Radiotagging a long-distance migratory Characiform fish: reproduction after surgery, tag losses, and effects in weight

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Although biotelemetry studies bring useful information, tagging is a highly invasive procedure. For this reason, we assess if intracoelomic tagging alters survivorship, weight gain and/or reproductive capacity of a neotropical migratory fish. In August 2016, 60 fish were equally and randomly distributed among 3 treatments: Control (anesthesia), sham surgery (anesthesia and surgery), and true surgery (anesthesia, surgery and tagging). Surveys for weight and tag expulsion were performed periodically from surgery through spawning (November/2016), with a final survey occurring in May/2017. Mortality was higher in true surgery (25% died) and for males. Twenty percent expelled tags, and initial weight loss followed by gain were verified for all treatments. Females of all treatments spawned, and fecundity and fertility were not different. Then, future studies should consider the losses due to death and tag expulsion when defining the sample number. We also encourage further investigations about differences in mortality between sex and weight variation, as well as any other factor that leads to increase in mortality. This is the first study that investigated tagging effects in reproduction of neotropical fishes, and since the methods and tags used are not species-specific, we infer that similar effects could occur in other species.

Keywords: Biotelemetry, Fecundity, Fertility, *Prochilodus lineatus*, Weight variation.

Submitted September 10, 2020
Accepted June 3, 2021
by Gerson Araújo
Epub July 9, 2021

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 19, no. 2, Maringá 2021

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Embora estudos de biotelemetria gerem informações úteis, processos de marcação são invasivos. Por isso, avaliamos se a marcação intracelomática altera sobrevivência, ganho de peso e/ou capacidade reprodutiva de um peixe migrador neotropical. Em agosto de 2016, 60 peixes foram distribuídos igualmente e aleatoriamente entre os tratamentos controle (anestesia), cirurgia-falsa (anestesia e cirurgia) e cirurgia-verdadeira (anestesia, cirurgia e marcação). Vistorias para pesagem e observação de expulsão de marcas foram realizadas periodicamente da cirurgia até a desova (novembro/2016), com uma vistoria final em maio/2017. A mortalidade foi maior nos peixes marcados (25% morreram) e entre machos. Vinte por cento expulsaram as marcas e, para todos os tratamentos, foi observado perda de peso inicial com posterior recuperação. Fêmeas de todos os tratamentos desovaram; fecundidade e fertilidade não foram diferentes. Novos estudos devem considerar as perdas de marcas por mortalidade e rejeição na definição do número amostral. Também recomendamos estudos que investiguem a mortalidade diferencial entre sexos e variação de peso, bem como fatores causadores de mortalidade. Este é o primeiro estudo que investigou efeitos da marcação na reprodução de peixes neotropicais e, uma vez que métodos e marcas utilizadas não são espécie-específicos, inferimos que efeitos similares podem ocorrer em outras espécies.

Palavras-chave: Biotelemetria, Fecundidade, Fertilidade, *Prochilodus lineatus*, Variação de peso.

INTRODUCTION

Biotelemetry data has revolutionized the study of ecology, management and conservation of wildlife fishes (Cooke *et al*., 2013), especially when considering migratory behavior. Most of such biotelemetry studies have aimed to assess migratory patterns in reproductive seasons (Rustadbakken *et al*., 2004; Godinho, Kynard, 2006; Godinho *et al*., 2006; Crossin *et al*., 2009; Lopes *et al*., 2018a) and assumed that the behavior of tagged fish is similar to non-tagged individuals. While many techniques exist for fish tagging, laparotomy – the surgical procedure to intracoelomically introduce a transmitter – is commonly the most appropriate (Jepsen *et al*., 2002; Brown *et al*., 2009). Despite this, the implantation of transmitters by intracoelomic surgery is highly invasive (Wilson *et al*., 2017).

The handling and surgery procedures can affect the health of the tagged fish, causing infections and physiological problems, altering behavior and even leading to death (Cooke *et al*., 2011; Lopes *et al*., 2016; Wilson *et al*., 2017). The vast majority of studies using fish tagging are conducted in northern hemisphere countries and target salmon species (Cooke *et al*., 2011), which has been called salmo-centric science (Birnie-Gauvin *et al*., 2019). Just a few studies have investigated the effects of intracoelomic fish tagging in tropical and less economically developed countries (Okland *et al*., 2003; Mitamura *et al*., 2006; Thorstad *et al*., 2009), with only two focusing on Neotropical fishes (Schulz, 2003; Lopes *et al*., 2016).

The knowledge available about tagging success reports a highly variable range of results, depending on the species studied. Mortality ranges from 0 – 100%, as well as tag expulsion, and conclusions about the best tagging technique are also highly variable (Close *et al*., 2003; Okland *et al*., 2003; Mitamura *et al*., 2006). Although the central knowledge generated from species of salmon could be used for adaptation of surgery techniques, it is fundamental to conduct studies about other species in different world regions and climates in order to refine tagging techniques and knowledge (Cooke *et al*., 2011).

Fish tagging procedures cause stress (Close *et al*., 2003), which can have negative consequences to reproduction in teleosts (Schreck *et al*., 2001). The presence of tags can compromise muscle contraction and block spawning, causing egg retention (Berejikian *et al*., 2007). Additionally, fecundity relies directly on space available in the coelomic cavity (Wootton, 1992), so reductions in fecundity of tagged fish is expected. However, fewer studies have investigated effects of laparotomy tagging in reproduction (Baras *et al*., 2000; Close *et al*., 2003; Berejikian *et al*., 2007), and none of them on Neotropical fishes. So, there is a need to investigate the effects of tagging in reproduction, especially in mature fish during the reproductive season, usually the focus of biotelemetry studies (Cooke *et al*., 2011).

Data about fish ecology and reproductive behavior are very important for planning management and conservation measures, so any partiality in information caused by preferred study techniques could lead to inadequate actions (Brown *et al*., 2010; Wilson *et al*., 2017). Thus, considering the importance of biotelemetry in understanding the life cycle of fish and possible physiologic changes resulting from intracoelomic implantation of transmitters, we aimed to investigate if: i) there are differences in mortality between fish subjected to surgery and/or due to the presence of transmitter; ii) tagging procedures interfere with weight gain, and iii) fecundity and fertility are altered by surgery and/or due to the presence of a transmitter.

MATERIAL AND METHODS

Target-species. The curimba *Prochilodus lineatus* (Valenciennes, 1837) is a native species from the Paraná River basin (Langeani *et al*., 2007). Considered long distance migrants (Agostinho *et al*., 2003; Lopes *et al*., 2018b), species of this genus usually represent most of the fish biomass in many South American river basins (Bowen, 1983). In the lower Paraná River basin, *P. lineatus* makes up to 95% of commercial fish catch (Espinach-Ros, Delfino, 1993). Although scientific publications on biotelemetry are yet rare in South America, many of these studies are related to *Prochilodus* spp. (Godinho, Kynard, 2006; Hahn *et al*., 2007; Pesoa, Schulz, 2010; Silva, 2012; Hahn *et al*., 2015; Lopes *et al*., 2018a,b, 2019). Voucher specimens (CI-UFLA 786–787) were deposited in the Coleção Ictiológica Universidade Federal de Lavras.

Experiment. The experiment was carried out at Estação Ambiental de Volta Grande hatchery (EAVG), located in the Volta Grande hydropower plant area, Grande River, Upper Paraná River basin. In August 16th 2016, 60 individuals of *P. lineatus* were removed with seine nets from a 200 m^2 pond and randomly distributed across six separate 2000

L tanks, without any weight or sex selection. Fishes were hatchery-reared in EAVG, so they were acclimated to water conditions including temperature and feed type. From this moment on, fish were not feed until they returned to the 200 m^2 ponds. After sorted for treatment, each fish was anesthetized with Eugenol (clove oil) (0.050 ml/L) in an individual 30 L anesthetic tank, with aeration. Fishes were kept in these individual anesthetic tanks until they reached stage IV of the anesthesia, characterized by no reaction to visual or mechanical stimulation and total loss of balance and muscular tonus (Summerfelt, Smith, 1990). The time spent to reach this stage was registered. All fish were weighed (g), measured (cm), and injected with intramuscular PIT-tags, between the dorsal fin and the lateral line, for further identification. Among these fishes, by sorting, 20 made up the control treatment (CO), in which fish were only anesthetized and measured; 20 made up sham-surgery treatment (SS), in which fish were anesthetized, measured and passed through surgery and suture without tagging; and 20 made up the true-surgery treatment (TS), which fish passed through the same proceedings of SS and also underwent transmitter insertion into the coelomic cavity. Such procedures were performed between August 16 and 18, 2016.

Surgery. Fish sorted for treatments SS and TS were removed from the 30 L anesthetic tanks with nets and placed on a cushioned foam where their gills were continuously irrigated with a solution of Eugenol (0.035 ml/L) or pure water, if necessary, by a plastic tube connected to a bucket. For fish in the TS treatment, an inactive (empty-battery) transmitter (Lotek model MCFT–3EM; 8.9 g weight in air, 11 mm width and 49 mm in length) was inserted through a left lateral incision of approximately 5 cm long, made 3 to 5 scales behind the pelvic fin. The antenna was guided with a catheter through the musculature and exited the skin and scales 2 – 3 cm after the end of the incision. Incisions were sutured with square knots (three throws in each knot), using 4 – 5 interrupted sutures approximately 1 cm apart of each other. The fish in the SS treatment were submitted to the same procedure of incision and suture, but without inserting the tag. Disposable gloves, blades, sutures of monofilament nylon (diameter of 0.2 mm), and curved needles of 20 mm was used. Non-disposable surgical-materials and transmitters were washed with filtered water and mild detergent and stored in a 70^o GL ethanol bath for approximately 30 min before each use, a sterilization procedure similar to those used in other field studies in Brazil (Lopes *et al*., 2016).

After tagging proceedings, fish from all treatments were kept together, by treatment type, in 2000 L tanks for at least 24 h recovery period. Then, on August 19th 2016, all fishes were transferred into a 200 m^2 pond and fed daily with commercial feed (36% crude protein). The pond was monitored for dead fish or any abnormal behavior.

Periodic survey. Following the tagging, periodic surveys were conducted after 7 days (S1), 43 days (S2), 65 days (S3), and 84 days (S4). The first survey happened earlier because this is a critical period for healing assessment (Lopes *et al*., 2016). During the surveys, all fish were removed from the ponds with seines nets and taken to the laboratory tanks where they were individually anesthetized with Eugenol (0.050 ml/L), identified by PIT-Tag number, measured, weighed, and checked for tag expulsion. On May 5, 2017, 259 days after the beginning of the experiment, the final survey (S5) was performed. The fish were removed from the 200 m^2 pond and euthanized with Eugenol

(0.1 ml/L). All fish were weighed and autopsied for sex determination, observation of tag expulsion, and presence of internal infections or any other abnormalities. Water temperature of tanks were monitored throughout the experiment at 9:00 a.m. each day.

Assessment of reproduction capacity. As in October 2016, fish were observed weekly for assessment of ideal gonadal maturation conditions in order to receive hormonal induction, the usual practice for migratory fish reproduction in EAVG. In females that showed abdomen bulged and soft when touched, and reddish urogenital papilla, it was performed a cannulation for verifying the presence of light green eggs. Males were massaged in abdomen, from head to urogenital papilla sense, in order to verify semen extrusion. At November 18, 23 and 30 in 2016, males and females of all treatments that showed the required condition for reproduction were taken to the laboratory, weighed, and identified by the PIT-Tag number. Then, females and males received the first dose of 0.5 mg of hypophysis/kg of body weight. About 10 – 12 h after the first dose, males and females received a second dose of 5.0 mg of hypophysis/kg. After completing hormonal induction, two males and one female were joined in 2000 L tanks for natural spawning. At this stage, other males from EAVG not used in the experiment were also taken to the reproduction tanks. These 2000 L tanks had water entrance (from the same source of 200 m^2 pond) and exit at the top, keeping full time water renovation and circulation, with a screen at water exit to avoid egg losses. After spawning, all eggs were recovered with appropriated nets in a beaker, and a sample of 10 ml was taken. Three repeated counts of this sample were performed, for estimation of the average number of eggs by ml. Fecundity by female was estimated by multiplying the total volume off recovered eggs, measured in the beaker, by the average number of eggs counted by ml. After removed the 10 ml samples, eggs were transferred to the egg incubators. About 8 – 9 h after fertilization, three samples of 260 eggs were collected from egg incubators and transferred to a Bogorov plate; here, fertilized and non-fertilized eggs were counted. The fertilization rate was calculated by multiplying the average fertilized eggs of the three counts by 100 and dividing this value by 260.

Data analysis. Mortality in relation to treatment, sex and weight, and effects of the anesthetic and surgery time in mortality were assessed by a generalized linear model (GLM), considering binomial distribution (linkfunction = logit). Effects of treatments on fecundity were assessed by a GLM using Gamma distribution (linkfunction = inverse). Given the widely known effects of weight on fecundity (Vazzoler, 1996), number of oocytes per gram of fish was used as a response variable. The weight of transmitters was subtracted from the total weight for fishes of treatment TS. Fertility was assessed in relation to treatment by GLM using values between 0 – 1 and binomial distributions (linkfunction = logit). Overdispersion was calculated by the ratio between residual deviance and degrees of freedom. Variables contributing to the final model were chosen by likelihood ratio test (LRT) (function drop1), using test F for Gamma and Chi test for binomial distribution. Non-significant variables were excluded from the model.

Since the weight of each fish was measured six times (tagging and S1 – S5), it is expected to have repeated design measures, which lead to a mixed model analysis. In this model, logarithmic weight was used as a response variable, and treatment, sex and event of measurement as a predictor variable with fixed effect, and the individual as a random effect variable. The model was fitted considering the restricted maximum likelihood (REML) criterion, using the function lmer. Afterwards, Wald Test type II was performed for p-values. Non-significant variables were excluded from the final model for better fit of the model. Only fish that remained alive until the end of the experiment were analyzed.

The residual distributions of all models were graphically analyzed and all analysis were performed in the software R Studio (R Core Team, 2019).

RESULTS

Water temperature ranged from 20 ºC in August 2016 to 29 ºC in February 2017 ($\overline{X}$ = 24.7 ± 1.9 ºC). One TS fish disappeared and was not considered in the analysis. Weight of all fish ranged from 475 to 1290 g, and tag weight in air as a percent of fish tagged weight ranged from 0.69–1.65%.

Mortality and tag expulsion. One fish from CO died (5%) six months after the experiment began. Four fish from SS died (20%), three of them within eight days after surgery. Five TS fish died (25%), three of them within six days after tagging. For SS, 40% of the males, but no females, died while 37.5% and 18% of the TS males and females died, respectively. All SS fishes without sex determination died (Tab. 1); these were fish found in the 200 m^2 pond within a few days of death but could not have their sex identified due to decomposition. One tag expulsion was detected in S3 and another three in S5, and these tag expulsion started three months after surgery. All fish that expelled the tags (two females and two males) remained alive in the tanks until S5, and none of them were found with any problems regarding post-surgery healing or infections.

The weight was not a significant predictor for mortality and was excluded from the model (AIC: 45.31; Dispersal ratio: 0.7). Fish of TS showed higher mortality than CO (Standard error = 1.2; z = -1.97; P = 0.048), but the same did not occur for fish of SS (Standard error = 1.33; z = -1.13; P = 0.26). The mortality was also higher between males (Standard Error= 0.92; z = -2.09; P = 0.04) (Fig. 1). The anesthesia time ranged

TABLE 1 | Total number of fish (N), number of dead fishes (Deaths), number of days after surgery each death occurred (Days), and average weight (g), by treatment and sex (ND = fish without sex identification).

Sex	Control				Sham-Surgery				True Surgery			
	N	Deaths	Days	Weight	N	Deaths	Days	Weight	N	Deaths	Days	Weight
Males	12	1	228	665.4	5	2	4, 8	748.3	8	3	4, 5, 228	800
Females	8	0	–	696.9	13	0	–	850.4	11	2	6, 167	902
ND	0	0	–	-	2	2	4, 38	527.5	0	0	–	–
Total	20	1	–	678	20	5	–	831.2	19	5	–	863.7

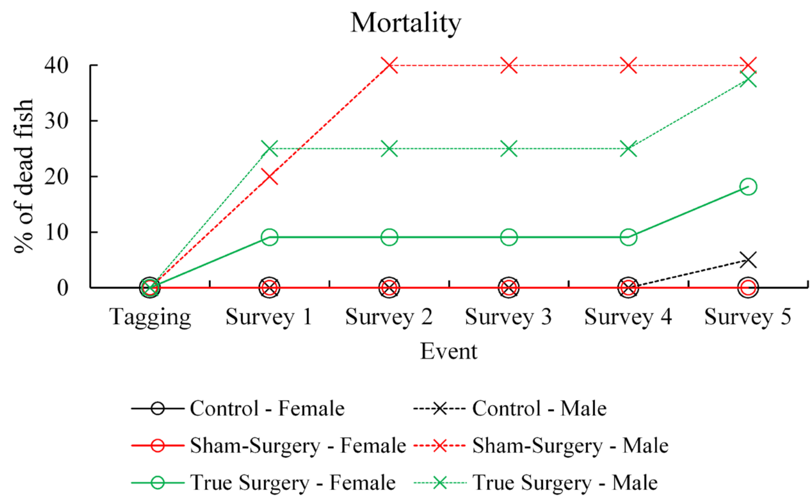

FIGURE 1 | Mortality by event, treatment, and sex. Tagging occurred in 16–18/11/2016, survey 1 in 24/08/2016, survey 2 in 28/09/2016, survey 3 in 21/10/2016, survey 4 in 09/11/2016, and survey 5 in 03/05/2017. Reproduction occurred between survey 4 and survey 5. We excluded fishes with unidentified sex from the figure.

from 2:41 min to 9:50 min ($\overline{X}$= 4:59 ± 2:10 min), surgery time ranged from 5:37 min to 13:12 min ($\overline{X}$ = 8:42 ± 1:39 min), and there were no differences in anesthesia (AIC: 57.6; Dispersal ratio: 0.09, P = 0.47) or surgery time (AIC: 46.5; Dispersal ratio: 1.1; P = 0.73) among fish that died or remained alive.

Weight variation. The difference in weight between treatments was not significant (Standard error-SS: 0.04; t-SS: 2.64; SE-TS: 0.04; t-TS: 2.56; P = 0.051) but the difference in the weight between surveys was (SE: < 0.01; t: 7.07; P < 0.01). The interaction between the two variables was also significant (SE: 0.002; t: -1.26; P < 0.01), indicating different weight variations across surveys among treatments. As a variable, sex did not have a significant effect and was excluded from the final model (REML: -994.7; Variance of random effect: 0.01; Residual variance of random effect: < 0.1; SD of random effect: 0.1; Residual SD of random effect: 0.03) (Fig. 2).

Reproductive capacity. Considering all treatments, 17 females were hormonal-induced for gonadal maturation (Tab. 2). One TS female did not prepare the gonads and did not spawn. When compared with females of CO, we observed a reduction in the median fecundity (Fig. 3), but this difference was not significant for SS (Standard error: <0.01; t: 1.6; P: 0.13) nor for TS (Standard error: < 0.01; t: 0.78; P: 0.45) (Final Model – AIC: 179.8; Dispersion ratio: 0.12). The fertility was not different among treatments for SS (SE: 1.53; t: 0.56; P: 0.58) nor for TS (SE: 1.42; t: -0.43; P: 0.66) compared with treatment CO (Final Model - AIC: 19.6; Dispersal ratio: 0.21; R^2: 0.31).

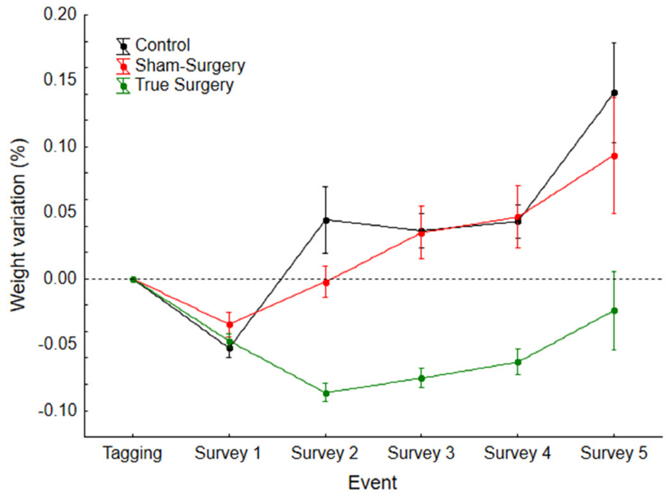

FIGURE 2 | Percentage weight variation of fish that remained alive in the pond until the end of the experiment. Tagging occurred in 16–18/11/2016, survey 1 in 24/08/2016, survey 2 in 28/09/2016, survey 3 in 21/10/2016, survey 4 in 09/11/2016, and survey 5 in 03/05/2017. Reproduction occurred between survey 4 and survey 5.

TABLE 2 | Fecundity and fertility values for female of *Prochilodus lineatus* subjected to induced reproduction with natural spawning.

Reproductive capacity	Control	Sham-Surgery	True-Surgery
N-Females	4	7	6
Mean oocytes number	172.30	137.03	155.92
Standard deviation	77.76	59.90	75.00
Fertility	71%	85%	57%
Standard deviation	21	10	30

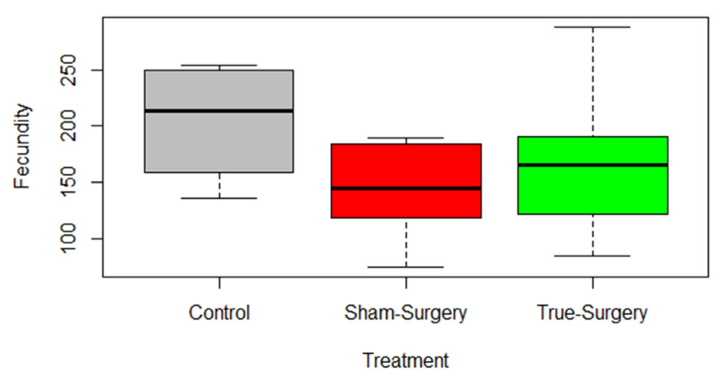

FIGURE 3 | Fecundity (oocytes by gram of body weight) compared among treatments.

DISCUSSION

Our results point out that surgery and/or tagging cause significant losses due to death and tag expulsion as well as higher mortality between males, although the experiment was not completely conclusive about the differences between sexes. Fishes from all treatments showed weight loss in the first week after experiment, followed by a gain, but recovering weight patterns were not the same among treatments. The capacity of spawning, the fecundity, and the fertility were not altered by handling, surgery, and/or tagging.

Mortality and tag expulsion. Considering the total of 10 deaths in all treatments, 60% occurred less than 10 days after surgery, all of these were individuals of the surgery treatments (SS and TS). The number of deaths were very similar between the two treatments that includes surgery (SS and TS), especially if considering those which occurred within a few days after surgery. This indicates that these deaths occurred due to surgery and not the tags. Lopes *et al*. (2016), studying intracoelomic radiotagging effects in *P. lineatus*, but for 30 days, found less mortality (1.5%), although 15% of fish had internal infections by the end of the experiment, meaning that many of those fish could have potentially died or expelled the tags if the experiment had continued any longer. One difference between the two studies is the position of the incision: we performed lateral incisions and Lopes *et al*. (2016) did ventral incisions. In addition, studies have pointed that trailing antennas exiting body wall, as used in this study, could increase mortality in relation to antenna coiled within body cavity (Collins *et al*., 2002; Isely *et al*., 2002). However, detectability should be considered, since internal antenna attenuate signal (Cooke, Bunt, 2001). Therefore, we strongly recommend further studies to analyze effects of incision location and antenna placement in Neotropical fishes.

Usually, mortality after surgery in fish species from colder waters is lower than in tropical ones (Baras *et al*., 2000; Close *et al*., 2003; Smircich, Kelly, 2014). Okland *et al*. (2003) observed 100% mortality when tagging common carp in a Namibian reservoir, when temperatures ranged between 24 and 25ºC, and proposed that higher temperatures could increase mortality rates. Higher temperatures favor microbial outbreaks (Baras *et al*., 2000) which can increase infection and mortality rates. So, the use of antibiotics may be considered to reduce infections immediately after surgery (Isely *et al*., 2002). For example, the antibiotic oxytetracycline have been successful in preventing post-surgical infections in fish living at warm waters (Hanzen *et al*., 2020), and should be considered in surgical protocols for tagging Neotropical fishes. Once handling protocol was the same for all treatments, we assume no additional effects of these factors in mortality difference among treatments. Further studies should investigate if the combination of handling and surgery could harm or delay the healing and increase mortality.

Only mortality of TS fishes was significantly greater than CO, which indicates that a combination between surgery and tagging increased mortality. The negative consequences of tagging are widely known (Close *et al*., 2003; Jadot *et al*., 2005; Hahn *et al*., 2011), but differ among species (Jepsen *et al*., 2005). For example, predatory fish generally have larger body cavities and more flexible bodies than omnivorous or planktivorous species (Jepsen *et al*., 2002), which could help to fit the tag. Additionally, while Siluriform fishes have dorsally flattened bodies and larger celomatic cavities (some exceptions must be observed, such as *Pinirampus pirinampu* (Spix & Agassiz, 1829), a more elongated Siluriform), *Prochilodus* spp. are detritivorous fishes with laterally flattened bodies and less flexible body walls, which could explain their greater tag losses and mortality among wild Neotropical fishes.

While average tag losses in Neotropical biotelemetry studies amount to 4.6% for Siluriforms and/or carnivores fishes (Godinho *et al*., 2006; Hahn *et al*., 2011; Silva, 2012), it has been estimated as high as 17% for *Prochilodus* species (Godinho, Kynard, 2006; Pesoa, Schulz, 2010; Silva, 2012; Lopes *et al*., 2018a). The same driver could explain observed differences between sexes since less flexible body walls are expected in males, presenting lower variation in gonad volume along the maturation cycle compared to females (Vazzoler, 1996). When considering the two treatments that included surgery, 8% of females and 38% of the males died.

Weight variation. We verified that fishes from all treatments lost weight in the first week after the experiment. From S1 to S2, CO and SS fishes started to recover and kept increasing weight until the final of the experiment. Baras *et al*. (2000) also reported that fishes lost weight some days after handling and surgery, but compensated these losses with compensatory growth. Not only surgery and tagging, but also handling, could lead to stress in fishes (Jepsen *et al*., 2001; Almeida *et al*., 2018) which can reduce feeding activity during the days after the experiment proceedings.

However, TS fishes started recovering weight only after S2 and did not reach the same weight from CO and SS fishes until the final survey. According to Jepsen *et al*. (2001), growth is indicator of fish well-being and stress due to handling and tagging is the cause of such lower growth. Baras *et al*. (2000) also reported clear differences between the growth of perch with tag ratios in the water above and below 1.25%, so it is possible that higher tag burdens can lead to impacts in growth. It is also possible that *Prochilodus* species have worse tag “fits” in relation to carnivorous and/or Siluriform species due to body shape which can lead to difficulties in feeding. For example, Mitamura *et al*. (2006) reported that weight gains of the tagged *Pangasianodon gigas* Chevey, 1931 did not differ from control fishes. In this way, we suggest that when and if possible, smaller tags should be considered for *Prochilodus* species. Since stress levels are highest in the hours or days after tagging (Jepsen *et al*., 2001; Close *et al*., 2003), we suggest the use of analgesics like morphine prior to the release of tagged fishes. Although pain in fishes is a question under discussion (Rose *et al*., 2014), the use of analgesic could improve recovery and stress after invasive procedures (Sneddon, 2012).

Reproductive capacity. Fecundity, fertility and spawning were not affected by surgery and presence of tags. In opposition, Berejikian *et al*. (2007) reported higher mortality and egg retention and mortality in intracoelomic tagged females of Chinook Salmon *Oncorhynchus tshawytscha* (Walbaum, 1792) after spawning, arguing that water intrusion and loss of ovarian fluid caused this effect. An important difference in this study is the time of tagging: those female Chinook Salmon were tagged very close to spawning (60% of females were ovulated when tagged). In our study, tagging took place three months before spawning, when gonads were still in the resting stage. In this case, gonads are smaller and likelihood of damage during surgery from tagging is probably lower. For example, Close *et al*. (2003) and Baras *et al*. (2000) also verified that Pacific

Lamprey *Lampetra tridentata* (Richardson, 1836) (= *Entosphenus tridentatus*) and European Perch *Perca fluviatilis* Linnaeus, 1758 were able to mature the gonads without problems within four and six months, respectively, after tagging. So, we suggest that it may be not suitable to proceed laparotomy tagging at mature fishes, a common procedure in biotelemetry studies with Neotropical fishes. Berejikian *et al*. (2007) recommends the use of subdermal tagging to reduce negative effects in spawning in these cases.

An essential assumption in biotelemetry is that the tagged sample is representative of the population, so it is desirable that the presence of the tag does not alter the performance, physiology or survival of tagged fish (Smircich, Kelly, 2014). Despite of the use of hormonal induction for final gonad maturation – a necessary procedure to reproduce Neotropical migratory fishes in captivity, the spawning was natural. Therefore, we assume that in a natural condition, the tagged females would not have the reproductive behavior altered by tagging or handling procedures.

According to our results, within the nine months' time scale, almost half of the tagged fish would not return information in a field study due to mortality and tag expulsion. These tag losses should be considered when defining sample number of future studies. We identified that reproduction capacity, represented in this study by occurrence of spawning, fecundity and fertility, were not significantly altered by tagging procedures and suggest that, after healing from surgery, tagged fish are able to show natural reproduction behavior in nature. Improvements in tagging procedures, especially in surgery and transmitter manufacturing, must be encouraged to reduce loss of information due to death and tag expulsion. Although our study was conducted in non-natural conditions, it represents the first results regarding the effects of tagging in Neotropical fish reproduction. Additionally, our results indicate that intracoelomically tagged fish could provide reliable information about migration and reproduction behavior. Since tagging and surgery techniques generally are the same for a wide range of species, we consider plausible to infer that the same effects observed in this study could occur with other species and tag types. Finally, we strongly encourage future investigation about differential mortality between males and females, as well as any other factors that could lead reduce mortality and tag expulsion, such as antibiotics and antenna configuration.

ACKNOWLEDGMENTS

We are thankful to the staff of the Estação Ambiental de Volta Grande (EAVG) for cooperation in all steps of the experiment. Companhia Energética de Minas Gerais (CEMIG) and Peixe Vivo Program provided administrative and financial support for this experiment (CEMIG/Aneel Research and Development Program 455). We also thank Dr. Gustavo Heringer for help in statistical analysis. SP was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; PQ- 303548/2017-7), and AP was granted a PhD schoolarship by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

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ETHICAL STATEMENT

The voucher specimens were collected by SISBIO license number 10.327-1.

COMPETING INTERESTS

The authors no declare competing interests.

HOW TO CITE THIS ARTICLE

- **Peressin A, Lopes JM, Bedore AG, Alves CBM, Prado IG, Pompeu PS.** Radiotagging a long-distance migratory Characiform fish: reproduction after surgery, tag losses, and effects in weight. Neotrop Ichthyol. 2021; 19(2):e200097. https://doi.org/10.1590/1982-0224-2020-0097

ERRATUM

In the article "Radiotagging a long-distance migratory characid fish: reproduction after surgery, tag losses, and effects in weight", with DOI: https://doi.org/10.1590/1982-0224-2020-0097, published in the journal Neotropical Ichthyology, 19(2):e200097, page 1 (Title):

Where read: Radiotagging a long-distance migratory characid fish: reproduction after surgery, tag losses, and effects in weight
Should read: Radiotagging a long-distance migratory Characiform fish: reproduction after surgery, tag losses, and effects in weight

And the page 15 (How to cite this article):

Where read: Peressin A, Lopes JM, Bedore AG, Alves CBM, Prado IG, Pompeu PS. Radiotagging a long-distance migratory characid fish: reproduction after surgery, tag losses, and effects in weight. Neotrop Ichthyol. 2021; 19(2):e200097. https://doi.org/10.1590/1982-0224-2020-0097
Should read: Peressin A, Lopes JM, Bedore AG, Alves CBM, Prado IG, Pompeu PS. Radiotagging a long-distance migratory Characiform fish: reproduction after surgery, tag losses, and effects in weight. Neotrop Ichthyol. 2021; 19(2):e200097. https://doi.org/10.1590/1982-0224-2020-0097

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