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Use of visible fluorescent elastomer to monitor Chironius flavolineatus (Serpentes: Colubridae) in the Atlantic Forest

Studies of monitoring animal populations using capture-mark-recapture require reliable methods of marking, mainly to obtain data on both population size and growth, as well as on individual movement and displacement in the long term. From May 2019 to April 2021, we marked 111 individuals of Chironius flavolineatus from a population found in the Atlantic Forest of Northeast Brazil. The markings were made using four colours of the visible implant elastomer (VIE), obtaining a total of 40 recaptures that varied from three days to 696 days after the marking and release. We analysed the efficiency and persistence of the coloured polymers, and our findings indicate that the use of VIE is an effective method for marking arboreal snakes in both short- and long-term studies. All the colours worked successfully; however, the green-coloured polymer needs to be used with caution in long-term studies. Because of the consistency in the recapture of the snakes and the long period of recapture recorded during the research, the use of the visible implant fluorescent elastomer is recommended for future studies on the ecology of snake populations.

Keywords: Population ecology; marking technique; polymer; neotropical snakes.

Uso de elastômero fluorescente visível para monitorar Chironius flavolineatus (Serpentes: Colubridae) na Mata Atlântica

Estudos de monitoramento de populações animais por captura-marcação-recaptura requerem métodos confiáveis de marcação, principalmente para obter dados sobre tamanho e crescimento populacional, bem como sobre movimentação e deslocamento individual a longo prazo. De maio de 2019 a abril de 2021, marcamos 111 indivíduos de Chironius flavolineatus de uma população encontrada na Mata Atlântica do Nordeste do Brasil. As marcações foram feitas utilizando quatro cores do implante visível de elastômero (VIE), obtendo-se um total de 40 recapturas que variaram de três dias a 696 dias após a marcação e liberação. Analisamos a eficiência e persistência dos polímeros coloridos e nossos achados indicam que o uso de VIE é um método eficaz para marcação de cobras arbóreas em estudos de curto e longo prazo. Todas as cores funcionaram com sucesso; no entanto, o polímero de cor verde precisa ser usado com cautela em estudos de longo prazo. Devido à consistência na recaptura das serpentes e ao longo período de recaptura registrado durante a pesquisa, o uso do elastômero fluorescente de implante visível é recomendado para futuros estudos sobre a ecologia de populações de serpentes.

Palavras-chave: Ecologia de População; técnica de marcação; polímero; serpentes neotropicais.

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INTRODUCTION

Reliable marking techniques are of Major importance for the development of population ecology studies to estimate parameters such as population size, density, demographics, home range or behaviours (WALSH et al., 2004). These methods need to meet the following criteria: the markings should remain visible for the entire duration of the study, the marking techniques should not influence the animal habits, they should cause as little stress as possible, and they should be easy to observe and to be applied to different sizes of the animal (OTIS et al., 1978). The methodological techniques for capture-mark-recapture (CMR) make it possible to individualise each specimen of the studied population, allowing the monitoring of individuals over a long-term period, and providing information on population growth and longevity (BENTES et al., 2017). Different techniques have been used to mark and recapture snakes, such as clipping of ventral and subcaudal scales (BROWN et al., 1976), radiotelemetry and passive integrated transponder (PIT) tags (MACGREGOR et al., 2001), scale cauterisation markings (WINNE et al., 2006), identification of natural markings, such as pigmentation pattern and scalation configuration (SAZIMA, et al., 1988), and visible implant elastomers (HUTCHENS et al., 2008).

The use of visible implant elastomer (VIE) as a marking technique has been used in studies on the population ecology of diverse vertebrates, such as fishes (LEAL et al., 2012), amphibians (BAILEY et al., 2004), and lizards (FREITAS et al., 2013). VIE is a coloured polymer in liquid form that can cure a flexible solid with the addition of a curing agent. Once the polymer and curing agent are mixed, it can be injected subcutaneously, in different places of the animal body, using a small-bore needle. VIE forms a non-toxic mark that can be detected using a UV flashlight. VIE has been successfully tested, presents good retention, and does not interfere with the survival rate or behaviour of the marked species (FITZGERALD et al., 2004). Regarding snakes, few studies have used VIE for population monitoring, and some questions remain unanswered, such as whether the body growth rates influence the position and persistence of the polymer, and if all colours of the polymer have the same effectiveness in long-term studies.

REPORT

Here, we evaluated the use of a visible implant fluorescent elastomer to monitor a population of Boettger's Sipo Chironius flavolineatus Jan 1863 in the Atlantic Forest of Northeast Brazil. Chironius flavolineatus (Colubridae) is a moderate-sized species (maximum length, approximately 1.2 m), with arboreal habits and diurnal activity. It has been observed resting on vegetation at night, at heights ranging from 1 m to > 8 m. Chironius flavolineatus is a harmless snake that feeds on amphibians (PINTO et al., 2008). Its litter can range from three to eight eggs (PINTO et al., 2010). It is widely distributed in Brazil, occurring in the Atlantic Forest, Cerrado, Caatinga, Pantanal, and Amazon Forest (NOGUEIRA et al., 2019).

The study was conducted between May 2019 and April 2021 (during the period from April to September 2019, all fieldwork was cancelled due to the pandemic of coronavirus disease 2019 – COVID-19). The survey was carried out on a forest patch (−6.84977ºS; −34.9281ºW; WGS 84; 31 m elev.), Rio Tinto

municipality, Paraíba State, Northeast Brazil. The snakes were found during active searches between 1900 and 0200 hours and were captured by hand.

A total of 111 individuals were marked with VIE (Northwest Marine Technology Inc., Shaw Island, WA, USA) in four colours: red, green, orange, and yellow. Each specimen was marked by injecting the polymer subcutaneously, between the ventral scales, and between subcaudal scales. In the ventral scales, the markings were made at intervals of five in five scales. The first captured snake was marked between the first and second scales after the precloacal scale, the second individual was marked between the fifth and sixth scales, the third between 10th and 11th scales, and so on. The subcaudal scales were marked from the second scale after the cloaca until the end of the tail, without intervals. Each specimen of Chironius flavolineatus was marked with a unique combination of markings on the ventral scales along with the marking on the subcaudal scales (FIGURE 1-B). We also partially cut the ventral scales to facilitate the injection of the polymer and visualisation of the marks (FIGURE. 1-C) (BARBOSA et al., 2020). These cuts are temporary, and the scales grow back after a few ecdyses.

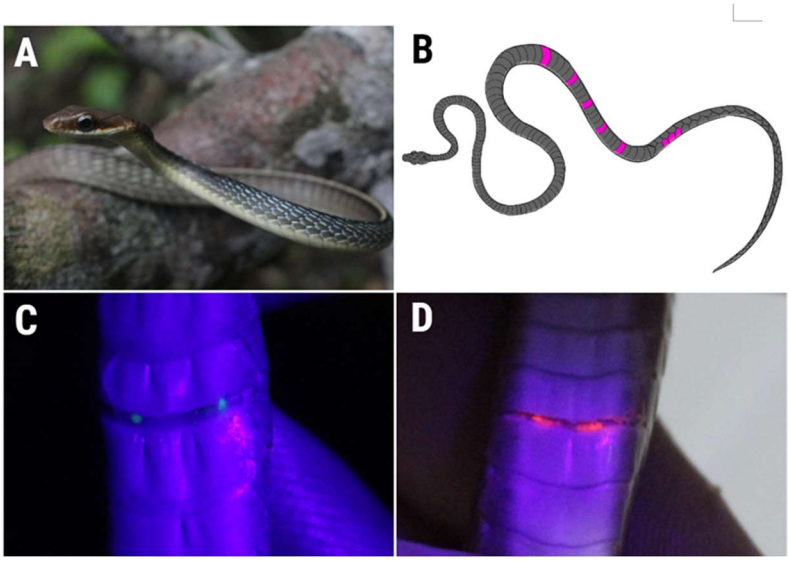

Figure 1: The use of visible implant fluorescent elastomer in Boettger's Sipo Chironius flavolineatus. (a) An adult Chironius flavolineatus in Atlantic Forest; (b) Schematic drawing of the marking methodology in ventral and subcaudal snake scales; (c) Highlight for the partial clipping of the ventral scale and the example of residual mark with green-coloured visible implant elastomer (VIE) under UV-B light, 106 days after capture and release; (d) Mark with red-coloured VIE under UV-B light, 696 days after capture and release, showing also the total growth of the ventral scale.

For each specimen, we recorded biometric data using a digital calliper (snout-vent length, tail length, head length, head width, head height, body width, and body height), mass with a pesola scale, and sex. Each mark was verified using UV-B light before the specimens were released into the wild. All snakes were released at the same locations where they were captured. Of the 111 marked specimens, 19 were recaptured once over the study period, nine specimens were recaptured twice and one (a male nº 44) was recaptured three times, totalling 40 recaptures (Table 1). Nine recaptured specimens were marked with red polymer (from 37 specimens marked in red), ten with green (from 20), eight with orange (from 39), and two with yellow (from 15). The time intervals between release and recapture varied from three days to 696 days. The specimen nº

5 had the longest recapture (recaptured after 696 days of being marked and released), followed by snakes nº 15 (591 days), nº 7 (567 days), and nº 6 (511 days) (Table 1).

Table 1: Data of recaptures of Chironius flavolineatus in Northeast Brazil marked with visible implant elastomer (VIE) presenting variation in weight (Mass in g) and snout-vent length (SVL in mm). R-Days are the days between capture and each recapture. Mass i- starting mass; Mass f- last mass; SVL i- starting snout-vent length; SVL f- last snout-vent length.

individual	color	Mass i	Mass f	SVL i	SVL f	Days
5	Red	69	115	695	720	52
			175		750	696
6	Red	29	74	524	631	511
7	Red	47	63	570	612	105
			87		709	567
15	Red	61	59	626	637	591
18	Green	11	11	302	302	3
19	Green	130	130	788	788	3
			90		788	7
22	Green	63	56	590	650	105
23	Green	30	29	451	451	4
			34		501	57
24	Green	6	19	273	404	106
25	Green	13	57	395	636	391
			53		656	521
28	Green	90	84	739	775	286
30	Green	15	27	412	495	49
			71		680	334
31	Green	34	33	543	570	45
			67		610	280
34	Green	23	61	502	614	221
39	Orange	29	69	502	606	285
43	Orange	21	60	405	606	211
44	Orange	74	99	702.5	742	184
			95		750	216
			91		750	284
45	Orange	70	105	609	722	216
47	Orange	63	66	620	624	32
48	Orange	75	76	665	665	27
50	Orange	59	62	591	602	27
			57		602	32
51	Orange	48.5	51	580	590	27
57	Yellow	19	21	435	435	5
			28		527	203
58	Yellow	59	60	625	641	73
71	Red	16	27	441.5	529	130
74	Red	56	58	631	644	130
81	Red	76	128	704	713	129
85	Red	61	66	670	675	129
86	Red	61	70	696	696	129

DISCUSSION

We found that the red-coloured VIE remained with good visibility throughout the study, and even in the specimen recaptured after 696 days, the colour remained visible with the use of UV light (FIGURE 1-D). This specimen (nº 5) grew up to 73 mm between the capture and recapture, and the polymer remained visible. Similarly, nº 15, which was recaptured after 591 days, grew up to 43 mm and his red marking migrated from the 85º scale to the 84º scale, but remained visible. Major et al. (2020) also observed that the markings with red polymer remained visible in a study with seven individuals of the Aesculapian Snake Zamenis

longissimus (Laurenti, 1768), a semi-arboreal species that was recaptured at intervals of 306 to 434 days. The authors reported that most marks remained intact in the same place in the body of the snake; however, one of these marks stretched dorsolaterally.

The orange-coloured VIE was as efficient as the red-coloured polymer. Eight individuals were recaptured at intervals ranging from 5 to 285 days and remained completely visible with the aid of UV light. In one individual (nº 50), the mark migrated from the 55º scale to the 56º scale. This specimen was recaptured after 32 days and grew by up to 22 mm. For the others, even the nº 39 that was recaptured after 285 days and grew by up to 201 mm, the marks remained intact at their initial application site.

On the other hand, marking with green coloured VIE did not present a good long-term efficiency. In six recaptured snakes nº 23, nº 24, nº 25, nº 28, nº 30, and nº 34, the polymer became quite fragmented and residual, and even under UV light, it was very difficult to identify the marks (Figure 1-C). For these individuals, it was necessary to reapply the VIE before they were released.

The yellow coloured VIE was the least used during the research period when compared to the other colours, presenting two recaptures with intervals of five days and 73 days. However, it seems to be efficient because of the easy visualisation of the marks in the recaptured specimens. Hutchens et al. (2008) used yellow VIE in a study with Red Cornsnake Pantherophis guttatus (LINNAEUS, 1766) and found that out of 18 marked individuals, only three markings in the pre-caudal region were lost in intervals from 23 to 310 days.

FINAL CONSIDERATIONS

Our results corroborate those of previous studies that show that the use of VIE (HUTCHENS et al., 2008) is an effective method for monitoring snake populations, and specifically, in our study, for arboreal snakes. We were able to recapture snakes after more than 690 days and recognise the markings, indicating that VIE is maintained for a long time. Despite some differences between the colours of the polymer used and the possibility of fragmentation or stretching of the markings due to the growth of individuals (MAJOR et al., 2020), we were still able to easily recognise and identify each marked individual. In this way, VIE can contribute to future studies on the population ecology of Chironius flavolineatus, on the displacement and growth of individuals, population size, and birth rate in the Brazilian Atlantic Forest.

REFERÊNCIAS

BAILEY, L. L.; SIMONS, T. R.; POLLOCK, K. H.. Spatial and temporal variation in detection probability of plethodon salamanders using the robust capture–recapture design. **Journal of Wildlife Management**, Washington, v.68, p.14–24, 2004. DOI: https://doi.org/10.2193/0022-541X(2004)068[0014:SATVID]2.0.CO;2

BARBOSA, V. N.; AMARAL, J. M. S.; GUSMÃO, R. A. F.; LIMA, L. F. L.; SOUZA, J. V. M.; AGUIAR, I. D. S.; SANTOS, E. M.. **Serpentes de uma área de proteção urbana da Floresta Atlântica nordestina brasileira**. Cuadernos de Herpetologia, Buenos Aires, v.34, n.2, p.201-209, 2020. DOI: https://doi.org/10.31017/CdH.2020.(2020-003)

BAUWENS, D.; CLAUS, K.; MERGEAY, J.. Genotyping validates photo-identification by the head scale pattern in a large population of the European adder (Vipera berus). **Ecology and Evolution**, London, v.8, n.5, p.2985-2992, 2018. DOI: https://doi.org/10.1002/ece3.3917

BENTES, A. P.; BENTES, S. S.; SANTOS-JR, A. P.. Utilização de marcas naturais para individualização da Cobra-d'água Helicops polylepis Gunther, 1861 (Dipsadidae, Xenodontinae). **Biota Amazônia**, Amapá, v.7, n.3, p.69-73, 2017.

BROWN, W. S.; PARKER, W. S.. A ventral scale clipping system for permanently marking snakes (Reptilia,

Serpentes). **Journal of Herpetology**, São Paulo, v.10, n.3, p.247-249, 1976.

DI-BERNARDO, M.; BORGES-MARTINS, M.; OLIVEIRA, R. B.; PONTES, G. M. F.. Taxocenoses de serpentes de regiões temperadas do Brasil. In: Nascimento, L. B.; Oliveira, M. E.. **Herpetologia no Brasil II**. Curitiba: Brasil, p.200-241, 2007.

FREITAS, P. R. S.; MESQUITA, D. O.; FRANÇA, F. G. R.. Uso do Implante Visível de Elastômero Fluorescente (IVE) para marcação de lagartos Phyllopezus pollicaris (Squamata: Phyllodactylidae). **Biotemas**, Florianópolis, v.26, n.4, p.271-276, 2013. DOI: https://doi.org/10.5007/2175-7925.2013v26n4p271

FITZGERALD, J. L.; SHEEHAN, T. F.; KOCIK, J. F.. Visibility of visible implant elastomer tags in Atlantic salmon reared for two years in marine net-pens. **North American Journal of Fisheries Management**, Bethesda, v.24, p.222-227, 2004. DOI: https://doi.org/10.1577/M02-138

GOLDSMITH, R. J.; CLOSS, G. P.; STEEN, H.. Evaluation of visible implant elastomer for individual marking of small perch and common bully. **Journal of Fish Biology**, United Kingdom, v.63, p.631–636, 2003. DOI: https://doi.org/10.1046/j.1095-8649.2003.00176.x

HAMDAN, B.; FERNANDES, D. S.. Taxonomic revision of Chironius flavolineatus (Jan, 1863) with description of a new species (Serpentes: Colubridae). **Zootaxa**, New Zealand, v.4012, n.1, p.97-119, 2015. DOI: https://doi.org/10.11646/zootaxa.4012.1.5

HARTMANN, P. A.; HARTMANN, M. T.; Giasson, L. O. M.. Uso do habitat e alimentação em juvenis de Bothrops jararaca (Serpentes, Viperídae) na Mata Atlântica do Sudeste do Brasil. **Phyllomedusa**, Piracicaba, v.2, n.1, p.35-41, 2003. DOI: https://doi.org/10.11606/issn.2316-9079.v2i1p35-41

HUTCHENS, J. S.; DEPERNO, C. S.; MATTHEWS, C. E.. Visible Implant Fluorescent Elastomer: A Reliable Marking Alternative for Snakes. **Herpetological Review**, v.39, n.3, p.301–303, 2008.

HYSLOP, N. L.; STEVENSON, D. J.; MACEY, J. N.; CARLILE, L. D.; JENKINS, C. L.; HOSTETLER, J. A.; OLI, M. K.. Survival and population growth of a long-lived threatened snake species, Drymarchon couperi (Eastern Indigo Snake). **Population Ecology**, Kyoto, v.54, p.145-156, 2012. DOI: https://doi.org/10.1007/s10144-011-0292-3

KAPFER, J. M.; COGGINS, J. R.; HAY, R.. Spatial ecology and habitat selection of bullsnakes (Pituophis catenifer sayi) at the Northern periphery of their geographic range. **Copeia**, Lawrence, v.2008, n.4, p.805-826, 2008. DOI: https://doi.org/10.1643/CE-07-104

LEAL, M. E.; BARBOSA, A. S.; SCHULZ, U. H.. Uso de Implante Visual Fluorescente de Elastômero (VIFE) na marcação de pequenos peixes de água doce tropicais. **Biotemas**, Florianópolis, v.25, n.3, p.311-315, 2012.

MACGREGOR, G. A.; REINERT, H. K.. The Use of Passive Integrated Transponders (PIT Tags) in Snake Foraging Studies. **Herpetological Review**, v.32, n.3, p.170-172, 2001.

MAJOR, T.; ALKINS, D. R.; JEFFREY, L.; WUSTER, W.. Marking the un-markable: visible implant elastomer in wild juvenile snakes. **Herpetological Journal**, Montrose, v.30, p.173-176, 2020. DOI: https://doi.org/10.33256/hj30.3.173176

NOGUEIRA, C. C.; ARGÓLO, A; J. S.; ARZAMMENDIA, V.; AZEVEDO, J. A.; BARBO, F. E.; BÉRNILS, R. S.; BOLOCHIO, B. E.; MARTINS, M. B.; GODINHO, M. B.; BRAZ, H.; BUONONATO, M. A.; HEREDIA, D. E. C.; COLLI, G. R.; COSTA, H. C.; FRANCO, F. L.; GIRAUDO, A.; GONZALEZ, R. C.; GUEDES, T.; HOOGMOED, M. S.; MARQUES, O. A. V.; MONTIGELLI, G. G.; PASSOS, P.; PRUDENTE, A. L. C.; RIVAS, G. A.; SANCHEZ, P. M.; SERRANO, F. C.; SILVA, N. J.; STRÜSSMAN, C.; ALENCAR, J. P. S. V.; ZAHER, H.; SAWAYA, R. J.; MARTINS, M.. Atlas of Brazilian Snakes: verified point-locality maps to mitigate the Wallacean Shortfall in a megadiverse snake fauna. **South American Journal of Herpetology**, São Paulo, v.14, n.1, p.1-274, 2019. DOI: https://doi.org/10.2994/SAJH-D-19-00120.1

OLSEN, E. M.; GJØSÆTER, J.; STENSETH, N. C.. Evaluation of the use of Visible Implant tags in age-0 Atlantic cod. **North American Journal of Fisheries Management**, Bethesda, v.24, n.1, p.282-286, 2004. DOI: https://doi.org/10.1577/M02-190

OTIS, D. L.; BURNHAM, K. P.; WHITE, G. C.; ANDERSON, D. R.. **Statistical inference from capture data on closed animal populations**. Wildlife Monographs, Hoboken, v.62, p.3-135, 1978.

PINTO R. R.; FERNANDES, R.; OTAVIO, A. V. M.. Morphology and diet of two sympatric colubrid snakes, Chironius flavolineatus and Chironius quadricarinatus (Serpentes: Colubridae**). Amphibia-Reptilia**, v.29, n.2, p.149–160, 2008. DOI: https://doi.org/10.1163/156853808784125027

PINTO, R. R.; MARQUES, O. A. V.; FERNANDES, R.. Reproductive Biology of two simpatric colubrid snakes, Chironius flavolineatus and Chironius quadricarinatus, from the Brazilian Cerrado domain. **Amphibia-Reptilia**, v.31, n.1, p.463-473, 2010. DOI: https://doi.org/10.1163/017353710X518423

ROSE, J. P.; WYLIE, G. D.; CASAZZA, M. L.; HALSTEAD, B. J.. Integrating growth and capture–mark–recapture models reveals size-dependent survival in an elusive species. **Ecosphere**, Washington, v.9, n.8, p.1-18, 2018. DOI: https://doi.org/10.1002/ecs2.2384

SAZIMA, I.. **Um estudo de biologia comportamental de jararaca, Bothrops jararaca, com uso de marcas naturais**. Memórias do Instituto Butantan, Vila Butantan, v.50, n.3, p.83-99, 1998.

SILVA, R. C. C.; FREITAS, M. A.; SANT'ANNA, S. S.; SEIBERT, C. S**.. Serpentes no Tocantins** – Guia ilustrado. 1 ed. São Paulo: Brazil, 2019.

WALSH, M. G.; WINKLEMAN, D. L.. Anchor and Visible Implant Elastomer tag retention by hatchery rainbow trout stocked into na Ozark stream. **North American Journal of Fisheries Management**, Bethesda, v.24, p.1435–1439, 2004. DOI: https://doi.org/10.1577/M03-246.1

WINNE, C. T.; WILLSON, J. D.; ANDREWS, K. M.; REED, R. N.. Efficacy of marking snakes with disposable medical cautery units. **Herpetological Review**, v.37, n.1, p.52-54, 2006.

WOODS, C. M. C.; MARTIN-SMITH, K. M.. Visible Implant Fluorescent Elastomer tagging of the big-bellied seahorse, Hippocampus abdominalis. **Fisheries Research**, v.66, p.363–371, 2004. DOI: https://doi.org/10.1016/S0165-7836(03)00183-8