting that extended acclimation throughout the colder months preceding their surgeries may have contributed to no mortality. Knights and Lasee (1996) reported no surgery-related mortality of bluegill when reducing water temperatures from 20 C to 6 C in 12 days, but surgeries were conducted at water temperatures considerably colder than our study. In addition, fungal virulence could be reduced at 6 C, however *Saprolegnia* spp. can tolerate temperatures as low as 3 C (Willoughby and Roberts 1992, Aly and El-Ashram 2000).

Cooke et al. (2003) found that experienced surgeons can significantly improve survival when compared with inexperienced surgeons when working with juvenile largemouth bass. All surgeries at Toledo Bend Reservoir and the Sam Rayburn Reservoir trials were conducted by the author, and those at Lake Fork Reservoir and the Lake Athens trial by the coauthor. Prior to this study, the two surgeons were unexperienced. Both surgeons conducted extensive research on methods, consulted with fish health experts and experienced surgeons, practiced sutures on foam medical kits, and honed the entire procedure on numerous moribund and live largemouth bass until each was comfortable with the entire surgical process. Total training for each surgeon was approximately 15 h. Overall, our post-operative mortalities did generally decline with the increasing number of surgeries each surgeon conducted. However, we propose the reduction in mortality was likely unrelated to increased experience. Both surgeons were equally confident with each completed surgery, and all were conducted with identical methodology. Further, mortality rates were similar between surgeons (e.g., 100% for 2019 Toledo Bend and Lake Athens surgeries; ≤ 20% for subsequent field studies), suggesting equal surgical abilities. Therefore, surgical methodology or surgeon experience likely had little effect on mortality of tagged fish.

This study is the first to observe high largemouth bass mortality during tagging surgery in colder, declining water temperatures. However, our three surgical trials should be viewed as preliminary, as they were exploratory in nature and conducted prior to awareness of the potential for declining water temperatures to cause immunosuppression in fishes when fungal virulence could be high. Sample sizes were also low and inconsistent which prevented any statistical analyses. Additional trials should be conducted to examine largemouth bass immunosuppression and related post-operative mortality, particularly to determine water temperatures, trends required to instigate immunosuppression, and acclimation time required for immunorecovery. Also, necropies are needed to confirm the identity of the primary fungi/water molds causing mortality, as the *Aphanomyces* water mold is also virulent at colder water temperatures (Hawke et al. 2003) and has caused mortality in numerous freshwater species, including largemouth bass (Sosa et al. 2007).

Our findings should caution future investigators about conducting surgeries during similar conditions that may result in unnecessary loss of fish, additional costs, and sample-size reductions due to high post-surgical mortality. In addition, our results suggest that other studies are needed to determine if the immunosuppression or temperature acclimation dynamic could also influence mortality of largemouth bass during other common but stressful handling events, such as at fish hatcheries or tournaments. Finally, little if any research has been done on susceptibility of other black bass species to immunosuppression or fungal infection. Some of these fishes support popular and economically important fisheries, and some evidence exists that they may have different responses to stressors than largemouth bass (e.g., Hartley and Moring 1995, Ricks 2006).

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