

Effect of anesthetic, tag size, and surgeon experience on postsurgical recovering after implantation of electronic tags in a neotropical fish: *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae)

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Implantation of telemetry transmitters in fish can be affected by different parameters. This study aimed to evaluate the effect of type of anesthetic, tag size, and surgeon experience on surgical and postsurgical wound healing in the neotropical fish *Prochilodus lineatus*. In total, eighty fish were surgically implanted with telemetry transmitters and forty fish were kept as controls. Forty fish were implanted with a small tag and other forty were implanted with a large tag. Similarly, forty fish were anesthetized with eugenol and forty fish were anesthetized by electroanesthesia, and forty surgeries were performed by an expert surgeon and forty surgeries were performed by novice surgeons. At the end of the experimental period seventeen (21.3%) tagged fish had postsurgical complications, including death (1.3%), tag expulsion (2.5%), antenna migration (2.5%), and infection (15%). Tag size was the key determinant for postsurgical complications. Surgical details and postsurgical wound healing were not affected by type of anesthetic. Incision size, duration of surgery, and wound area were significantly affected by tag size and surgeon experience, and the number of sutures was significantly affected by tag size only. The results indicate that successful implantation of telemetry transmitters is dependent upon surgeon experience and tag size.

A implantação de transmissores em peixes pode ser afetada por diversos aspectos. Este estudo objetivou avaliar a influência do tamanho do transmissor, do tipo de anestésico e da experiência do cirurgião em parâmetros cirúrgicos e recuperação pós-cirúrgica do peixe neotropical *Prochilodus lineatus*. Foram marcados oitenta indivíduos com transmissores de telemetria, enquanto outros quarenta foram utilizados como controle. Quarenta indivíduos foram marcados com um transmissor pequeno e outros quarenta com um transmissor maior. Foram utilizados dois tipos de anestésico sendo quarenta indivíduos anestesiados por eletroanestesia e outros 40 por eugenol e as cirurgias foram realizadas por dois grupos de cirurgiões, experientes e inexperientes com quarenta peixes em cada grupo. Dezesete peixes (21,3%) apresentaram impactos diretos resultantes da cirurgia como o óbito (1,3%), perda do transmissor (2,5%), migração da antena (2,5%) e infecções internas (15%). Estes efeitos estão ligados ao tamanho do transmissor utilizado. A experiência do cirurgião possui relação direta em três parâmetros cirúrgicos (tamanho da incisão, tempo de cirurgia e área de cicatrização). O tamanho dos transmissores utilizados possui relação com quatro parâmetros (tamanho da incisão cirúrgica, número de suturas utilizadas, tempo de cirurgia e área de cicatrização). O anestésico não teve relação com os parâmetros cirúrgicos. Os resultados indicam que tanto a dimensão dos transmissores quanto a experiência dos cirurgiões têm impacto direto no sucesso de implantação dos transmissores.

Keywords: Incision, Infection, Postsurgical complications, Telemetry, Wound Healing.

Introduction

Studies using acoustic and radio telemetry to evaluate the migratory dynamics of fish are becoming increasingly common around the world (Lucas & Barras, 2000; Block, 2005; Chomyshyn *et al.*, 2011; Cooke *et al.*, 2011a; Thiem *et al.*, 2011; Hockersmith & Beeman, 2012) and in Brazil (Hahn, 2012). Telemetry can remotely and precisely

transfer information from tagged fish with a radio or acoustic transmitter across spatial and temporal scales to study the behavior of Neotropical freshwater fishes and their relationship with environmental variables (Koehn, 2012). In recent years, affordable technologies and an increase in the number of international collaborations with Brazilian research institutions have broadened the Brazilian regions covered by these studies from

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river basins in southern and southeastern states since the early 2000s (Godinho & Kynard, 2006; Schulz & Leuchtenberger, 2006; Alves *et al.*, 2007; Godinho *et al.*, 2007; Hahn *et al.*, 2007; Andrade-Neto, 2008; Pesoa & Schulz, 2010; Hahn *et al.*, 2011; Alves, 2012; Silva, 2012; Ribeiro, 2013; Pérez, 2014; Suzuki, 2014) to other large river basins in central and north regions of the country.

Biotelemetry has a number of advantages over conventional methods used for monitoring freshwater fish in Brazil, especially mark-recapture methods. In telemetry studies, detection rates are considerably higher, animals can be studied in their natural environment, and more complex movement patterns can be monitored as animals can be tracked continuously over time (Chomyshyn *et al.*, 2011; Hockersmith & Beeman, 2012; Koehn, 2012; Liedtke & Rub, 2012). However, mark-recapture methods often rely on external tags that are attached directly to the body surface, whereas intracoelomic implantation of telemetry transmitters is inherently more invasive and involves surgical procedures that may adversely affect the physiology and behavior of tagged individuals (Thoreau & Baras, 1997; Cooke *et al.*, 2011a; Thiem *et al.*, 2011).

Undesirable and harmful effects resulting from the surgical implantation of telemetry transmitters including mortality, altered physiology and behavior (*e.g.*, reduced feed intake and movement rates), morphological changes, and tag expulsion have been widely reported (Marty & Summerfelt, 1986; Koehn, 2012; Liedtke & Rub, 2012). To alleviate or suppress such complications, several studies have investigated the factors associated with the adverse effects from surgical tagging, including type of anesthetic used for anesthesia and immobilization (King *et al.*, 2005; Carter, 2011; Vandergoot *et al.*, 2011; Javahery *et al.*, 2012; Trushenski *et al.*, 2012; Kanani, 2013), transmitter type and size and its relationship with fish size (Penne *et al.*, 2007; Thorstad *et al.*, 2009), surgeon experience (Smith *et al.*, 2009; Brown *et al.*, 2011; Cooke *et al.*, 2011b), as well as sterilization of transmitters and surgical instruments, attachment methods, incision type and location, and suture material and pattern (Cooke *et al.*, 2003; Wagner *et al.*, 2011; Brown *et al.*, 2013).

Despite the growing number of telemetry studies in South America, to date no systematic review has summarized the effects of transmitter attachment on Neotropical fish. Most studies in Brazil were not aimed at evaluating the effects of transmitter presence on fish behavior and physiology, but have focused on migratory patterns and followed established surgical procedures (Jones, 1979; Knights & Lasee, 1996). Only Schulz (2003) has specifically examined the adverse effects from surgical tagging on fish behavior in Brazil. Moreover, few studies have examined tagging effects in tropical fish species in less economically developed countries (Okland *et al.*, 2003; Schulz, 2003; Mitamura *et al.*, 2006; Thorstad *et al.*, 2009). Neotropical fish species may experience adverse effects from surgical tagging similar to those observed in

temperate species. Some complications that may reduce the number of active individuals or adversely affect the behavior of tagged individuals, causing researchers to make erroneous conclusions about the sample population, may be more severe in tropical fish due to differences in physiology, morphology, and environmental conditions (Cooke *et al.*, 2011a).

Studies about the adverse effects of the surgical implantation of telemetry transmitters on fish behavior and physiology are essential given the increasing number of dams constructed in developing countries such as Brazil (Pompeu *et al.*, 2012; Pelicice *et al.*, 2015) and the existing knowledge gaps in fish migratory dynamics and life history strategies in these regions (Hahn, 2012; Makrakis *et al.*, 2015). Without addressing potential telemetry limitations, the data gathered about the migratory patterns of tropical fish may be unreliable and incomplete. Nevertheless, an underlying assumption in all tagging studies is that the behavior of tagged fish is similar to that of nontagged conspecifics (Collins *et al.*, 2013).

This study aimed to evaluate the effect of the surgical implantation of radio transmitters on postsurgical wound healing in a Neotropical freshwater fish species. Specifically, the following factors were evaluated: i) type of anesthetic agent: eugenol and electroanesthesia, the two most frequently used fish anesthetics in Brazil (Hahn, 2012); ii) tag size (1-g and 10-g transmitters); and iii) the effect of surgeon experience on transmitter attachment, surgical details, and wound healing.

Material and Methods

Tagging effects were evaluated in curimba *Prochilodus lineatus*, a medium-sized, rheophilic, long-distance migratory species (Godoy, 1959; Capeleti & Petrere Jr., 2006). The genus *Prochilodus* is distributed across virtually every major South American river basin (Castro & Vari, 2004), and thus have been used in most telemetry studies in Brazil (Godinho & Kynard, 2006; Pesoa & Schulz, 2010; Alves, 2012; Silva, 2012; Hahn, 2012; Ribeiro, 2013; Suzuki, 2014). In total, 120 curimba reared at the Volta Grande Hatchery, located in the Grande River basin, upper Paraná River basin, state of Minas Gerais, Brazil, were used in the study. The fish were randomly transferred from hatchery ponds into five 2000-L tanks regardless of size and sex at a stocking rate of 12 fish per tank. The tanks were provided with continuous aeration and water flow. Water temperature in the tanks was monitored throughout the experiment. The experiments were conducted over two four-week periods in 2013: from August 28 to September 25 and September 24 to October 21.

Fish were assigned to one of seven treatments: control; eugenol; electroanesthesia; small tag; large tag; experienced surgeon; and novice surgeon. The treatments were chosen regarding their importance for future

field studies to be executed in Minas Gerais State. The results of this experiment will help the research team to choose the better anesthetic, training program for novice surgeons and tag characteristics. Forty fish were kept as controls. All fish were first anesthetized with eugenol (Biodinâmica, USP Degree 99 to 100.5%) diluted to reach concentration of 0.035 ml/L. The time at which fish achieved stage IV anesthesia, which is characterized by total loss of equilibrium, muscle tone, and responsiveness to tactile and visual stimuli (Summerfelt & Smith, 1990), standard length, and body weight were recorded. During anesthesia, a cannula was inserted into the genital orifice for gonadal tissue sampling. Following sampling, the material was examined under a stereomicroscope for sex determination. Additionally, one passive integrated transponder (PIT) tag was inserted in the dorsal musculature of all experimental fish for identification of individual fish. Next, control fish were placed into a 2000-L recovery tank and transferred into a 200-m² hatchery pond the following day. Postsurgical recovery times and the time required to resume normal swimming (horizontal swimming and normal caudal motion) were recorded immediately after fish were placed into the tank.

The remaining six treatments evaluated the effects of type of anesthetic, tag size, surgeon experience, and their interaction on postsurgical wound healing. The same procedures described above were repeated for the remaining 80 fish. Following the initial procedures, biometric measures were taken (less than 3 min) and fish were deployed at the surgical area where they were immobilized using eugenol (0.035 ml/L) or electroanesthesia (0-30 V). Electroanesthesia is not considered a true anesthetic because it does not affect the ability of fish to perceive pain and only causes their immobilization (Liedtke & Rub, 2012; Trushenski *et al.*, 2012). Nevertheless the term “anesthesia” will be used for both treatments here. In total, 40 fish were anesthetized with eugenol and 40 fish were anesthetized by electroanesthesia. Anesthetic-containing water was circulated across the gills continuously in fish anesthetized with eugenol, which was replaced by freshwater if opercular movements became too low or non-existent. Fish anesthetized by electroanesthesia were partially submerged in a tub with electric current (0-30 V) being applied by a power inverter. The anesthesia tank was continuously supplied with aerated water and fish were able to breathe normally via involuntary muscle contractions.

The effect of tag size on postsurgical wound healing was also investigated. Forty fish were surgically implanted with 0.8-g in air, 6-mm width x 4-mm height x 15-mm length tags (NTC-3-1, Lotek Wireless, Inc., Newmarket, ON, Canada) and 40 fish were implanted with 8.9-g in air, 11-mm width x 49-mm length tags (MCFT-3EM, Lotek Wireless Inc., Newmarket, ON, Canada). Differences in tag weight were purposely high

to highlight the effects of different tag size on surgery and recovery of tagged fish. Tag weight did not exceed the 2% tag/fish body relationship. Lastly, 40 fish were surgically implanted by an expert surgeon with over 10 years' experience and 40 fish were implanted by recently trained surgeons with less than a month's experience in the surgical procedure. Novice surgeons were taught how to conduct surgeries in a similar manner to that used by the expert surgeon: incision was made in the ventral body surface along the *linea alba*, just off the midline, and the transmitter was then placed into the body cavity. Transmitters were inserted through the incision and placed in the coelom anterior to the incision. The antenna was placed posterior to the incision site by transfixation of the body wall. Treatments were randomized and their sequence was determined by a draw before the start of the experiment to prevent that the same procedure was performed in sequence, which could bias the results (*e.g.*, the same surgeon performing multiple surgeries in a row, which could lead to fatigue).

Surgeries were performed using disposable blades, monofilament nylon sutures (diameter from 0.200 to 0.249-mm), 20-mm curved needles, and non-sterile disposable gloves for aseptic reasons. Incisions were closed with square knots with three throws in each knot. The length of the knot tail was standardized in about 1 cm. The number of knots was chosen by the researcher performing surgery. Surgical materials and transmitters were washed with filtered water and mild detergent and kept in a 70° GL ethanol bath for approximately 30 min before use. These sterilization procedures are similar to those used in field studies in Brazil.

During surgery, the following variables were recorded: water temperature, fish weight, standard length, sex, and time at which fish achieved stage IV anesthesia following the initial anesthesia with eugenol. The following surgical details were compared across treatments: i) duration of surgery (from taking the fish to the surgical area until completion of suturing); ii) recovery time from surgery (after fish were transferred into the recovery tank); iii) mean suture spacing; iv) number of sutures; and v) size of the incision. The later three parameters were evaluated by photographs taken immediately following surgery. Recovery from surgery was assessed by the following parameters: i) wound area and ii) number of sutures retained.

Following surgery, fish were placed into 2000-L recovery tanks for 24 h and then transferred into three 200-m² hatchery ponds at a stocking rate of 20 fish per pond. Fish were fed commercial feed (36% crude protein; Laguna, Ocialis, Paulínia, São Paulo, Brazil) daily and ponds were checked for dead fish or abnormal behavior. Fish were taken to the laboratory at seven-day intervals for assessment of postsurgical wound healing. Fish were anesthetized with eugenol (0.035 ml/L), identified by the PIT-TAG, measured, and weighed. Photographs of the

incision site from all tagged fish were taken for analysis of the following parameters: general appearance, wound area, and number of sutures retained. After four weeks, fish were collected, assessed for healing by the same procedures described above, euthanized with an overdose of eugenol (0.05 ml/L), and dissected for examination of internal morphology, signs of infection, macroscopic tissue changes, and confirmation of sex. Voucher specimens were deposited in the Coleção Ictiológica Universidade Federal de Lavras (CI-UFLA 786-787).

The general aspect of surgery and postsurgical recovery one and four weeks post-surgery was assessed qualitatively on a scale of poor to good. The criteria and examples for the surgical and postsurgical rankings are presented in Figure 1. Wound area, defined as the area around the incision site showing macroscopic tissue changes, was measured one week post-surgery using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA).

Differences in biometric data and induction time to stage IV anesthesia with eugenol between the two experimental periods were compared using the Student's t test. The effects of treatments on surgical details and wound healing parameters were tested using Factorial ANOVA. Data were screened for normality using the Shapiro-Wilk test. Similar comparisons were made for all surgical details and postsurgical wound healing parameters. The relationship between wound area *versus* duration of surgery and wound area *versus* incision size was determined using multiple regression analysis. All analyses were performed using Statistica software (StatSoft Inc., Tulsa, OK, USA). Differences were considered significant at $p < 0.05$.

Results

There were no significant differences in fish standard length and weight between the two four-week experimental periods (Table 1). Similarly, mean body weight in both experimental periods was not significantly different during the experimental periods (Table 1), indicating that feed intake was not adversely affected by intracoelomic implantation of telemetry transmitters. More individuals had their sex determined at the second ($n = 50$) than at the first ($n = 40$) experimental period because most fish did not exhibit advanced gonadal development in August, making it difficult to determine the sex of fish by laparoscopy. Additionally, the time required to achieve stage IV anesthesia was longer for eugenol at the second experimental period, when water temperature was higher, even though the difference in induction time between the two periods was not significant (Table 1).

Table 1. Biometric data of *Prochilodus lineatus*, water temperature, and induction time to stage IV anesthesia (mean, \pm SD, range) at the two experimental periods.

	August	September
Water temperature (°C)	20.7 \pm 1.2 (19.0-21.5)	22.8 \pm 1.1 (22.0-23.5)
Standard length (cm)	35.4 \pm 3.1 (28.2-43.8)	35.8 \pm 3.1 (29.9-46.6)
Weight (g)	943.0 \pm 254.0 (420.0-1,750.0)	933.0 \pm 253.0 (510.0-2,100.0)
Sex		
Male	27	32
Female	13	18
Unknown	20	10
Time to stage IV anesthesia (s)	186 \pm 46 (113-350)	204 \pm 64 (122-365)

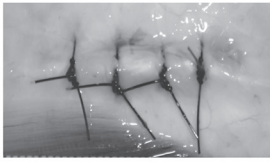
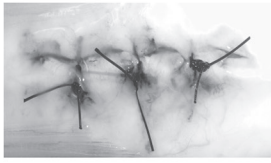
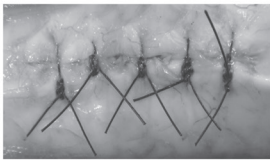
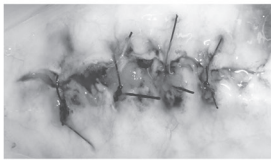
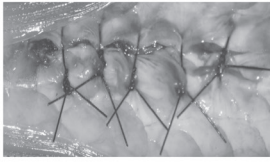
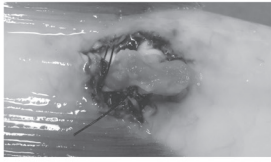
Surgical ranking		Example	Postsurgical ranking		Example
Good	.Incision completely closed.		Good	.Incision closed.	
	.No incision opening.			.All sutures retained (applies to one week post-surgery only).	
	.Apposed wound edges.			.No signs of infection.	
Average	.Incision completely closed.		Average	.Incision not fully closed.	
	.Presence of blood on the incision wound area.			.Most sutures retained (applies to one week post-surgery only).	
	.Wound edges not perfectly apposed.			.No signs of infection.	
Poor	.Presence of blood on the incision wound area.		Poor	.Large incision opening.	
	.Incision opening.			.Most sutures lost (applies to one week post surgery only).	
	.Poor wound apposition.			.Signs of infection and necrotic tissue around the incision site.	

Fig. 1. Criteria and examples for the surgical and postsurgical rankings.

Of the 80 tagged fish, 17 (21.3%) individuals presented some direct impact from surgery like death, tag expulsion, antenna migration or internal infections. Only one individual died during the experiment (Table 2). This fish died seven days post-surgery and exhibited no external signs of trauma, indicating that mortality was likely related to postsurgical complications from tag implantation; it had been anesthetized with eugenol and implanted with a small tag by a novice surgeon. Tag expulsion was observed in two individuals and the antenna migrated to the incision site in other two individuals, which may be a sign of imminent tag expulsion; novice surgeons had performed surgery on these four individuals, three of which had been implanted with large tags (Fig. 2). Twelve fish had signs of infection on necropsy. Infections were identified by the strong odor and necrotic aspect of tissues around the tag (Fig. 3), and most infected fish were implanted with large tags (Table 2). Necropsies revealed no signs of damage to organs or tissue adhesion to transmitters. Most transmitters were encapsulated by fibrous tissue four weeks post-surgery.

Table 2. Postsurgical complications of *Prochilodus lineatus*. Eug: eugenol; Elec: electroanesthesia; Small: small tag; Lar: large tag; Exp: expert surgeon; and Nov: novice surgeon.

Complication	Treatment	Sex
death	Eug-Small-Nov	female
tag expulsion	Eug-Lar-Nov	male
tag expulsion	Elec-Small-Nov	male
antenna migration	Elec-Lar-Nov	female
antenna migration	Eug-Lar-Nov	female
infection	Eug-Lar-Exp	male
infection	Elec-Lar-Exp	male
infection	Elec-Lar-Exp	male
infection	Elec-Lar-Nov	female
infection	Eug-Lar-Exp	male
infection	Elec-Small-Nov	male
infection	Eug-Lar-Exp	female
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infection	Eug-Lar-Nov	male
infection	Elec-Lar-Exp	male
infection	Elec-Lar-Nov	female
infection	Eug-Lar-Nov	male

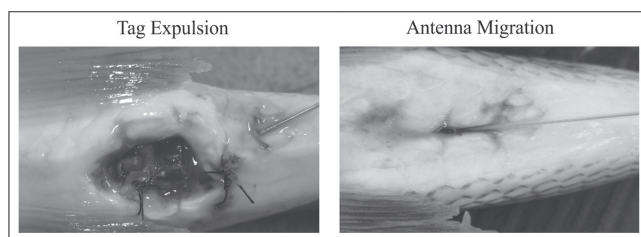


Fig. 2. Examples of tag expulsion (left) and antenna migration (right) of *Prochilodus lineatus*.

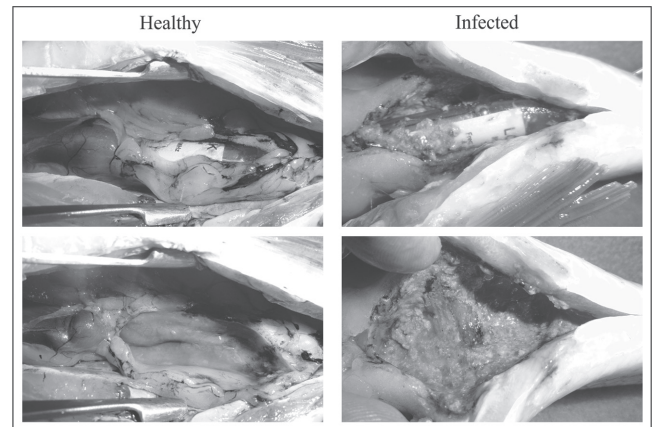


Fig. 3. Healthy (left) and infected (right) viscera on necropsy of *Prochilodus lineatus*.

Surgery details were not significantly different between fish anesthetized with eugenol or electroanesthesia, except for recovery time (Table 3), or for wound area when in interaction with tag size and surgeon experience. Fish anesthetized with electroanesthesia resumed their normal swimming immediately after being placed into the tank, whereas fish anesthetized with eugenol required 342 s (≈ 6 min) on average to resume normal swimming following anesthesia (Table 3).

Tag size and surgeon experience had the largest effect on surgical details. Mean incision size, number of sutures, and duration of surgery (10.5 min for large tags vs. 8 min for small tags) were significantly greater in fish implanted with large tags than with small ones. Additionally, wound area was significantly larger in fish implanted with large tags (2.8 ± 0.19 cm) than with small ones (1.73 ± 0.13 cm; Table 3) one week post-surgery.

There was a single significant effect of surgeon experience on incision size, suture spacing, duration of surgery (expert surgeon: 6 min vs. novice surgeon: > 12 min per surgery), wound healing (wound area one week post-surgery), and on number of sutures when in interaction with tag size (Table 3). Similarly, recovery time was longer in fish implanted with large tags and in surgeries performed by novice surgeons, even though the differences were not significant. In addition, there was a significant positive relationship between incision size and duration of surgery and wound area one week post-surgery (Fig. 4).

Suture retention was high (79-85%) one week after tag implantation and decreased to 40% four weeks post-surgery (Table 3). Nevertheless, incisions were covered by connective tissue at week four in all fishes and sutures were no longer effective in protecting the incision site.

A large proportion of surgical procedures were rated as good immediately after surgery (Fig. 5). However, postsurgical ranking scores were considerably worse one week after tag implantation. Postsurgical recovery was considered poor in a significant proportion of fish one week

post-surgery, which may indicate that even if the surgical procedure was satisfactory, wound healing may exhibit an undesirable appearance. Nevertheless, the proportion

of fish with average or good scores increased four weeks after tag implantation, indicating that postsurgical wound healing improved four weeks post-surgery.

Table 3. Effect of type of anesthetic, tag size, and surgeon experience of *Prochilodus lineatus* on surgical details and postsurgical wound healing parameters (mean ± SD) one and four weeks post-surgery tested by Factorial ANOVA. Mean values in bold indicate single significant treatment differences. In parenthesis are indicated significant treatment differences when in interaction.

Surgical detail	Treatment					
	Type of anesthetic		Tag size		Surgeon experience	
	eugenol	electroanesthesia	small	large	novice	expert
Incision size (cm)	2.66 ± 0.11	2.60 ± 0.10	2.18 ± 0.08	3.07 ± 0.09	2.84 ± 0.11	2.41 ± 0.09
Number of sutures	3.23 ± 0.15	3.18 ± 0.13	(2.60 ± 0.11)	(3.80 ± 0.10)	(3.35 ± 0.14)	3.05 ± 0.13)
Suture spacing (cm)	1.22 ± 0.03	1.23 ± 0.04	1.20 ± 0.04	1.25 ± 0.03	1.19 ± 0.03	1.27 ± 0.04
Duration of surgery (s)	560.8 ± 41.1	575.4 ± 33.1	501.4 ± 32.6	634.7 ± 38.7	763.4 ± 25.78	372.7 ± 12.7
Recovery time (s)	342.1 ± 172.3	0.0	286.2 ± 24.5	398.0 ± 46.0	387.6 ± 39.0	296.6 ± 36.1
One week post-surgery						
Suture retention (%)	84.34	80.42	85.75	79.08	85.00	79.79
Wound area (cm ²)	(2.38 ± 0.21)	2.18 ± 0.16)	(1.73 ± 0.13)	2.80 ± 0.19)	(2.76 ± 0.20)	1.82 ± 0.14)
Four weeks post-surgery						
Suture retention (%)	38.3	37.8	38.4	37.7	41.7	34.6

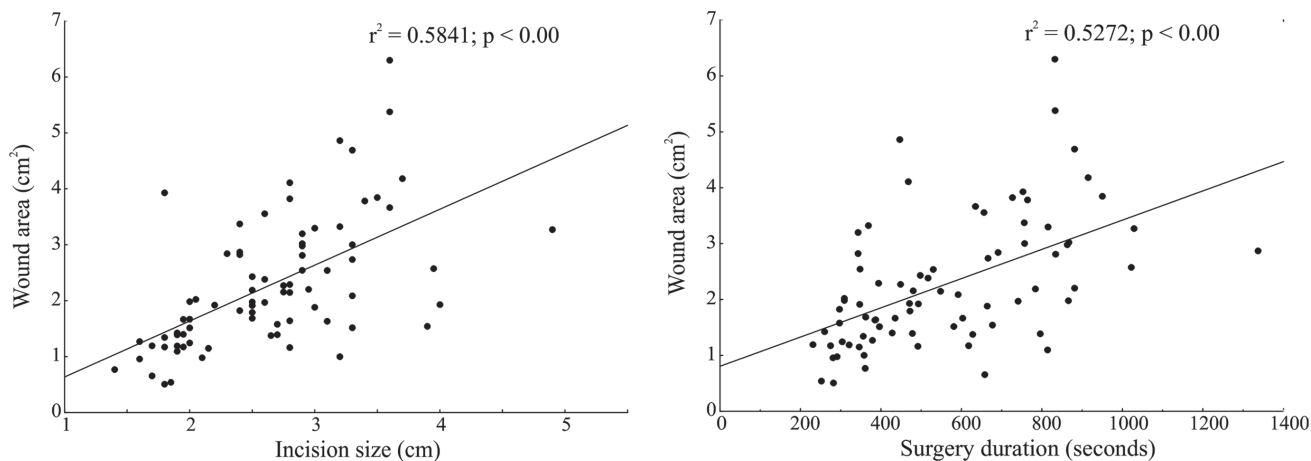


Fig. 4. Effect of incision size (cm) and duration of surgery (s) on wound area (cm²) one week after surgical implantation of telemetry transmitters in *Prochilodus lineatus*.

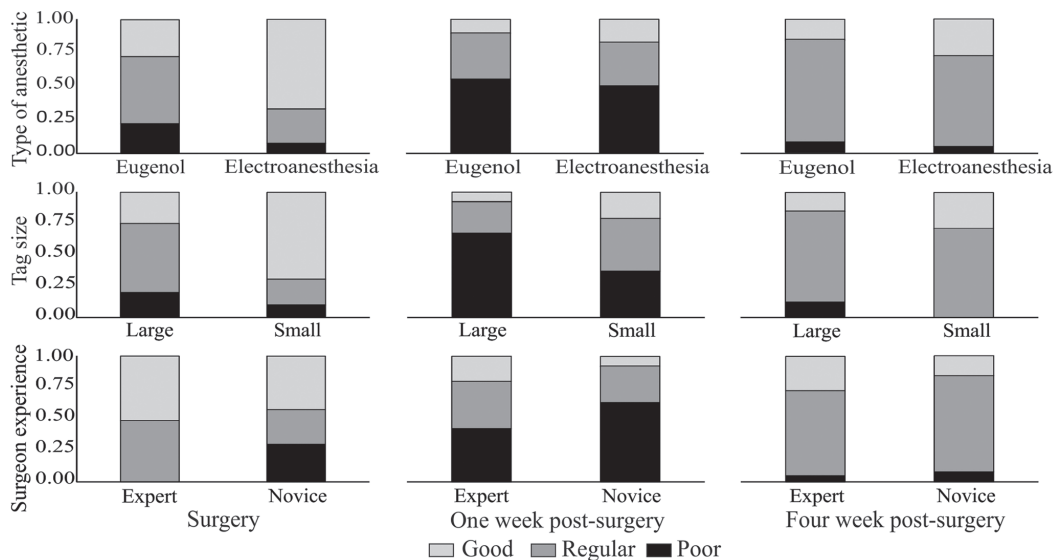


Fig. 5. Surgical/postsurgical ranking of *Prochilodus lineatus* over four weeks. Black: poor; gray: average; and light gray: good.

Regarding the effect of treatments in the surgical and postsurgical recovery, tag size and surgeon experience seem to be more responsive than anesthetic. A higher proportion of fish anesthetized by electroanesthesia had good post-surgery scores compared to fish anesthetized with eugenol, but postsurgical ranking scores were similar for eugenol and electroanesthesia at one and four weeks post-surgery. Postsurgical ranking was worse in fish implanted with large tags than with small ones: wound healing one week post-surgery was considered poor in approximately 68% of fish implanted with large tags compared to only 37% in fish implanted with small tags. In addition, surgery quality and recovery time were likely affected by surgeon experience: no surgeries performed by experienced surgeons were considered poor and postsurgical wound healing was better in surgeries performed by expert surgeons (Fig. 5).

Discussion

A high incidence of postsurgical complications was observed in our study. Even though, mortality was low throughout the experiment (1/80, 1.3%), approximately 21% of all tagged fish had some complication following surgery. Of the 17 postsurgical complications, 14 (14/17, 82.4%) occurred in fish implanted with large tags. Therefore, 35% of fish with large tags presented a surgery problem. This result indicates that tag size is the key determinant for postsurgical complications: large tags require larger incisions and longer surgical times and have a larger contact area with tissues and viscera, which can potentially increase the risk of infection. We strongly recommend the use of aseptic and sterilization techniques and the use of disposable surgical materials to reduce the risk of infection for tagged neotropical fish in the wild (Marty & Summerfelt, 1986; Mulcahy, 2003; Liedtke & Rub, 2012), despite the difficulty of keeping a sterile environment in the field and the need to avoid disinfection procedures to cause more harm than benefits if not used in a proper manner (Cooke *et al.*, 2015).

The encapsulation of most transmitters by fibrous tissue four weeks post-surgery may have improved tag retention and reduced the likelihood of tag expulsion. This encapsulation protected the transmitter, preventing tag expulsion through the incision site or transintestinal expulsion. In fact, the rapid encapsulation of the transmitter has been shown to play a role in retention by limiting its mobility and minimizing the risk of organ damage (Thoreau & Baras, 1997; Penne *et al.*, 2007; Luo *et al.*, 2015). Additionally, the effect of encapsulation processes and incision site on wound healing and tag retention should also be investigated in future studies. In the current study, incisions were made ventrally along the *linea alba*, which is preferable compared to lateral incisions because of the lower risk of bleeding and internal organ damage (Schramm & Black, 1984; Marty & Summerfelt, 1986; Liedtke & Rub, 2012). However, because *Prochilodus lineatus* is a

benthic species, the incision site and sutures may rub the bottom of the pond (as in this study) or riverbed (in the wild), which may result in less healing, reopening of the incision, and entry of pathogens. Moreover, the exit point of trailing antennas should also be considered, because the exit site of the antenna could serve as an entry point for pathogens in tagged fish (Adams *et al.*, 1998; Mulcahy, 2011). Thus, future studies should investigate the effect of incision location on postsurgical wound healing and tag retention in curimba and other benthic species.

Tag losses via mortality or loss of signal have been reported in all telemetry studies in Brazil. The tag/signal loss rates of 16% (Godinho & Kynard, 2006), 19.5% (Andrade-Neto, 2008), 40.5% (Hahn *et al.*, 2011), 18% (Silva, 2012), 20% (Ribeiro, 2013) and 10% (Suzuki, 2014) estimated in these studies are comparable to the complication rates (21%) observed in this study. Some of that loss may be attributed to adverse effects from surgical tagging, including tag expulsion and changes in behavior that may reduce movement rates or increase the chance of capture by fishermen, predation, or death of tagged individuals.

In this study, the type of anesthetic had no marked single effect on most surgical details. In fact, only recovery times were significantly affected by type of anesthetic: recovery was significantly longer for eugenol than for electroanesthesia. Because eugenol acts on the central nervous system (Vandergoot *et al.*, 2011; Javahery *et al.*, 2012), longer recovery times are expected. Electroanesthesia blocks muscle movements and induces an anesthetic effect not by chemical means, but by physical means via electric current, and fish return to a normal state and resume normal swimming when the current is withdrawn. Thus, both anesthetics used in this study meet the requirements for use of anesthetics in fish proposed by Liedtke & Rub (2012): rapid induction and recovery times and lack of long-term effects on fish physiology and behavior. In fact, eugenol and electroanesthesia are appropriate anesthetics for studies that require surgical implantation of transmitters in the field, and choice of anesthetic should be based on logistical aspects and on the welfare status of tagged fish (Jepsen *et al.*, 2013; Mulcahy, 2013; Jepsen *et al.*, 2014; Mulcahy, 2014).

Incision size and duration of surgery, parameters that affect postsurgical wound healing, are usually shorter in surgeries performed by experienced surgeons. Surgical time has been used as an endpoint of surgical success or measure of surgeon experience, and shorter surgery times are associated with shorter sedation times and reduced stress responses in tagged fish (Chomyshyn *et al.*, 2011; Cooke *et al.*, 2011b; Liedtke & Rub, 2012). In this study, surgeries performed by inexperienced surgeons took on average twice longer to complete than surgeries performed by the expert surgeon. In addition, the general appearance of fish was better in surgeries performed by the expert surgeon than by novice surgeons. The reduced

occurrence of bleeding, non-apposed wound edges, and incision openings also appeared to be associated with better postsurgical wound healing, because wound area was significantly smaller one week post-surgery in fish implanted by the expert surgeon. Small internal injuries caused by errors in surgical technique increase the chances of bleeding and bruising, which may increase wound area and recovery times (Cooke *et al.*, 2011b). In fact, several studies have demonstrated the importance of surgeon training on surgical outcomes (Cooke *et al.*, 2003; Smith *et al.*, 2009; Cooke *et al.*, 2011b). Thus, electronic tagging studies in Neotropical fish should incorporate a training program for researchers performing surgical tagging to ensure that the data from tagged fish are representative of untagged conspecifics.

Tag size can also significantly affect surgical details and wound healing. Implantation of large tags requires larger incisions and subsequently more sutures to close the incision, which can cause greater damage to tissues and increase the risk of infection by microorganisms (Brown *et al.*, 2013). In addition, duration of surgery is longer for implantation of large tags than for small ones. In fact, differences in these parameters, some of which are related to surgeon experience, also affect wound healing one week post-surgery. For instance, Collins *et al.* (2013) reported less healing of the incision and suture holes in fish implanted with large tags than fish implanted with small ones, which may have been a result of the heavier tag weight on the incision. It should be noted that a large wound area or poor wound healing may not result in short-term mortality of tagged fish in a controlled environment such as fish tanks. Conversely, in the natural environment, where fish are more exposed to predators and pathogens and have to search for food, higher mortality rates are expected during healing of the incision compared to untagged conspecifics, and changes in behavior are more likely. Thus, smaller tags should be favored over larger ones whenever possible and the trade-off between the longer operating life of large tags and the increased incidence of postsurgical complications should be carefully addressed when selecting telemetry transmitters for fish studies. Another alternative would be tagging with injected transmitters, without sutured incisions. As tags become smaller it must become a suitable solution to avoid a lot of postsurgical problems (Cook *et al.*, 2014) like the ones observed in this study.

Surgical details and postsurgical wound healing, expressed as postsurgical wound scores, were also evaluated in our study. Even though a large proportion of surgical procedures was rated as good immediately after surgery, a large proportion of individuals (> 50%) exhibited poor postsurgical wound healing on visual inspection one week post-surgery. A large proportion of incisions was open and had necrotic tissue around the edges, indicating poor surgical technique. This reduced skin integrity may damage the scales and disrupt the protective mucous layer,

providing an entryway for pathogens and significantly increasing the risk of infection (Bauer *et al.*, 2005; Liedtke & Rub, 2012). Similarly, poor wound healing may expose tagged fish to an increased risk of predation and parasitism in the wild. Moreover, wild and hatchery-reared fish may exhibit different responses to transmitter attachment procedures (Peake *et al.*, 1997).

Postsurgical wound healing was completed four weeks post-surgery with the apposition of connective tissue layers and scale growth on the incision site. Wound healing is likely affected by water temperature, because higher temperatures are associated with higher metabolic rates and smaller wound healing periods (Panther *et al.*, 2011). This study was conducted before the start of the breeding season of curimba in the study area, which coincides with increased rainfall and water temperatures in October. Future studies should investigate the effect of water temperature and time of year on postsurgical wound healing to determine the advantages and disadvantages of delaying tag implantation in areas where water temperature increases with the approaching breeding season of target species.

Our results demonstrate the need to carefully evaluate the surgical procedures used for implantation of electronic tags in telemetry studies of Neotropical fish. Further studies that evaluate the effects of the factors investigated in this study, as well as other factors including origin of fish (hatchery vs. wild), time of year of implantation, sexual maturation, incision location, changes in behavior and swimming performance, sterilization procedures, changes in physiology indicative of stress, and water where fish are released are crucial to improve the reliability of field telemetry studies of Neotropical fish.

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