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RECORD OF VISIBLE IMPLANT ALPHA TAG LOSS IN THE CATFISH *RHAMDIOGLANIS TRANSFASCIATUS* (SILURIFORMES: HEPTATERIDAE) INHABITING AN ATLANTIC FOREST STREAM

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Abstract: We report the number of recaptured individuals and the loss of visible implant alpha tags in the Neotropical catfish *Rhamdioglanis transfasciatus* during a mark-recapture study conducted in Atlantic Forest streams, Brazil. We recaptured 1.8% and 29% of the marked individuals in the rainy and dry seasons, respectively. The lowest number of recaptured labels occurred in the rainy, warm season, which could be attributed both to higher movement events of individuals outside the monitored area or because the inflammatory reaction that leads to tag loss, which is favored in warmer conditions. These findings highlight the need to improve the application of VI Alpha tags, whether through refining tagging protocols and materials, so as to enhance tracking accuracy and minimize resource loss in ecological studies.

Key-words: Atlantic Forest; freshwater fish; mark-capture; stream; tagging.

Marking fish individuals is fundamental to understanding ecological patterns related to movement, growth, survival, and habitat use. Common techniques include fin clipping, chemical or dye marking, external tags, passive integrated transponders, and elastomer-based markers. Those methods are associated to different levels of physical injuries (*e.g.* Ivasauskas *et al.* 2012, Collins *et al.* 2013), fitness reduction (*e.g.* Gauthier-Clerc *et al.* 2004, Wilson *et al.* 2011; Ouedraogo *et al.* 2014), predation risks (*e.g.* Carlson & Langkilde 2013), and mortality rates (*e.g.* Økland *et al.* 2001, Gauthier-Clerc *et al.* 2004, Wilson *et al.* 2011). In stream environments, where fish are often small-bodied and habitats are structurally complex, researchers frequently use visible implant elastomer (VIE) tags and visible implant alpha (VI Alpha) tags to individually mark fish (*e.g.*, Mazzoni & Iglesias-Rios 2012, Mazzoni *et al.* 2018). VIE tags pose issues for individualize members of the population, thus hampering the obtention of individual-level information on growth and condition (Jungwirth *et al.* 2019). In turn, externally VI Alpha tags are small fluorescent tags inscribed with unique alphanumeric codes (Knapp *et al.* 2023), making them suitable for tagging small-sized fishes given their size, easiness of application, and low cost (Turek *et al.* 2014).

In addition to the negative effects to the organism (*e.g.*, mortality, injuries), there are many records of expulsion of internally implanted markers, such as subcutaneous labels and passive integrated transponders (Jepsen *et al.* 2002). Studies assessing the tag retention rates of VI alpha tags (Northwest Marine Technology, Inc.) have shown a wide range of variation in retention (Turek *et al.* 2014). Tag expulsion does not compromise the organism fitness and survival (Jepsen *et al.* 2002), but poses a loss of time, material, resources and information to the population study. Herein, we report the loss of VI alpha tags in the Neotropical catfish *Rhamdioglanis transfasciatus*, a heptapterid inhabiting Atlantic Forest streams, following a mark-recapture experiment. We carried out four expeditions in the rainy season and four

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expeditions in the dry season of 2016, each consecutive expedition within the season in a 20-day interval.

We captured fish by electrofishing (Mazzoni *et al*. 2000) within a 375 m stretch in the "Mineiro-Branco" Stream (Figure 1; 22° 25' 18.26" S, 42° 44' 20.90" W), Guapiaçu River basin, within a preserved Atlantic Forest area in the Rio de Janeiro State, southeastern Brazil. We used an alternate current generator (Honda EU10i, 1.000W, 220V, 4.1A), with two electrified poles. After stunning, individuals were captured from the water column using nonelectrified hand nets. Captured individuals were kept in floating cages, with water circulation from the stream itself, for total recovering from electronarcosis. More details on sampling techniques can be found in Soares *et al.* (2020). Sampled individuals were anesthetized with Eugenol and marked through an incision in the ventral region of the caudal peduncle with VI Alpha Tags, biocompatible labels individualized by color and alphanumeric code. More details are available in the manufacturer's website (http://www.nmt.us/vi-alpha/). We used a total of 842 labels (Table 1) and did not record any mortality due to anesthesia, handling and/or marking procedures.

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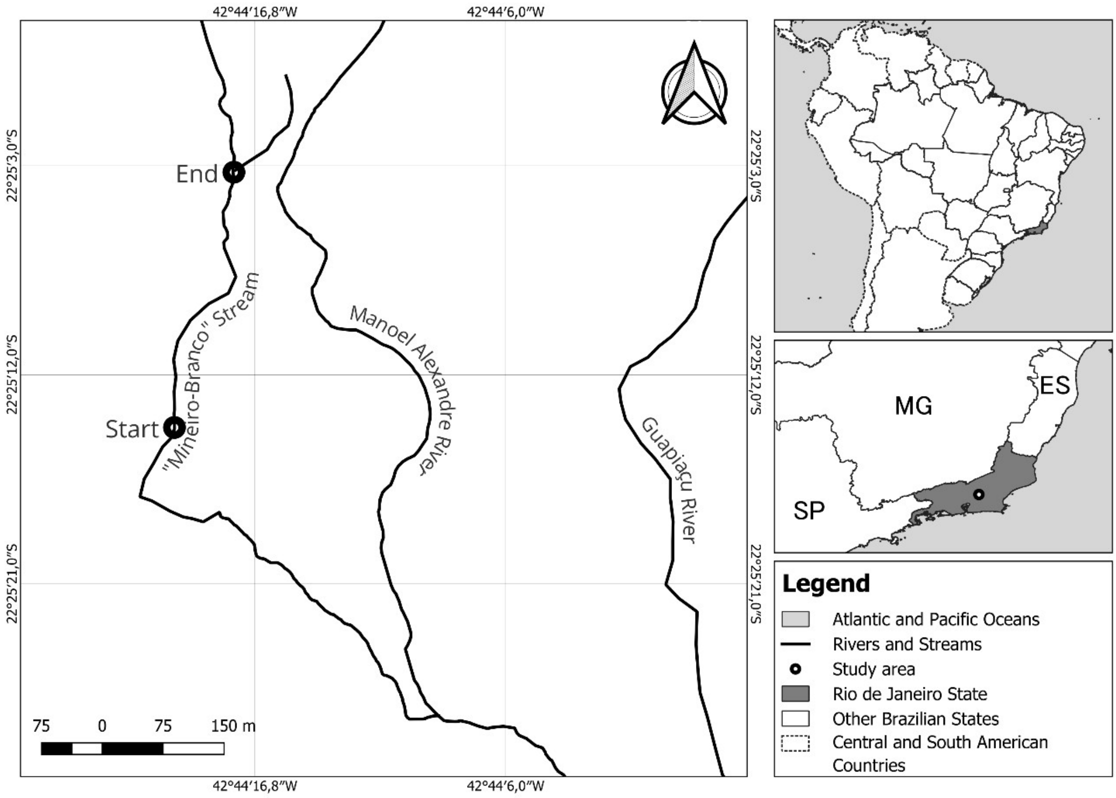

Figure 1. Location of the Mineiro-Branco Stream, located in the Guapiaçu River basin, southeastern Brazil.

Table 1. Number of individuals caught at each mark-recapture event in the Rainy and Dry seasons of 2016 in the Mineiro-Branco Stream, Rio de Janeiro State, southeastern Brazil.

Event	Rainy/2016	Dry/2016
Day 0	109	104
Day 20	94	89
Day 40	136	101
Day 60	114	95

During the field expeditions, we noticed that many fish have lost their labels by the presence of a round-whitish scar in the incision point. Some fish presented advanced inflammatory reactions in the incision location and still conserved their tags (Figure 2). We counted all the times we observed the inflammation process in the incision point for the following descriptive statistics (Table 2): the total number of used labels, the number of individuals recaptured with labels, the number of never-recovered labels (= the total number of used labels minus the recovered ones). Proportions of these numbers were calculated as percentages from the total number of used labels. We also calculated the estimated number of

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labels used per individual within a season by dividing the total number of labels used by the sum of individuals recaptured with labels. One should note that those calculations consider that all individuals displaying labels were marked and recaptured just once, which potentially introduce biases in this description, since some individuals were probably marked more than once.

Figure 2. Perceptible inflammatory reaction as a purple-colored skin lesion (circle) around the label in an individual of *Rhamdioglanis transfasciatus* collected in the Mineiro-Branco Stream, Rio de Janeiro State, southeastern Brazil.

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Table 2. Numbers of recovered and not recovered labels, proportions based on the total number of labels used and the average number of labels used per individual in each season in the Mineiro-Branco Stream, Rio de Janeiro State, southeastern Brazil. The second and last sampling expedition occurred in the dry season, so there is no record of individuals recaptured in its next season.

Season	**Rainy**		**Dry**	
	N	% from total	N	% from total
Individuals recaptured with labels	8	1.8%	83	29.0%
Individuals recaptured with labels in the next season	3	0.7%	-	-
Never-recovered labels	431	97.5%	203	71.0%
Maximum number of individuals marked in a season	136	31.6%	104	51.2%
Used labels (Total)	442	100.0%	286	100.0%
Average number of labels used per individual	3.01		1.53	

During the rainy season (*i.e.*, summer in Brazil), we deployed approximately 442 labels, but only 11 tagged individuals were recaptured, representing a recapture rate of 2.5%. Given the low recapture rate, we estimate that more than three labels were used, on average, for each individual fish captured in this season. As the rainy season corresponded to the first sampling expedition, the low recapture rate raised concerns about the feasibility and validity of continuing this study. We estimate that about US$500 was lost in material during this season alone. Differently, the dry season (*i.e.*, winter in Brazil) was more promising in relation to the maintenance of labels in individuals, since 29.0% of all used labels were indeed recovered. The tag losses posed some methodological limitations to the application of the data to understand population dynamics and movement ecology. First, we were unable to determine the exact number of marked individuals during the study. Some individuals may have lost their tags and, without visible signs of tagging or inflammation, were indistinguishable from unmarked individuals. Second, due to the tag loss, we could not accurately calculate population sizes and other population-level descriptors, as necessary parameters (total number of recaptured individuals at each sampling event) were compromised by low tag retention rates.

Cases of implanted markers' expulsion have already been recorded for other species, including other catfishes such as *Ictalurus punctatus* (Marty & Summerfelt 1986) in the United States and *Rhamdia quelen* (Schulz 2003) in Brazil. Encapsulation prior to expulsion occurs due to the organism's inflammatory reaction to a foreign body, and its subsequent expulsion usually occurs via the incision point or through the body wall (Coleman *et al*. 1974, Moore *et al*. 1990, Jepsen *et al*. 2002). The risk of expulsion is usually reduced if the marker is cylindrical or has rounded tips but increased if it has sharp edges (Thorstad *et al*. 2013). VI Alpha Tags have a rectangular shape with rounded edges, but are flat and slightly hardened, which gives them relatively sharp edges, thus increasing the risk of expulsion. In addition, we also consider that the lower temperatures during the winter potentially decreased the incidence of inflammatory processes and subsequent tag expulsion. The water temperature ranged from 22.2 to 22.8 °C (average = 22.5 °C, SD = 0.29) in the rainy season and from 16.3 to 18.7 °C (average = 17.8 °C,

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SD = 1.06) in the dry season. It is reported in the literature that greater infection risk may be linked to the release of fish in relatively warmer waters (Jepsen *et al*. 2002). Also warmer temperatures promoted tag expulsion in the bluegill sunfish *Lepomis macrochirus* (Knights & Lasee 1996), the striped bass *Morone saxatilis* × white bass *M. chrysops* hybrid (Walsh *et al*. 2000), and the chinook salmon *Oncorhynchus tshawytscha* (Deters *et al*. 2010) in North America.

Studies regarding tag expulsion in Neotropical fish have been conducted under manipulative conditions (*e.g.* Schulz 2003, Lopes *et al*. 2016). Although recent studies using biocompatible markers in Neotropical stream fishes (*e.g.* Mazzoni & Iglesias-Rios 2012, Espírito-Santo *et al*. 2016, Celestino *et al*. 2017, Mazzoni *et al*. 2018) have not reported marker expulsion, several of them were conducted under similar environmental conditions to our study. Therefore, we do not attribute the high tag loss in our study to abiotic factors alone. Instead, we suggest potential explanations: (1) inflammation or scaring may have gone unnoticed by researchers, and (2) interspecific differences in tag retention. Tagging methods exhibit varying levels of tag loss, but they commonly present some losses. Passive integrated transponder tags have shown to present low retention rate in body cavity (Mamer & Meyer 2016) while visible implant elastomer exhibits higher retention rates (Hohn & Petrie-Hanson 2013, Bushon & Rash 2021). While the latter exhibits higher retention rates than the method we used, there are limitations on the number of individuals that can receive a unique combination of colors that allow the recognition of different individuals.

One way to avoid possible premature study interruptions and hindrances caused by loss of time, material, resources, and marked individuals is to use alternative methods of individual identification. Methods such as the detection of electric fields (*e.g.,* Henninger *et al*. 2020 with Gymnotiformes) or the use of natural skin marks (*e.g.* Dala-Corte *et al*. 2016 with Loricariidae) can be easily applied because of currently available technological resources. Despite this, these methods are taxon-dependent and rarely applied, requiring more investigation. Other suggestions would be combining both VI Alpha tags and VIE for better controlling the number of marked individuals and carrying out a pilot tagging program in a more controlled environment to assess retention rates in the focal species. Finally, despite the high loss of labels, the number of marked individuals allowed us to continue the study and promoted novel findings regarding the movement of Neotropical stream-dwelling fish.

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