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Article

A funnel trap adapted for sampling semi-aquatic snakes and cylinder-shaped fish in densely vegetated aquatic environments

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Abstract: Aquatic environments are among the most difficult environments to sample, and this reflects in the limited knowledge about the biology of some aquatic organisms. Adapting existing collection methods or creating new equipment is thus essential for collecting new species or increasing knowledge about habitats that have not yet been properly sampled. Given this context, this study proposes an adaptation of the terrestrial funnel trap and the covo-fish trap used in aquatic environments. This new trap, called aquatic funnel trap, allows the capture of both semi-aquatic and aquatic organisms. Its efficiency was tested between June, 2011 and April, 2012 (during the dry and rainy seasons) in five first and second order streams. The trap proved to be efficient both in the capture of semi-aquatic snakes and of *Synbranchus marmoratus*, a fish species largely undersampled due to the lack of specific equipment.

Keywords: Aquatic funnel trap; Sampling effort; Rarefaction curve; Semi-aquatic snakes; *Synbranchus marmoratus*.

Resumo: Ambientes aquáticos estão entre os ambientes mais difíceis de serem amostrados, e isso reflete no conhecimento limitado sobre a biologia de alguns organismos aquáticos. Adaptar métodos de coleta existentes ou criar novos equipamentos é essencial para coletar novas espécies ou aumentar o conhecimento sobre habitats que ainda não foram adequadamente amostrados. Diante desse contexto, este estudo propõe uma adaptação da armadilha terrestre tipo funil e da armadilha para peixes tipo covo utilizadas em ambientes aquáticos. Essa nova armadilha, chamada de armadilha aquática tipo funil, permite a captura tanto de organismos semiaquáticos quanto aquáticos. Sua eficiência foi testada entre junho de 2011 e abril de 2012 (durante as estações seca e chuvosa) em cinco riachos de primeira e segunda ordem. A armadilha mostrou-se eficiente tanto na captura de serpentes semiaquáticas quanto de Synbranchus marmoratus, uma espécie de peixe amplamente subamostrada devido à falta de equipamento específico.

Palavras-chave: Armadilha aquática tipo funil; Esforço de amostragem; Curva de rarefação; Serpentes semiaquáticas; *Synbranchus marmoratus*.

1. Introduction

The Amazon is considered the most diverse tropical forest on earth (Silva *et al.*, 2005), but the loss and fragmentation of natural habitats caused by deforestation and forest-burning are among the main threats for preserving biodiversity in the region (Fonseca *et al.*, 2019). To minimize the negative impacts from human actions and maximize biodiversity preservation, it is important to collect data in the field, which allows generating information on species distribution, preferably in places or habitats yet to be studied, to provide subsidies for actions aimed at biodiversity conservation (Frederico *et al.*, 2014).

Despite the great effort employed in recent years in describing new species for different biological groups, we still lack studies on biodiversity for much of the Amazon (Antonelli *et al.*, 2018). This is due, among other factors, to its geographical extent, its high diversity, hard-to-sample environments, and methodological limitations. All this requires constant improvements and innovations in collection methods, as a result, combining different capture techniques is key to assist in sampling processes, allowing the evaluation of species diversity of understudied sites (Duarte *et al.*, 2019).

In the Amazon, aquatic environments are the most challenging to sample among the studied environments due to the vast hydrographic network and the dimensions of Amazonian rivers basin (Lessmann *et al.*, 2016). Despite the increased sampling effort for some groups such as fish (Jézéquel *et al.*, 2020), the aquatic herpetofauna remains undersampled, and the most commonly used methodological procedures for sampling this group are pitfall traps, effective in capturing amphibians and terrestrial reptiles such as lizards and frogs (Greenberg *et al.*, 1994; Bernarde and Abe, 2006), and funnel traps, commonly used to capture terrestrial snakes (Thompson and Thompson, 2007). Techniques have been proposed for capturing aquatic vertebrates such as turtles (Vogt, 1980), snakes and salamanders (Luhring and Jennison, 2008). Although all these methods are effective, some escape rates have been observed, particularly for aquatic snakes (Willson *et al.*, 2005).

Due to habitat variability and species characteristics and habits, new collection gears are constantly being proposed with the aim of increasing sample efficiency and obtaining a better representation of different biological groups (Ribeiro and Zuanon, 2006; Thompson and Thompson, 2007; Luhring and Jennison, 2008). Proposing new methods or adaptations to existing ones is made considering their efficiency, the most cost-effective material for preparing traps and limiting factors, such as size and weight of the devices, that can hinder the transport and use of the equipment.

In the Amazon, there are about 150 snake species (Bernardes et al., 2012), and only a small proportion from the genera *Erythrolamprus* (tree species), *Eunectes* (one species), *Helicops* (four species), *Hydrops* (two species), *Hydrodynastes* (two species), *and Micrurus* (nine species) may be considered aquatic or semi-aquatic (Best, 2012), and some of the species from the *Micrurus* genus are medically significant. As for fish, there are 2716 fish species (Dagosta and de Pinna, 2019), and only a few of them exhibit a cylindrical body shape. Among these, the most common are from the families Ertytrinidae (19 species) (Guimarães *et al.*, 2022) and Synbranchidae (three species) (Sabaj *et al.*, 2022). Some species from these families are exploited as bait for sports and commercial fishing.

In the present study, we propose an adapted methodology to capture semi-aquatic snakes and cylinders-shaped fishes in environments with dense aquatic vegetation and evaluate its efficiency in the wet and dry seasons. More precisely, we posed two research questions: (i) Is the aquatic funnel trap effective for capturing semi-aquatic snakes and cylinders-shaped fish species?; and (ii) Is the new trap efficient during dry and rainy periods??

2. Material and Methods

2.1 Aquatic funnel trap (AFT)

The trap proposed here is an adaptation of the “covo-fish trap” method used for fish collection (Ribeiro and Zuanon, 2006) and the funnel trap used for terrestrial snake collection (Thompson and Thompson, 2007). We designed and built its structure based on informal conversations with local amateur fishermen who use similar traps, made with PET bottles to capture cylindrical shape-fishes, mainly *Synbranchus marmoratus* (Bloch, 1795) (locally known as "mussum") in environments with dense aquatic vegetation. The captured individuals are marketed as live baits in the region for fishing large catfish.

The aquatic funnel trap consists of a main structure made of a PVC pipe of 10 cm in diameter and 70 cm in length. One of the ends has is a funnel-like opening of 2.5 cm in diameter. For this trap component we can use a disposable PET bottle, or some plastic container of consistent material, attaching it to the main body of the trap. At the other end, we have a trap cover, also PVC, with two hooks: one for external attachment and another, on the inside, for fixing the bait. This cover is fitted to the main body of the trap (Figure1). The cost of this trap is approximately $3.70.

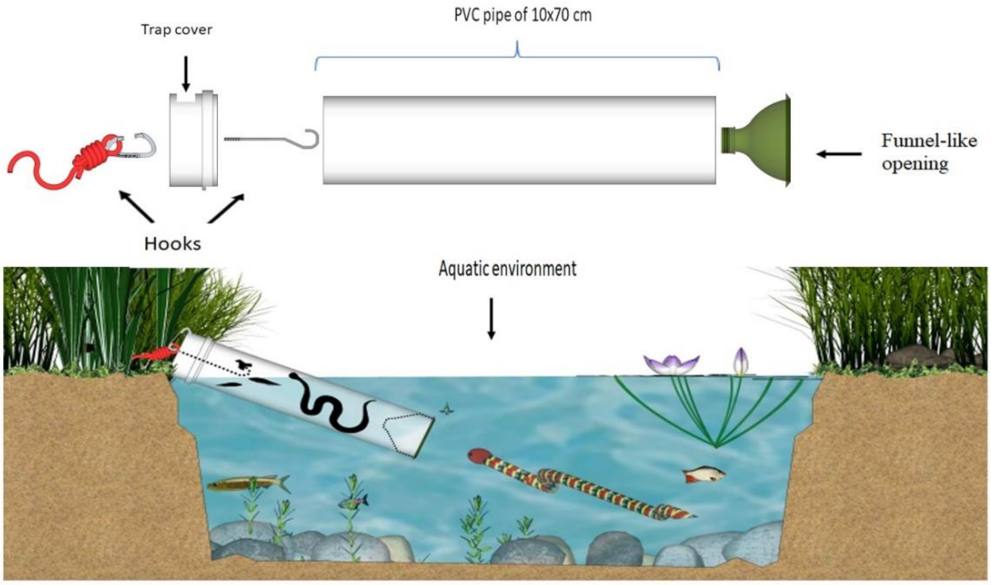

Figure 1. Different components of the Aquatic funnel trap (up) and Aquatic funnel trap installed in natural environment (bottom).

2.2 Aquatic funnel trap efficiency to capture semi-aquatic snakes and cylinders-shaped fishes

To test the Aquatic funnel trap efficiency to capture semi-aquatic snakes and cylinders-shaped fishes we conducted monthly samplings between June, 2011 and April, 2012 in five shallow, highly vegetated, and clear-water streams from Machado River, a Madeira River watershed, in the Brazilian Amazon (Table 1). Due to a problem with the aquatic funnel trap methodology, we discarded the February data, thus each stream was thus sampled for 10 months. The traps were installed in the morning and removed daily in the late afternoon, remaining exposed for 10 hours/day, for three days in each month, totaling 30 hours of sampling effort for each trap in each month. They were installed in areas containing aquatic plants, which served as a basis for fixing the structures. Its end with the funnel-like opening was placed down, leaving half of the trap out of the water so that the organisms captured could breathe and not drown (Figure 1). The traps were installed approximately 50 meters apart from each other, baited only in the morning, and the traps were checked only at the end of the day.

We installed a total of 17 traps. The number of traps and sampling effort in each locality depended on habitat availability for their installation and ranged from two to seven traps (Table 1). For example, site 1, with the highest number of baited traps (4 AFT), had a sampling effort of 600 hours during both the dry and rainy seasons, resulting in 1200 hours (4 AFT x 30 hours/months x 10 samples) of sampling; sites 3, 4 and 5, with only one trap installed each, had a sample effort of 150 hours at both seasons and resulted in 300 hours (1 AFT x 30 hours/month x 10 samples) of sampling effort (Table 1). To attract the species, we added baits with live or dead earthworms (*Lumbricina* sp.), ground beef or chicken offal in nine traps. The baits were placed inside a nylon net, tied and fixed inside the trap with galvanized metal wire. The other eight traps were installed without baits. All captured individuals were identified in the field and returned alive to the natural environment. Snakes nomenclature flowed Costa and Bérnilis (2018), while fish nomenclature followed Reis *et al*., (2003) and Fricke *et al.,* (2017). Sampling was conducted under a sample license from the Chico Mendes Institute for Biodiversity Conservation (ICMBio 33240).

Table 1. Geographical coordinates of the sites where the Aquatic Funnel Trap (AFT) was tested, number of baited and unbaited AFT and total sampling effort.

Sites	Latitude	Longitude	Aquatic funnel trap			
			Baited	Sampling effort (hours)	Unbaited	Sampling effort (hours)
Site 1	11º 41' 46.7'' S	61º 46' 23.2'' W	4	1200	3	900
Site 2	11º 41' 35.6'' S	61º 47' 46.6'' W	2	600	2	600
Site 3	11º 42' 37.2'' S	61º 48' 01.8'' W	1	300	1	300
Site 4	11º 42' 47.1'' S	61º 48' 26.6'' W	1	300	1	300
Site 5	11º 44' 08.0'' S	61º 46' 43.0'' W	1	300	1	300

2.3 Data analysis

Data from June to October (five months) were considered as dry season and data from November to April (excluding February - five months) were considered as rainy season. We combined data from the two biological groups (semi-aquatic snakes and cylinders-shaped fishes) to compute the capture rate and species richness by sites. Data from aquatic funnel traps from the same site were combined. Rarefaction curve and extrapolation specie richness (Chao1) were used to assess the aquatic funnel trap efficiency and the required sampling effort in capturing semi-aquatic snakes and cylinders-shaped fish species (Thompson *et al.,* 2007), as well as to assess if the capture rates vary between dry and rainy seasons (Gotelli and Chao, 2013). These curves were calculated using the iNext function from the iNext package (Hsieh *et al.,* 2016) for Hill numbers of q = 0 (species richness) with the maximum reference sample size (Chao *et al.,* 2014). All analyses were performed using R Program (R Core Team, 2022).

3. Results

We captured a total of 1,687 individuals, distributed in seven species. Of these, 87 individuals were from the class Reptiliia (snakes) and 1699 individuals belonged to the subclass Actinopterygii (fishes) (Table 2). Among the most captured snake species were *Helicops angulatus* (70.11%), *Micrurus lemniscatus* (19.54%), *Hydrops Triangularis* (4.59%), *Micrurus Surinamensis* (3.44%), and *Erythrolamprus taeniogaster* (2.29%). Among fish species, we captured only *Synbranchus marmoratus* (94.45%) and *Hoplias malabaricus* (5.55%) (Table 2).

Table 2. Semi-aquatic snakes and cylinder-shaped fishes captured using funnel trap in five streams during dry and rainy seasons.

Phylum/Class/Order/Family/Species	Dry	Rainy	Total
Phylum Chordata			
Class Reptiliia			
Order Squamata			

Family Colubridae			
Erythrolamprus taeniogaster (Jan, 1863)	1	1	2
Helicops angulatus (Linnaeus, 1758)	21	53	61
Hydrops triangularis (Wagler in Spix, 1824)	3	1	4
Family Elapidae			
Micrurus lemniscatus (Linnaeus, 1758)	2	15	17
Micrurus surinamensis (Cuvier, 1817)	0	3	3
Class Actinopterygii			
Order Characiformes			
Family Erithrinidae			
Hoplias malabaricus (Bloch, 1794)	3	9	12
Order Synbranchiformes			
Family Synbranchidae			
Synbranchus marmoratus (Bloch, 1795)	698	989	1687

Among the 17 traps installed, only the baited traps succeeded in capturing individuals. Thus, having some kind of lure inside the device is key for its effective operation. Although the number of species captured differed between the five sites sampled, the capture rate was similar between the five streams (Figure 2). Except for site 4, where extrapolation shows no trend of stabilization, in all other locations the sampling of the traps showed limitations in the capture of new species (Figure 2).

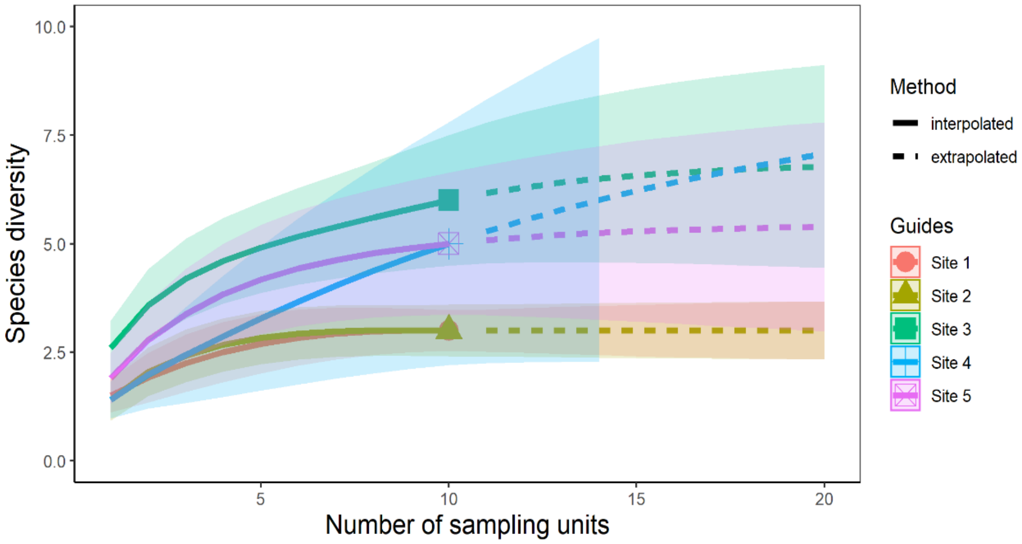

Figure 2. Smoothed rarefaction and extrapolation curve of sites. X-axis is the number of sampling units (months) and the y-axis is the observed number of species. The solid curve represents the rarefaction curve interpolated from the reference sample, and the dashed curve the extrapolation, which extends to a minimum asymptotic estimator (Chao1) of seven species.

Although the number of baited traps and sampling effort were higher in site 1 (4 AFT and 1200 hours) and site 2 (2 AFT and 600 hours) when compared to sites 3, 4 and 5 (1 AFT and 150 hours), the number of species sampled in the first two was lower than in the latter (Figure 2), showing that the trap captures can vary with the site sampled.

The rarefaction curve and the richness estimator showed that species richness is similar between the two seasons and that AFT is efficient both in the rainy and dry seasons (Figure 3). Although the trap captured five species of semi-aquatic snakes, only two fish species were captured, showing efficaciousness for capturing predatory fish with cylindrical body shape, such as *S. marmoratus* and young individuals of *H. malabaricus*.

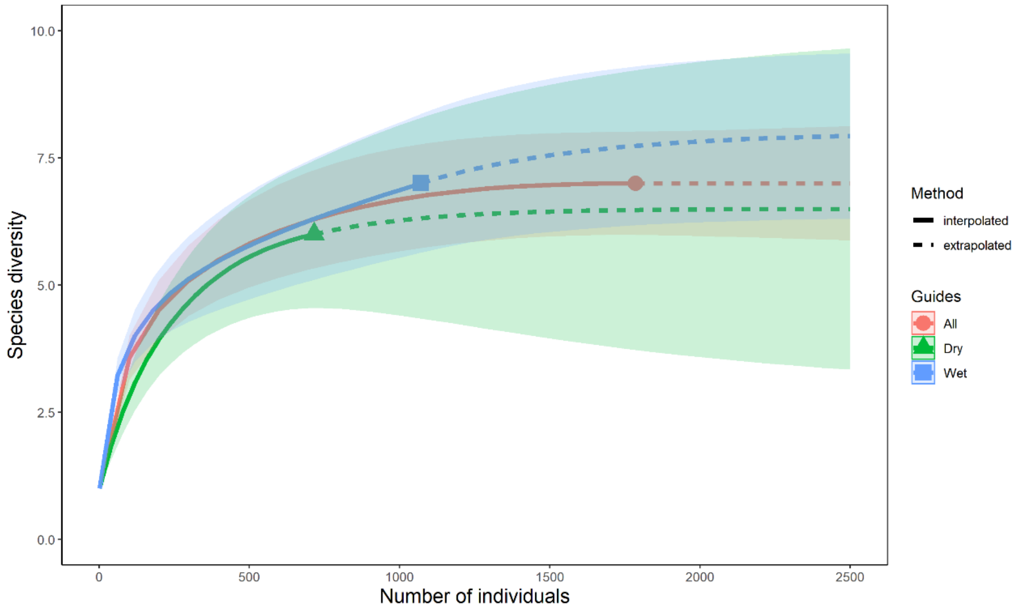

Figure 3. Smoothed rarefaction and extrapolation curve of dry (6 species and 698 individual) and rainy (seven species and 989 individuals) seasons. X-axis is the number of individuals and the y-axis is the observed number of species. Total collection (the reference sample, filled circle) included seven species and 1,687 individuals. The solid curve represents the rarefaction curve interpolated from the reference sample, and the dashed curve the extrapolation, which extends to a minimum asymptotic estimator (Chao1) of seven species.

4. Discussion

The aquatic funnel trap proved to be efficient for capturing some semi-aquatic snakes and the cylinders-shaped fish species *S. marmoratus*; but the trap showed a slight variation between sampled sites and part of its effectiveness depends on the animals' seasonal activities, since we had a small difference in the number of species and individuals captured during the period of greater rainfall. The slightly lower capture rate of species during the dry season may be related to the characteristics of the studied environment, as small-order streams tend to drastically reduce water volume during the dry season, thus reducing the availability of aquatic habitat. Thus, our results showed that sampling that considers information both dry and rainy season is more efficient (Luhring and Jenninon, 2008).

The aquatic funnel trap proposed in this work showed efficiency in capturing snakes with semi-aquatic habits, just as the funnel trap was efficient for capturing terrestrial species (Campbell and Christman, 1981; Greenberg *et al.*,1994; Cechin and Martins, 2000). While pitfall traps have high installation costs (Thompson and Thompson, 2007) and are unfeasible in flooded environments, the aquatic funnel trap has low production costs (about $3.70), easy installation and allows sampling of aquatic environments, such as areas with dense aquatic vegetation. This type of environment is among the most difficult to sample, especially when active collection methods are used.

S. marmoratus is a fish species widely distributed throughout South America (Reis *et al.*, 2003), but rare in sampling, except in some studies of fish associated with aquatic plant beds that utilize active sampling methods such as sieves (Baginski *et al.*, 2007). One reason for the low sampling of this species in ecological studies is its preference for habitats associated with aquatic plants, such as macrophyte beds (Bulla *et al.*, 2011), and the limitation of the equipment used for fish collection (e. g., gillnets, castnet, and trawlnet), which hinders capturing this species (Zajicek and Wolter, 2018). However, our results showed that aquatic funnel trap is an efficient trap for sampling *S. marmortus*.

The limitation of the trap to capture other semi-aquatic snakes or fish species may be due to the diameter of the funnel-like opening used (Abensperg-Traun and Steven, 1995). Traps with small openings can limit the entry of large-bodied individuals or allow only the capture of small-bodied species, which can limit the number of species captured by the aquatic funnel trap (Uieda and Castro, 1999). Additionally, Ribeiro and Zuanon (2006) showed that, when set for a long period of time, fish escape and predation are two limitations of passive methods such as the aquatic funnel trap. The predation was not quantified in this study, but all fish species and semi-aquatic snakes captured were predators (Bernades and Abe, 2010; Montenegro *et al.*, 2012; Montenegro *et al.*, 2013). Thus, since the traps were checked only at the end of the day, small fish species might have been preyed upon inside the trap, which could explain why they were not detected.

5. Conclusion

In conclusion, the aquatic funnel trap was found to be effective for sampling both semi-aquatic snakes and *Synbranchus marmoratus* fish. These species are often undersampled. Snakes because most collection traps are designed for terrestrial environments, and fish because the collection gear used capture species with cylindrical and slippery body shapes. In this way, the use of this trap in fauna survey studies can contribute to more accurate inventories and, consequently, assist in the conservation of these species.

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