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**PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA**

**INFLUÊNCIA DO MÉTODO DE AMOSTRAGEM NAS ESTIMATIVAS DE  
DETECÇÃO E OCUPAÇÃO DE PEIXES ELÉTRICOS (GYMNOTIFORMES) EM  
IGARAPÉS DA RESERVA DUCKE, AMAZÔNIA CENTRAL**

**ANDRÉ LUIZ RAMOS HOLANDA DE ANDRADE**

Manaus, Amazonas

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André Luiz Ramos Holanda de Andrade

Influência do método de amostragem nas estimativas de detecção e ocupação de peixes elétricos (Gymnotiformes) em igarapés da Reserva Ducke, Amazônia Central

Orientador: Dr. Jansen Zuanon

Coorientador: Dr. Gonçalo Ferraz

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Dra Cristhiana Paula Röpke	INPA-BADPI	Aprovado
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### **Sinopse:**

Estudou-se as diferenças nas probabilidades de detecção e de ocupação de habitats por peixes Gymnotiformes em igarapés da Reserva Ducke, em Manaus, AM. Comparou-se essas probabilidades obtidas com e sem o uso de detectores de sinais elétricos, em séries de dados obtidos por meio de visitas sequenciais aos mesmos trechos de igarapés, entre os anos de 2005 e 2015.

**Palavras-chave:** detector de sinais elétricos, igarapé, modelagem ecológica, fatores ambientais

*Em memória de José Holanda de Andrade Sobrinho, meu pai,  
de quem herdei o amor a todas as formas de vida.*

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Agradeço primeiramente aos meus pais pelas lições de vida e apoio às minhas escolhas quando estas eram boas e faziam sentido para eu alcançar um futuro promissor.

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“Chuang Tzu e Hui Tzu  
Atravessavam o rio Hao pelo açude.

Disse Chuang:  
‘Veja como os peixes pulam e correm tão livremente.  
Isto é a sua felicidade’.

Respondeu Hui:  
‘Desde que você não é um peixe,  
Como sabe o que torna os peixes felizes?’

Chuang respondeu:  
‘Desde que você não é eu,  
Como é possível que saiba que eu não sei  
O que torna os peixes felizes?’

Hui argumentou:  
‘Se eu não sendo você,  
Não posso saber o que você sabe,  
Daí se conclui que você,  
Não sendo peixe,  
Não pode saber o que eles sabem’.

Disse Chuang:  
‘Um momento:  
Vamos retornar à pergunta primitiva.  
O que você me perguntou foi  
‘Como você sabe o que torna os peixes felizes?’  
Dos termos da pergunta  
Você sabe evidentemente que eu sei  
O que torna os peixes felizes.

Conheço as alegrias dos peixes no rio,  
Através da minha própria alegria,  
À medida que vou caminhando à beira do mesmo rio”.

(“A alegria dos peixes”. A via de Chuang Tzu, Thomas Merton, p. 126-127).

## Resumo

O registro da ocorrência de espécies em um dado habitat nem sempre é uma tarefa simples, pois está sujeito a falhas no processo de detecção. Peixes da ordem Gymnotiformes podem ser de difícil detecção, pois apresentam hábitos noturnos e criptobióticos. Por outro lado, Gymnotiformes emitem sinais elétricos, que podem ser captados com uso de um detector de sinais elétricos. Todavia, amostragens de peixes raramente incluem o uso do detector, podendo subestimar sua abundância e diversidade. Este estudo compara as probabilidades de detecção e de ocupação de habitats de Gymnotiformes em igarapés da Reserva Ducke, na Amazônia Central Brasileira, com e sem o uso de detectores de sinais elétricos, e avalia a influência de variáveis ambientais em tais probabilidades. Foram utilizados modelos de ocupação de sítios *single species*, *single season*, com a seleção de modelos baseada no critério de informação de Akaike. Amostragens foram realizadas em 31 igarapés em 2005, 2006 (2x), 2011 e 2015 (2x). O detector de sinais elétricos aumenta a probabilidade de detectar um gymnotiforme em quase 10 vezes, o que pode afetar fortemente as conclusões sobre riqueza de espécies e composição ictiofaunísticas em igarapés. Os principais fatores que interferem na detecção dos Gymnotiformes são o tipo de substrato e a velocidade da correnteza. Concluimos que o uso de detector de sinais elétricos é fundamental para a obtenção de estimativas mais confiáveis de abundância de espécies de Gymnotiformes em igarapés.

**Palavras-chave:** Detectabilidade, Modelos ecológicos, Detector de sinais elétricos, Fatores ambientais, Riachos de “terra firme”.



## **Abstract**

### **Influence of the sampling methodology on the probability of detection and occupancy of electric fish (Ostariophysi: Gymnotiformes) in streams of Ducke Reserve, Central Amazon**

Recording species occurrence is not always a simple task, because it is subject to flaws in the detection. Fishes of the Gymnotiformes can be difficult to detect, since they have nocturnal and cryptobiotic habits. On the other hand, gymnotiforms can send electrical signals, which can be detected using electrical signal detectors. However, fish sampling rarely includes the use of such detectors, which may underestimate its abundance and diversity. This study compares probabilities of detection and habitat occupancy of Gymnotiformes in streams of Ducke Reserve, Brazilian Amazon, from samplings with and without the use of electrical detectors, and evaluates the influence of environmental variables on such probabilities. Single species, single season models were employed, with model selection based on Akaike Information Criterion. Samplings were done in 31 streams in 2005, 2006 (twice), 2011, and 2015 (twice). Electrical signal detector increased the probability of finding a gymnotiform by nearly 10 times, which may strongly affect conclusions about species richness and species composition in streams. The main factors interfering with gymnotiform detection were substrate type and current velocity. We conclude that the use of electrical signal detector is of foremost importance to generating accurate estimates of abundance of Gymnotiformes in streams.

**Keywords:** Detectability, Ecological modelling, Electric signal detector, Environmental factors, Forest creek.

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## Apresentação

Estimativas da proporção de área ocupada por uma espécie (ou taxa de ocupação) começaram a surgir nos trabalhos científicos diante do longo prazo que levantamentos biológicos podem levar para mapear todas as espécies de uma área. Por não haver a necessidade da captura efetiva do material biológico para análise e identificação, mas apenas registros da presença como rastros, sons e outras evidências, as estimativas de ocupação não são um substituto para levantamentos mais extensos, mas podem ser um instrumento válido para se ter uma primeira noção dos organismos que se encontram em um determinado ambiente (Mackenzie e Nichols, 2004).

Entretanto, a tarefa de registrar a presença de organismos na natureza pode não ser fácil, pois eles podem ocorrer no local e não serem detectados (Mackenzie *et al.*, 2006). De fato, muitas espécies desenvolveram estratégias de ocultação no meio ambiente, seja como estratégia de defesa ou ataque (Edmunds, 1974), que as tornam pouco perceptíveis para outros organismos, incluindo seres humanos. As estratégias podem se refletir tanto na morfologia corporal como na fisiologia e padrão de atividade do animal (Kronfeld-Schor e Dayan, 2003). Uma ordem de peixes neotropicais em especial, Gymnotiformes, apresenta um padrão de atividade noturna e possui uma característica fisiológica admirável: pode gerar e detectar campos elétricos, o que os permite perceber com precisão o ambiente e organismos ao redor, mesmo na escuridão (Albert e Crampton, 2005). As descargas elétricas produzidas pelos Gymnotiformes podem ser convertidas em sons por aparelhos especializados, como detectores de sinais elétricos, os quais têm sido usados em estudos ictiofaunísticos diversos.

Em estudos de ecologia de comunidades, é importante que as amostragens representem adequadamente todas as espécies presentes nos ambientes amostrados, de forma a não enviesar análises quali- e quantitativas baseadas nas relações de abundância entre as espécies. Neste sentido, e considerando as dificuldades específicas envolvidas nas amostragens de peixes Gymnotiformes, é possível que a riqueza, diversidade e abundância relativa das espécies desse grupo de peixes estejam sendo subestimadas nos estudos que não têm utilizado detectores de sinais elétricos (Rangel Pereira, 2014). No presente estudo, buscamos comparar a eficiência de amostragens de peixes Gymnotiformes em riachos da Reserva Florestal Adolpho Ducke, em Manaus, com e sem o uso do detector de sinais elétricos. Além disso, avaliamos as estimativas de detecção e ocupação desses peixes por meio dos dois métodos de amostragem (com e sem

detector de sinais elétricos), durante visitas sequenciais a trechos de riachos. Finalmente, buscamos avaliar quais fatores ambientais podem influenciar a detectabilidade das espécies mais comuns e abundantes de Gymnotiformes nos riachos.

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## **Objetivos**

### **Objetivo geral**

Comparar a eficiência de amostragens de peixes Gymnotiformes em riachos amazônicos com e sem o uso do detector de sinais elétricos.

### **Objetivos específicos**

1. Avaliar as estimativas de detecção e ocupação desses peixes nos dois métodos de amostragem (com e sem detector de sinais elétricos), por meio de visitas sequenciais a trechos de riachos.
2. Avaliar quais fatores ambientais podem influenciar a detectabilidade das espécies mais comuns e abundantes nos riachos.

## Capítulo 1

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electric fish (Gymnotiformes) in streams of  
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*Neotropical Ichthyology*.

**Influence of the sampling methodology on the probability of detection and occupancy of electric fish (Ostariophysi: Gymnotiformes) in streams of Ducke Reserve, Central Amazon**

André Luiz R. H. de Andrade<sup>1</sup>, Jansen Zuanon<sup>2</sup> and Gonçalo Ferraz<sup>3</sup>

<sup>1</sup>Programa de Pós-Graduação em Ecologia, Instituto Nacional de Pesquisas da Amazônia, Avenida André Araújo, 2936, Manaus, AM, Brazil. 69067-375. E-mail: andrerhandrade@gmail.com (corresponding author)

<sup>2</sup>Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Avenida André Araújo, 2936, Manaus, AM, Brazil. 69080-971. E-mail: jzuanon3@gmail.com

<sup>3</sup>Departamento de Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves, 9500, Porto Alegre, RS, Brazil. 91509-900. E-mail: gferraz29@gmail.com

**Abstract**

Recording species occurrence is not always a simple task, because it is subject to flaws in the detection. Fishes of the Gymnotiformes can be difficult to detect, since they have nocturnal and cryptobiotic habits. On the other hand, gymnotiforms can send electrical signals, which can be detected using electrical signal detectors. However, fish sampling rarely includes the use of such detectors, which may underestimate its abundance and diversity. This study compares probabilities of detection and habitat occupancy of Gymnotiformes in streams of Ducke Reserve, Brazilian Amazon, from samplings with and without the use of electrical detectors, and evaluates the influence of environmental variables on such probabilities. Single species, single season models were employed, with model selection based on Akaike Information Criterion. Samplings were done in 31 streams in 2005, 2006 (twice), 2011, and 2015 (twice). Electrical signal detector increased the probability of finding a gymnotiform by nearly 10 times, which may strongly affect conclusions about species richness and species composition in streams. The main factors interfering with gymnotiform detection were substrate type and current velocity. We conclude that the use of electrical signal detector is of foremost importance to generating accurate estimates of abundance of Gymnotiformes in streams.



## Resumo

O registro da ocorrência de espécies em um dado habitat nem sempre é uma tarefa simples, pois está sujeito a falhas no processo de detecção. Peixes da ordem Gymnotiformes podem ser de difícil detecção, pois apresentam hábitos noturnos e criptobióticos. Por outro lado, Gymnotiformes emitem sinais elétricos, que podem ser captados com uso de um detector de sinais elétricos. Todavia, amostragens de peixes raramente incluem o uso do detector, podendo subestimar sua abundância e diversidade. Este estudo compara as probabilidades de detecção e de ocupação de habitats de Gymnotiformes em igarapés da Reserva Ducke, na Amazônia Central Brasileira, com e sem o uso de detectores de sinais elétricos, e avalia a influência de variáveis ambientais em tais probabilidades. Foram utilizados modelos de ocupação de sítios *single species*, *single season*, com a seleção de modelos baseada no critério de informação de Akaike. Amostragens foram realizadas em 31 igarapés em 2005, 2006 (2x), 2011 e 2015 (2x). O detector de sinais elétricos aumenta a probabilidade de detectar um gymnotiforme em quase 10 vezes, o que pode afetar fortemente as conclusões sobre riqueza de espécies e composição ictiofaunísticas em igarapés. Os principais fatores que interferem na detecção dos Gymnotiformes são o tipo de substrato e a velocidade da correnteza. Concluimos que o uso de detector de sinais elétricos é fundamental para a obtenção de estimativas mais confiáveis de abundância de espécies de Gymnotiformes em igarapés.

**Keywords:** Detectability, Ecological modelling, Electric signal detector, Environmental factors, Forest creek.

**Running Head: Sampling methods and detection of electric fishes**

## Introduction

The task of finding species in nature might not be easy, and very often even when they occur in a region they are not detected (Mackenzie *et al.*, 2002, 2006). Occurrence or occupancy refers to the presence or absence of the species in a particular location (Mackenzie & Nichols, 2004; Mackenzie *et al.*, 2006). Detectability is an aspect of the sampling, i.e., the probability of detecting the species when it is present (Mackenzie *et al.*, 2002; Royle & Nichols, 2003; Mackenzie *et al.*, 2006). The detection probabilities vary among species, and may be influenced by many factors including the species' habits (Nichols *et al.*, 2007; Lele *et al.*, 2012). Many species have developed strategies to conceal themselves in the environment, either as a defense or as an attack strategy (Edmunds, 1974). Strategies employed by many cryptic species may be reflected in both body morphology and physiology, and in the animal's activity pattern (Kronfeld-Schor & Dayan, 2003).

The knife fishes of the order Gymnotiformes show a pattern of nocturnal activity and have a remarkable physiological characteristic: they can generate and detect electric fields, which allow them to perceive the environment and organisms around, even in complete darkness (Heiligenberg, 1973; Albert & Crampton, 2005). The Gymnotiformes occur in all major Neotropical river basins, from Argentina to southern Mexico (Reis *et al.*, 2003). The Amazon region is home to the greatest diversity of Gymnotiformes, housing as many as 80% of all known species (Crampton, 1996; Albert & Reis, 2011; Tagliacollo *et al.*, 2016).

Knife fishes stay almost inactive and sheltered during the day. Several species use submerged leaf litter, macrophyte stands, and roots of plants of the riparian zone, whereas others bury in the substrate (Albert & Crampton, 2005; Sazima *et al.*, 2006; Zuanon *et al.*, 2015). Some species hide so deep in the substrate that it is almost impossible to carry out adequate quantitative samplings using typical fish sampling gear, like hand nets, sieves and seine nets (Mendonça *et al.*, 2005; Rangel-Pereira, 2014).

All species of Gymnotiformes have specialized organs called electrocytes capable of producing weak (in the millivolt range) electrical discharges, which function in detecting objects in the environment (electrolocalization) and for intra- and interspecific communication (electrocommunication) (Hopkins & Heiligenberg, 1978; Moller, 1995; Kramer, 1996; Hopkins, 1999; Zuanon *et al.*, 2015). The electrical discharges produced by Gymnotiformes can be converted into sound by specialized devices, such as electrical signal detectors (hereinafter, detector). These detectors consist of a portable amplifier equipped with a speaker and connected to a cable with electrodes arranged in the distal end of a pole (Crampton *et al.*, 2007). Gymnotiform fishes have specific electrical discharge patterns, allowing the identification of individuals at species level (or groups of closely related species). However, these patterns can be modified along ontogeny, life cycle (e.g. during reproduction) and in response to abiotic factors (Moller, 1995; Crampton, 1998), which sometimes may difficult species identification.

In ecological studies of communities, it is important that the sample adequately represent all the species present in the environments, so as not to skew qualitative and quantitative analysis (Eckblad, 1991). In addition, considering the difficulties involved in Gymnotiformes sampling in streams using common fishing gear, it is possible that the richness, diversity, and relative abundance of species of this group of fish are being underestimated in studies conducted without signal detectors (Mendonça *et al.*, 2005; Rangel-Pereira, 2014). In the present study, we sought to compare the efficiency of Gymnotiformes sampling in Amazon streams with and without the use of detectors. In addition, we evaluate the estimates of occupancy and

detection of these fish in the two sampling methods (with and without detector) through sequential visits to stream stretches. Finally, we aimed to evaluate what environmental factors may influence the detectability of the most abundant gymnotiform species in streams.

### **Material and Methods**

#### **Study area and sampling methodology**

We sampled for Gymnotiformes in 50 m stretches of 31 headwater streams located within the Reserva Florestal Adolpho Ducke (hereinafter, Ducke Reserve), Manaus, Amazonas state, Brazil (02° 55 'and 03° 01' S; 59° 53 'and 59° 59' W; Fig. 1). The reserve has a central plateau that runs in the north-south direction, dividing two river microbasins (Oliveira *et al.*, 2008). Each stream was visited twice between March and July 2015. The choice of sampling method in a given visit to a stream stretch (i.e. including or not the detector) was randomly assigned. After an interval of one to two weeks the same stretch was sampled with the alternative method. Samplings were performed during the day while moving in a counter current direction. When using the detector the electrodes were immersed in the water and the stream bed was scanned. When an electric fish was detected, we used a fine mesh dip net (2 mm) to collect the individual for identification. When the catch was not possible, the individual's identity was established based on the sound signal produced by the detector, which was compared with a database containing recordings of electrical discharges of all species documented in the reserve (J. A. Alves-Gomes, unpublished data). Sampling without the use of detectors was done with hand nets only, thoroughly searching the stretch for gymnotiforms. All the gymnotiforms collected in a stream stretch were maintained alive in a plastic box with water and an aerator up to the end of the sampling visit. The fishes were identified, counted and returned alive to the stream. Just a few times a specimen was euthanized in eugenol solution and preserved for taxonomical identification by direct comparison to specimens deposited at INPA's Fish Collection (voucher specimens are presented in Table 1). Environmental variables (dissolved oxygen, pH, electrical conductivity, water temperature, current velocity, channel depth and width, substrate type, and rainfall) were recorded during each visit.

(Fig. 1)

### Data analysis

We used two different data sets to answer our questions. In order to compare the abundance, species richness and composition of electric fishes in the streams we used only the data collected in 2015 (paired visits to each sampling site, performed with and without the use of detectors). This was done aiming to avoid possible differences in sampling efficiency resulting from different collectors, fishing gear, or sampling effort. We used Student's *t*-test for paired samples to compare the number of individuals and species of gymnotiforms detected in each stream stretch when sampling with and without the detector. In these analyzes, one of the samples (corresponding to the stream TI22, at Tinga stream microbasin) was excluded because it was considered as an outlier, with an abnormally high abundance of *Microsternarchus cf. bilineatus*. We applied a non-metric multidimensional scaling ordination (NMDS) to assess changes in species composition of Gymnotiformes in the samples obtained from the two sampling methods.

To compare the occupancy and detection probability with and without the use of detectors we included survey data collected from the same streams in previous years (once in 2005, twice in 2006, and once in 2011). The use of these complementary data aimed to create a capture history of all occurrences and to minimize any faulty detection in the streams. In 2005, sampling was carried out without the use of the detector, and in 2011, the sampling was undertaken using the detector. In 2006, there were two sampling occasions where we sampled with and without using detectors, similar to the sampling undertaken in 2015.

The probabilities of occupancy ( $\psi$ ) and detection ( $p$ ) of gymnotiform fishes in the streams were estimated using the *single season* site occupancy model proposed by Mackenzie *et al.* (2002, 2006). This model estimates the occupancy probability of a species accounting for non-detection. The model assumes that the occupancy of a species in the sampled sites remains constant during the study period, i.e., the population is closed to immigration and emigration. The analysis uses presence-absence data and corrects the occurrence estimates for the probability of detecting the species.

A series of environmental characteristics of the site and of the sampling occasions may have influenced the detectability of species. We carried out a correlation analysis among the environmental variables to check for possible autocorrelations. Substrate composition was represented the first three axes of a principal components analysis (PCA) based on the frequencies of occurrence of substrate types along channel transects. Substrates that contributed most to the components were sand (PC1), fine litter (PC2), and fine roots (PC3) (Supplementary material, S1) We use width of the stream, a feature of the site, as a covariate

to model detection probability (Supplemental material, S2). Additionally, we used various features of the sampling occasion including average water current velocity (flow), rainfall, water temperature, conductivity, dissolved oxygen, substrate type, and the sampling method (i.e. with and without detector). The basin (East or West) was chosen as a covariate to model the species occupancy (Supplemental material, S2).

To investigate the effects of environmental variables on the probability of detecting a species and to determine if there is a difference in the probability of occupancy between the two microbasins (east or west), we used a *single species, single season* occupancy modeling approach. To estimate the occurrence and the probability of detection of a species, we used the approach proposed by MacKenzie *et al.* (2006) which consists of estimating the probability of detection and then using it to estimate the occupation parameters (two-step, *ad hoc* estimation procedure). For this, in the first step, we created a set of a priori models to identify potential factors influencing the probability of detecting a species (Supplemental material, S3). Covariates were analyzed individually and in combination, resulting in a total of 20 models (including a null model without covariates) for each species. In the second stage we used the best detection models (selected based on the Akaike information criterion and considering the weight of each model) to correct for the detectability bias (Supplemental material, S3). We compare them with the set of occupation models. The number of competing models varied in line with the number of models selected in the detection stage.

We established two analysis criteria for models of probability of occupancy and detection. In the first criterion, we considered the two sampling occasions undertaken in 2015 as independent of the sampling undertaken in 2006, thus considering each pair of samplings as if performed at different sampling points. The time interval between the samples sets (nine years) was potentially long enough for new fish populations to have colonized the sampling sites, thus enabling us to interpret them as independent points ( $31 + 31 = 62$  stream stretches, each sampled twice). This criterion allowed the analysis of a larger number of dummy sampling sites, providing better estimates of detection. In the second criterion, we grouped the visits performed in the samplings of 2005, 2006 (twice), 2011 and 2015 (twice), and ignored the time interval between them, enabling the development of a longer history of occurrences. We chose to disregard the time in this case because the study area is a pristine environment where fish streams do not disperse enough to create a metapopulation, and there are weak annual variations in the general limnological and environmental conditions (Zuanon *et al.*, 2015). The advantage of this second criterion is the increased probability of encounter with

the species or group of species of interest with each visit. We designated the first criterion as "paired visits" and the second criterion as "sequential visits."

The selection of models was based on the Akaike Information Criterion (AIC) corrected by the number of samplings (Burnham & Anderson, 2003). Further, we computed the Akaike weights ( $\omega_i$ ), which provide information on the weight of each model, given a set of candidate models (Johnson & Omland, 2004; Wagenmakers & Farrell, 2004). The models were constructed and analyzed using the packages 'unmarked' (Fiske & Chandler, 2011) and 'AICcmodavg' (Mazerolle, 2016) in RStudio (R Core Team, 2016).

### Results

During our sampling in 2015 using the detector, we recorded, on an average,  $10.24 \pm 8.34$  sd (standard deviation) of Gymnotiformes individuals per stream (Fig. 2), whereas the average number of individuals found without the use of the detector was lower than 1 individual ( $0.79 \pm 1.32$  sd). We detected 10 species of Gymnotiformes in the streams sampled in 2015 (Table 1), belonging to four families: Gymnotidae, Hypopomidae, Rhamphichthyidae, and Sternopygidae.

(Fig. 2)

(Table 1)

The use of a detector resulted in the location of a significantly greater number of species (Fig. 2). The maximum number of species found in a stretch without the use of the detector was one, while up to five species were found when detector was used (Fig. 2). The composition of the samples obtained with the use of the detector was conspicuously more homogeneous than when sampling was done without this equipment (Fig. 3).

(Fig. 3)

*Gymnotus* (Gymnotidae) showed the highest number of species recorded in our study ( $n = 411$ , 57.8%; Table 2). Owing to the difficulty in differentiating between various species of *Gymnotus* using the sound generated by the detector, and because of the relatively large number of juvenile fishes in our samples, which are hard to identify, we grouped all the species belonging to this genus for analyses. However, it is important to mention that the most abundant species of *Gymnotus* at Ducke Reserve is *G. coropinae* Hoedeman, 1962, which

should represent the majority of the records obtained in this study (Igarapés Project, data not shown). The probability of occurrence of *Gymnotus* spp. in the stream stretches was 1.0 when the analysis considered paired visits, and 0.97 when sequential visits were considered (Table 3).

(Table 2)

(Table 3)

The second family in number of occurrences was Hypopomidae (38.1%) mainly represented by *Microsternarchus* cf. *bilineatus* Fernandez Yépez, 1968, which constituted 34.2% (269 individuals) of the occurrence records (Table 2). The probability of occupancy of *M.* cf. *bilineatus* was 0.25 when we considered paired visits and 0.32 when we considered sequential visits (Table 3). The species occurred predominantly in the streams of east side of the reserve. Twenty-one of the detected hypopomids were *Hypopygus lepturus* Hoedeman, 1962, constituting 2.7% of our sample (Table 2). The probability of occupancy of this species was 0.23 in paired visits and 0.46 in sequential visits (Table 3).

*Gymnorhamphichthys rondoni* (Miranda Ribeiro, 1930) was the only species of Rhamphichthyidae detected. We detected 22 individuals of this species, representing 2.8% of the total sample and having a probability of occurrence of 0.22 and 0.38 in paired and in sequential visits, respectively (Tables 2 and 3).

We recorded very few individuals of *Electrophorus electricus* (Linnaeus, 1766), *Steatogenys duidae* (La Monte, 1929), *Eigenmannia* aff. *macrops* (Boulenger, 1897), and *Sternopygus macrurus* (Bloch & Schneider, 1801) (Table 1), and therefore, these could not be included in the detection and occupancy analyzes.

Detection probabilities of all species analyzed were higher with the use of the detector.

*Gymnotus* spp. showed a higher probability of detection in sequential visits. In contrast, *H. lepturus*, *M.* cf. *bilineatus*, and *G. rondoni* showed detection probabilities of 1.0 in the paired visits analysis (Fig. 4, Table 4).

(Fig. 4)

(Table 4)

Most models that explain the factors that affect the detection of a species, either positively or negatively, included the type of substrate (Table 5). For *Gymnotus* spp., the proportion of

sand comprised in the substrate was a significant variable common to both paired and sequential visits models. The sand substrate showed greater weight in models that consider paired visits (20%; Table 5) compared to the models of sequential visits (6%; Table 5). However, water current showed a strong influence on the detection probability (70%) in models that used the sequential visits criterion (Table 5). The variable current velocity was included in all models for *M. cf. bilineatus*, with a higher weight for sequential visits (59%) compared to paired visits (18%), while the substrate type showed little influence in the paired visit models (Table 5).

(Table 5)

### Discussion

Our study demonstrated that the use of an electrical signal detector significantly increases the probability of detection of electric fish (Gymnotiformes) in streams, both in terms of number of species (five times) and individuals (nearly 10 times). The Gymnotiformes are a group of species commonly recorded in ichthyofaunal studies in amazonian streams (Mago-Leccia, 1994; Alves-Gomes *et al.*, 1995; Lowe-McConnel, 1999; Mendonça *et al.*, 2005), and our results indicate that traditional sampling methods using only nets grossly underestimate the abundance and species richness of these fish in stream stretches.

However, finding an electric fish using the detector does not guarantee its capture. Many of the species that inhabit streams have small body size and the habit to take refuge deeply in leaf litter, dense root tangles or in the substrate. This is the case with species of *Gymnotus*, which take refuge during the day in submerged litter packs along the banks of streams, and with *G. rondoni*, which buries deeply in the sand layer that makes up much of the substrate of the streams (Zuanon *et al.* 2006; pers. obs.). This difficulty in capturing explains, at least in part, the likely underestimation of occurrence and abundance of this group of fish in most ichthyological surveys conducted without the use of electrical signals detectors (Mendonça *et al.*, 2005; Rangel-Pereira, 2014). In fact, the ordination showed the strong effect the use of the detector had on the species composition of the samples, i.e., the use of a detector provides greater accuracy in the representation of local assemblages of Gymnotiformes in streams. Finally, the possibility of detecting the electrical discharges emitted by the fish through electronic devices in the field is an alternative that increases the reliability of the results and allows the use of less invasive techniques, with minimal disruption to the environment, which is especially relevant in environmentally protected areas, such as the Ducke Reserve.



### *Factors affecting the detection of electric fish in streams*

Upland streams do not respond in the same way to the periodic flooding cycles that take place in the large amazonian rivers and their floodplains systems (Junk *et al.*, 1989; Lowe-McConnel, 1999; Mendonça *et al.*, 2005). Those streams change little during the year; the rainy season promotes a quick local increase in flow, but the volume of water in the stream usually returns to its normal after a few hours. So, our findings can be considered applicable to stream habitats independent of the time of the year when the sampling is conducted.

Our results showed that the type of substrate was the main factor affecting the models of paired visits, and environmental variables related to hydrodynamics of streams, such as the current, mostly influence the models of sequential visits. Changes in current velocity affect the stream conditions, loading particles and leaf litter that accumulate on the banks (Christofoletti, 1980; Tundisi & Tundisi, 2013), creating shelter sites for several species of Gymnotiformes (e.g. *Gymnotus* spp., *M. cf. bilineatus*, *Hypopygus lepturus*, and *Steatogenys duidae*) during the day (Zuanon *et al.*, 2015). It is likely that a strong preference of species for certain types of substrates (Sazima *et al.*, 2006; Zuanon *et al.*, 2006) explains the results obtained.

In conclusion, comparing the probabilities of detection of the two sampling methods clearly shows that the use of the electrical signal detector significantly increases the probability of detecting Gymnotiformes in the streams of Ducke Reserve. On the other hand, sampling with only nets showed no significant variation in the detection of species between paired and sequential visits. The detection probabilities were higher in the paired visits than in sequential visits for most species, except for *Gymnotus* spp., which showed a slight increase in detectability in sequential visits. *Gymnotus* species present in the reserve (largely represented by *G. coropinae*, a species that is widely distributed in the Amazon; Crampton & Albert, 2003) are those with a more homogeneous distribution (i.e., they showed a high probability of occupancy in the sampled stream stretches). Hence, increasing the sampling effort, by either increasing the number of sampling sites or the number of visits, is unlikely to improve the already high chance of detecting this genus. On the other hand, *M. cf. bilineatus*, *G. rondoni*, and *H. lepturus* have more restricted distributions and, therefore, increasing the number of sampling sites increases the chances of finding the species at the study site.

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### Table legends

**Table 1.** Families and species of Gymnotiformes, voucher specimens, and number of individuals (N) found with (wDT) and without (wthDT) the use of detector of electrical signals during surveys conducted in 2015 at Ducke Reserve, Brazil. \*Individuals of the genus *Gymnotus* that were not identified to species-level (n= 18) or not collected (n= 197).

**Table 2.** Absolute frequency (AF) and frequency of occurrence (FO) of Gymnotiformes species detected during surveys in streams conducted in the years 2005, 2006, 2011 and 2015 at Ducke Reserve. \*Individuals of the genus *Gymnotus* that were not identified to species-level or not collected.

**Table 3.** Gymnotiformes occupancy probability estimates ( $\psi$ ) and associated standard errors (in parentheses) between paired and sequential visits (with and without the use of electrical signal detector) and between east ( $\psi_E$ ) and west ( $\psi_W$ ) basins at Ducke Reserve, Brazil.

**Table 4.** Gymnotiformes detection probability estimates ( $p$ ) and associated standard errors (in parentheses) between paired and sequential visits (with and without the use of electrical signal detector) at streams of Ducke Reserve, Brazil.

**Table 5.** Summary of model selection procedures for species of Gymnotiformes in paired and sequential visits to streams at Reserva Ducke.  $\Delta AIC_c$  is the difference in the  $AIC_c$  value of a given model and that of the best model;  $\omega_i$  is the Akaike weight (Burnham & Anderson 2002).

### Figure legends

**Fig. 1.** Map of the study area at Ducke Reserve. Black dots in the larger map represent the sampled streams. Darker gray shading indicates higher altitudes, especially the central plateau that divides the two main hydrographic basins (East and West) in the reserve.

**Fig. 2.** Number of individuals (a) and species (b) of Gymnotiformes detected in 50-m stream stretches in the visits with and without the use of electrical signal detector.

**Fig. 3.** Graphic representation of the first two axes of an ordination by Non Metric Multidimensional Scaling (NMDS) representing the Gymnotiformes species composition (presence-absence data) in 50-m stretches of 31 streams at Ducke Reserve. Black and gray dots represent samples obtained with and without the use of electrical signal detector respectively.

**Fig. 4.** Detection probabilities for the most abundant species of Gymnotiformes in samples obtained in 50-m stretches of 31 streams at Ducke Reserve. Probabilities were estimated with models for paired and sequential visits to each stream stretch, according to sampling method (with and without the use of electrical signal detector): (a) *Gymnotus* spp.; (b) *Microsternarchus* cf. *bilineatus*; (c) *Gymnorhamphichthys rondoni* and (d) *Hypopygus lepturus*.

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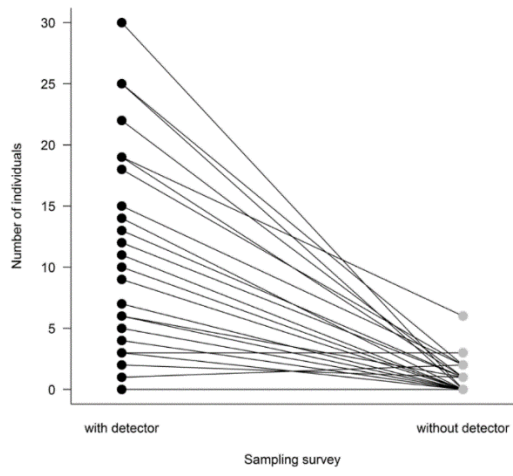
Figures

Fig. 1.

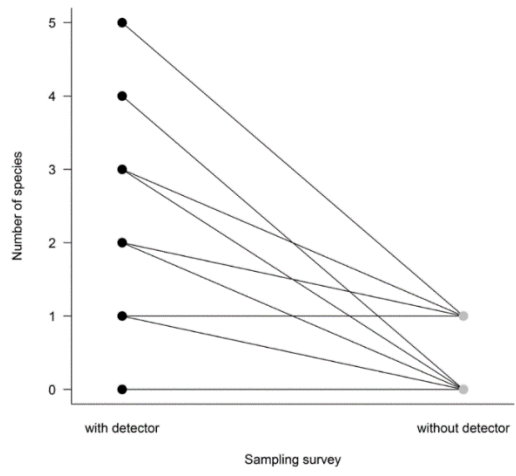




Fig. 2.



a



b

Fig. 3.

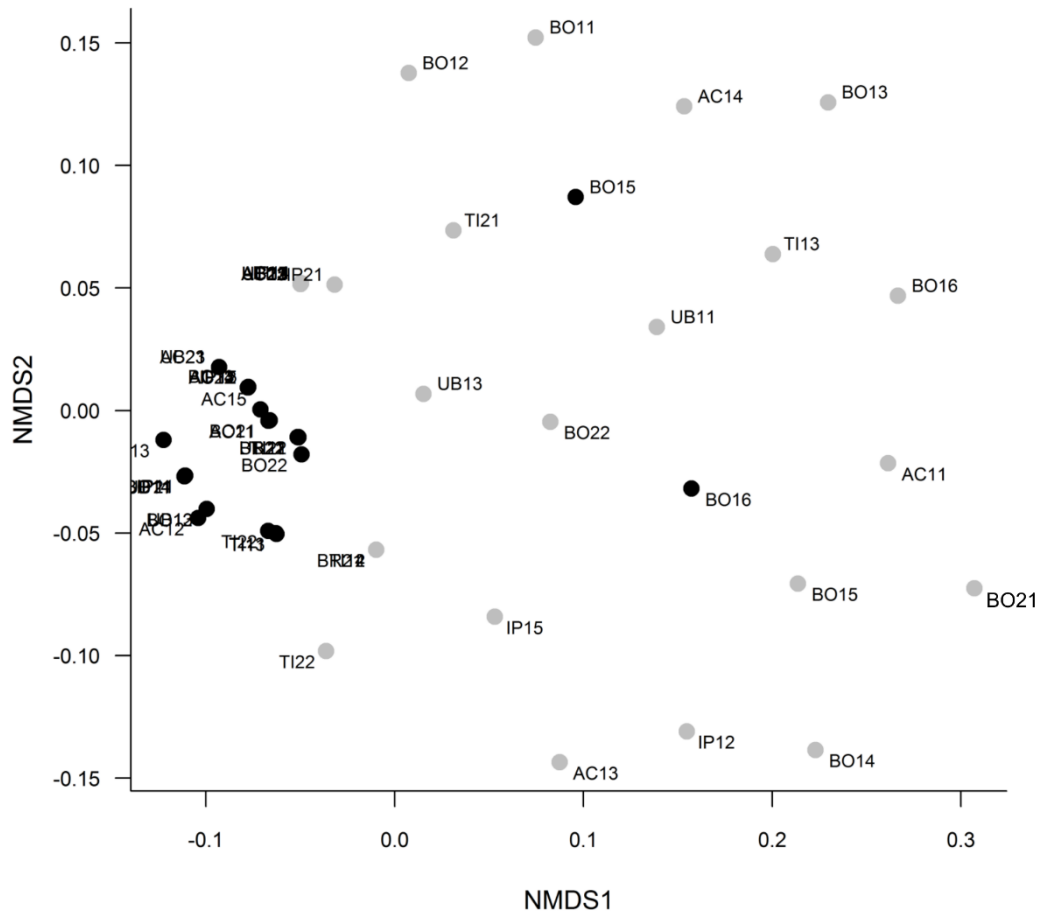
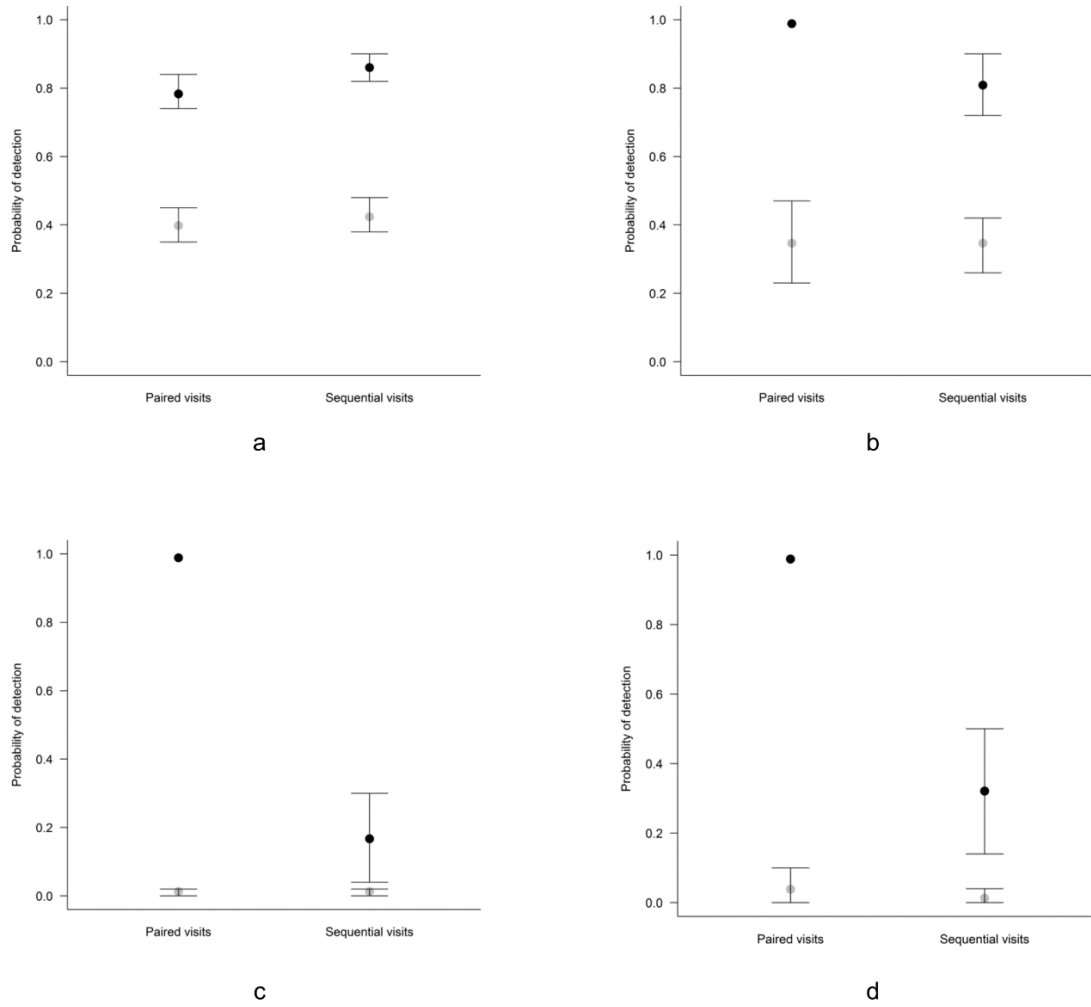


Fig. 4.



## Tables

Table 1.

Family/ Species	N (wDT)	N (wthDT)	Voucher INPA
GYMNOTIDAE			
<i>Electrophorus electricus</i> (Linnaeus, 1766)	-	1	-
<i>Gymnotus coropinae</i> Hoedeman, 1962	33	18	27810
<i>Gymnotus stenoleucus</i> Mago-Leccia, 1994	-	1	27812
<i>Gymnotus</i> spp.*	197	-	
HYPOPOMIDAE			
<i>Hypopygus lepturus</i> Hoedeman, 1962	10	-	27831
<i>Microsternarchus</i> cf. <i>bilineatus</i> Fernández Yépez, 1968	106	13	27790
<i>Steatogenys duidae</i> (La Monte, 1929)	5	-	27822
RHAMPHICHTHYIDAE			
<i>Gymnorhamphichthys rondoni</i> (Miranda Ribeiro, 1920)	18	-	27841
STERNOPYGIDAE			
<i>Eigenmannia</i> aff. <i>macrops</i> (Boulenger, 1897)	4	-	-
<i>Sternopygus macrurus</i> (Bloch & Schneider, 1801)	5	-	27911

Table 2.

Family/ Species	AF	FO
GYMNOTIDAE		
<i>Gymnotus coropinae</i>	196	24.9%
<i>Gymnotus</i> spp.*	215	27.3%
HYPOPOMIDAE		
<i>Hypopygus lepturus</i>	21	2.7%
<i>Microsternarchus</i> cf. <i>bilineatus</i>	269	34.2%
RHAMPHICHTHYIDAE		
<i>Gymnorhamphichthys rondoni</i>	22	2.8%

Table 3.

Family/Species	Paired visits			Sequential visits		
	$\psi$	$\psi_E$	$\psi_W$	$\psi$	$\psi_E$	$\psi_W$
GYMNOTIDAE						
<i>Gymnotus</i> spp.	1.0	1.0	0.98 (0.02)	0.97 (0.03)	1.0	0.94 (0.06)
HYPOPOMIDAE						
<i>Hypopygus lepturus</i>	0.23 (0.07)	0.19 (0.09)	0.28 (0.11)	0.46 (0.24)	0.34 (0.22)	0.59 (0.33)
<i>Microsternarchus</i> cf. <i>bilineatus</i>	0.25 (0.05)	0.46 (0.09)	0.03 (0.03)	0.32 (0.08)	0.57 (0.12)	0.07 (0.06)
RHAMPHICHTHYIDAE						
<i>Gymnorhamphichthys</i> <i>rondoni</i>	0.22 (0.06)	0.13 (0.07)	0.31 (0.09)	0.38 (0.14)	0.24 (0.14)	0.48 (0.17)

Table 4.

Family/Species	Paired visits		Sequential visits	
	With Detector	Without Detector	With Detector	Without Detector
	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>
GYMNOTIDAE				
<i>Gymnotus</i> spp.	0.79 (0.05)	0.40 (0.05)	0.86 (0.04)	0.43 (0.05)
HYPOPOMIDAE				
<i>Hypopygus lepturus</i>	1.0	0.05 (0.05)	0.32 (0.18)	0.02 (0.02)
<i>Microsternarchus</i> cf. <i>bilineatus</i>	1.0	0.35 (0.12)	0.81 (0.09)	0.34 (0.08)
RHAMPHICHTHYIDAE				
<i>Gymnorhamphichthys</i> <i>rondoni</i>	1.0	0.01 (0.01)	0.17 (0.13)	0.01 (0.01)

Table 5.

Family/Species	Model Selection					
	Paired visits			Sequential visits		
	Model	$\Delta AICc$	$\alpha$	Model	$\Delta AICc$	$\alpha$
GYMNOTIDAE						
	psi(.)p(Det+PC1)	0.00	20%	psi(.)p(Det+Vel.Med)	0.00	56%
<i>Gymnotus</i> spp.	psi(.)p(Det*Tmp)	0.86	13%	psi(.)p(Det*Vel.Med)	2.84	14%
	psi(.)p(Det+Tmp)	1.31	10%	psi(.)p(Det+PC1)	4.36	6%
HYPOPOMIDAE						
	psi(.)p(Det*PC3)	0.00	48%	psi(.)p(Det)	0.00	17%
<i>Hypopygus lepturus</i>	psi(.)p(Det)	3.74	7%	psi(.)p(Det+Vel.Med)	0.44	14%
				psi(.)p(Det+PC3)	1.25	9%
	psi(.)p(Det+Vel.Med)	0.00	18%	psi(.)p(Det+Vel.Med)	0.00	43%
<i>Microsternarchus</i> cf.	psi(.)p(Det)	0.78	12%	psi(.)p(Det*Vel.Med)	1.97	16%
<i>bilineatus</i>	psi(.)p(Det+Chv)	1.12	10%	psi(.)p(Det+PC1)	3.45	8%
	psi(.)p(Det+OD)	1.12	10%			
RHAMPHICHTHYIDAE						
	psi(.)p(Det+PC3)	0.00	50%	psi(.)p(Det*Cnd)	0.00	93%
<i>Gymnorhamphichthys</i>	psi(.)p(Det*Cnd)	1.29	26%	psi(.)p(Det+PC3)	7.67	1%
<i>rondoni</i>	psi(.)p(Det*PC3)	2.06	18%	psi(.)p(Det*PC3)	9.07	1%



## Supplementary material tables

**Table S1.** Principal Components Analysis (PCA) of substrates types found during Gymnotiformes sampling in streams of Ducke Reserve.

Substrate	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Sand	0.656646	0.135798	-0.16315	-0.11059	-0.31174	0.172568	-0.62014
Fine litter	-0.39714	0.511955	-0.377	0.038787	0.148704	-0.48322	-0.42537
Coarse litter	-0.57305	-0.22092	0.039289	0.219164	-0.16638	0.577738	-0.46018
Large roots	0.033828	-0.5006	-0.29777	-0.44642	0.638371	0.034673	-0.22711
Fine roots	-0.13099	-0.0322	0.768166	-0.44167	-0.06618	-0.30041	-0.31941
Rock	0.041513	-0.63842	-0.09268	0.407053	-0.28635	-0.55709	-0.15513
Stems	0.250364	0.106918	0.377327	0.614961	0.599276	0.033771	-0.21227

**Table S2.** Selected covariates to model the probability of detection and occupation of five species of electric fish (Gymnotiformes) in streams in Ducke Reserve.

<b>Covariates</b>	<b>Variable</b>	<b>Variable Type</b>	<b>Description</b>
<b>Detection</b>			
Constant	p	None	Detection assumed to be constant
Detector device	Det	Categorical	Use or not use of the detector device during survey
Temperature	Tmp	Continuous	Mean temperature measured during fish survey ( $^{\circ}\text{C}$ )
Conductivity	Cnd	Continuous	Mean conductivity measure during fish survey ( $\mu\text{S}/\text{cm}$ )
Dissolved oxygen	OD	Continuous	Mean dissolved oxygen measure during fish survey (%)
Current velocity	Cur.Vel	Continuous	Mean flow of water measure during fish survey (m/s)
Width	Width	Continuous	Mean width measure during fish survey (m)
Rain	Rain	Categorical	Presence of rain during the survey
Sand	PC1	Continuous	Presence of sand on substrate during the survey
Fine litter	PC2	Continuous	Presence of leaf litter on substrate during the survey
Fine roots	PC3	Continuous	Presence of fine root on substrate during the survey
Detector and rain	Det*Rain	Categorical	Interaction between detector device and rain during survey
Detector and current	Det*Cur.Vel	Continuous	Interaction between detector device and flow during survey
Detector and conductivity	Det*Cnd	Continuous	Interaction between detector device and conductivity
Detector and temperature	Det*Tmp	Continuous	Interaction between detector device and temperature
Detector and oxygen	Det*OD	Continuous	Interaction between detector device and dissolved oxygen
<b>Occupancy</b>			
Drainage basin	East/West	Categorical	Main drainage basin

**Table S3.** Set of models chosen for the first and second steps of analysis.

<b>First step: detection models</b>		
1 $\text{psi}(\cdot)\text{p}(\cdot)$	8 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{Cur.Vel})$	15 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{PC1})$
2 $\text{psi}(\cdot)\text{p}(\text{Det})$	9 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{Cnd})$	16 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{PC2})$
3 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{Rain})$	10 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{Cnd})$	17 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{PC3})$
4 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{Rain})$	11 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{OD})$	18 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{PC1})$
5 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{Width})$	12 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{OD})$	19 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{PC2})$
6 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{Width})$	13 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{Tmp})$	20 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{PC3})$
7 $\text{psi}(\cdot)\text{p}(\text{Det}+\text{Cur.Vel})$	14 $\text{psi}(\cdot)\text{p}(\text{Det}*\text{Tmp})$	
<b>Second step: occupancy models</b>		
$\text{psi}(\cdot)\text{p}(\cdot)$	$\text{Psi}(\cdot)\text{p}(\text{models selected in first step})$	
$\text{psi}(\text{East})\text{p}(\cdot)$	$\text{Psi}(\text{East})\text{p}(\text{models selected in first step})$	

## **Considerações finais e conclusão**

A utilização do detector de peixes elétricos proporcionou um aumento significativo do número de espécies e de indivíduos da ordem dos Gymnotiformes nos igarapés da Reserva Ducke. Neste sentido, recomendamos fortemente a utilização do aparelho detector de peixes elétricos em amostragens ictiofaunísticas na Amazônia, como forma de gerar estimativas acuradas da riqueza e abundância de Gymnotiformes nessa região.

O gênero *Gymnotus* foi o que apresentou o maior número de espécies e indivíduos nos igarapés amostrados, e se distribui amplamente em toda a Reserva Ducke. *Microsternarchus* cf. *bilineatus* também apresenta abundância significativa na Reserva, porém apresenta distribuição aparentemente restrita às microbacias da porção leste.

Comparando os dois critérios de análises de modelos escolhidos para estimar a ocupação e detecção de Gymnotiformes na Reserva Ducke, concluímos que os principais fatores ambientais que influenciaram a detecção das espécies foram a velocidade média da correnteza, a condutividade elétrica e o tipo de substrato. O tipo de substrato influenciou a detecção das espécies principalmente nos modelos de visitas pareadas, enquanto a velocidade média da correnteza e condutividade elétrica influenciaram as estimativas geradas pelos modelos de visitas sequenciais.

A hidrodinâmica sazonal dos igarapés promove o transporte periódico de sedimentos e substratos ao longo do canal, modificando a fisionomia desses ambientes aquáticos e constituindo um fator preponderante na distribuição espacial e ocupação de habitats pelos peixes Gymnotiformes.

## Ata de defesa



MINISTÉRIO DA  
CIÊNCIA, TECNOLOGIA,  
INOVAÇÕES E COMUNICAÇÕES



### PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

#### ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 12 dias do mês de agosto do ano de 2016, às 14h30min, no Auditório do PPG BADPI, Campus II, INPA/Aleixo. Reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Cristhiana Paula Röpke**, da Universidade Federal do Amazonas-UFAM, o(a) Prof(a). Dr(a). **Paulo Estefano Dineli Bobrowiec**, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o(a) Prof(a). Dr(a). **Fabricio Beggiato Baccaro**, da Universidade Federal do Amazonas- UFAM, tendo como suplentes o(a) Prof(a). Dr(a). Cláudia Pereira de Deus, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o(a) Prof(a). Dr(a). Rafael de Fraga, do Instituto Nacional de Pesquisas da Amazônia - INPA, sob a presidência do(a) primeiro(a), a fim de proceder a arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO de ANDRÉ LUIZ RAMOS HOLANDA DE ANDRADE**, intitulado: "INFLUÊNCIA DO MÉTODO DE AMOSTRAGEM NAS ESTIMATIVAS DE DETECÇÃO E OCUPAÇÃO DE PEIXES ELÉTRICOS (*Gymnotiformes*) EM IGARAPÉS DA RESERVA DUCKE, AMAZÔNIA CENTRAL", orientado(a) pelo(a) Prof(a). Dr(a). Jansen Alfredo Sampaio Zuanon do Instituto Nacional de Pesquisas da Amazônia - INPA e coorientada pelo(a) Prof(a). Dr(a) Gonçalo Nuno Côrte-Real Ferraz de Oliveira, da Universidade Federal do Rio Grande do Sul - UFRGS.

Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

APROVADO(A)  REPROVADO(A)

POR UNANIMIDADE  POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). CRISTHIANA PAULA RÖPKE

Prof(a).Dr(a). PAULO ESTEFANO DINELI BOBROWIEC

Prof(a).Dr(a). FABRICIO BEGGIATO BACCARO

Prof(a).Dr(a). CLÁUDIA PEREIRA DE DEUS

Prof(a).Dr(a). RAFAEL DE FRAGA

Coordenação PPG-ECO/INPA