THAIS SASSO LOPES

SPATIAL AND TEMPORAL OCCURRENCE OF STREAM FROGS IN THE ATLANTIC FOREST AND THEIR DETECTION THROUGH ENVIRONMENTAL DNA

PADRÕES ESPACIAIS E TEMPORAIS DE OCORRÊNCIA DE ANUROS EM RIACHOS DE MATA ATLÂNTICA E SUA DETECÇÃO POR MEIO DE DNA AMBIENTAL

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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na área de Ecologia.

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Prof. Dr. Marcio Roberto Costa Martins

Orientador

PARA A MINHA FAMÍLIA E A TODOS QUE TIVEREM CURIOSIDADE POR ANFÍBIOS E EDNA.

- Art by Tim Hopgood

Everybody should be quiet
near a little stream and listen

- by Ruth Krauss, 1982.

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OVERVIEW

Brazil ranks as the country with one of the highest amphibian species diversity. Streams in the Atlantic forest of southeastern Brazil have an important availability of microenvironments and harbors a particular richness in amphibian species. Monitoring herpetofauna and knowledge on their spatial and temporal dynamics provide primary information for ecological studies, and are essential to the development of other areas such as conservation biology. In this work we gather information on the occurrence and abundance of three torrent frogs, *Cycloramphus boraceiensis*, *Hylodes asper* and *Hylodes phyllodes* and examine the reliability of eDNA analysis to detect anuran communities. Samplings occurred within a 95 to 115 m transect in four streams in Núcleo Picinguaba, at the Parque Estadual da Serra do Mar, São Paulo, Brazil. Individual encounter number and their habitat were monthly recorded from January 2007 to December 2010 and every two months in 2011. We searched for post-metamorphic individuals while walking upstream for 30-60 min, checking all visually accessible spots in the streambed. The location of each active and inactive individual was mapped and its habitat use was characterized in relation to five ecological parameters. We collected eDNA samples at 16 sites on April, 2015. We used eDNA metabarcoding approach with a universal amphibian primer of a mitochondrial marker (12S) to detect amphibian communities. We recorded a total of 6335 visual observations. The three species abundance varied along and between streams and only *Hylodes phyllodes* were found in the stream 2. Abundance of *C. boraceiensis* and *H. asper* was significantly higher in the wet seasons. The three species were found active mainly in wet rocks, without moss and without cover. Inactive individuals of *Hylodes asper* and *H. phyllodes* were found mainly in dry leaves, without moss or cover. Through eDNA metabarcoding, we detected nine species, which were consistent with traditional survey results. DNA of riparian species and species with higher constancy in traditional surveys were detected in higher proportions. Our study showed that traditional survey and DNA metabarcoding results can be complementary and both methodologies can be combined in future ecology studies.

RESUMO GERAL

O Brasil apresenta uma das maiores diversidade de espécies de anfíbios, sendo reconhecidas em torno de 500 espécies endêmicas no país, as quais são encontradas predominantemente em área de Mata Atlântica. O monitoramento da herpetofauna e conhecimento da dinâmica espacial e temporal destas espécies são informações básicas, porém, fundamentais ao desenvolvimento de outras áreas de pesquisa e conservação. Neste trabalho reunimos informações sobre ocorrência e abundância de três espécies típicas de riacho, *Cycloramphus boraceiensis, Hylodes asper* e *Hylodes phyllodes* e testamos o uso de DNA ambiental para detecção de comunidades de anuros. As amostragens ocorreram em um transecto de 100 a 115 m em quatro riachos no Núcleo de Picinguaba, localizado no Parque Estadual da Serra do Mar, São Paulo, Brasil. Coletas de abundância e uso do habitat ocorreram mensalmente de janeiro de 2007 a dezembro de 2010 e em meses alternados em 2011. Indivíduos pós-metamórficos foram amostrados por procura visual a montante de cada riacho, verificando-se todos os locais ao longo do leito. A localidade de cada indivíduo ativo e inativo foi mapeada e o uso do ambiente foi caracterizado em relação a seis parâmetros ecológicos. As amostras de DNA ambiental foram coletadas em 16 pontos em Abril de 2015. eDNA *metabarcoding* foi realizado com primer universal de anfíbios para uma região do gene mitocondrial (12S). Registramos um total de 6335 observações visuais. A abundância das três espécies variou entre e ao longo dos riachos, sendo que apenas a espécie *Hylodes phyllodes* foi registrada no riacho 2. Houve uma sazonalidade na abundância de *Cycloramphus boraceiensis* e *Hylodes asper*, sendo ambas espécies encontradas em maior número na estação chuvosa. As três espécies foram encontradas ativas majoritariamente em rochas úmidas ou molhadas, sem musgo e sem cobertura. Indivíduos inativos de *Hylodes asper* e *H. Phyllodes* foram encontrados majoritariamente em folhas secas sem musgo e sem cobertura. Por meio da técnica de eDNA metabarcoding, detectamos nove espécies, compatíveis com a amostragem tradicional. O DNA de espécies com fases do ciclo de vida atreladas aos riachos e com maior constância na amostragem tradicional foi detectado em maior proporção. Nossos estudos demonstraram que os resultados da amostragem tradicional e de eDNA *metabarcoding* fornecem informações fundamentais e complementares, sendo uma combinação de ambas metodologias potencialmente útil a futuros estudos de ecologia.

GENERAL INTRODUCTION

Understanding the structure and dynamics of communities is a central goal in ecology. Achieving this goal entails describing local diversity patterns – such as species composition and abundance – and also temporal and spatial patterns of species distribution. The more detailed and thorough these descriptions are, the more diverse their applications can be, ranging from systematics and biogeography to conservation biology.

Brazil is a world leader in amphibian diversity, with 1026 recognized species (Silvano and Segalla, 2005; SBH, 2014). Almost 500 of these species are endemic to Brazil (True *et al.*, 2010) and predominantly recorded in the Atlantic Forest domain, even after the heavily destruction of its vegetation coverage (Ribeiro *et al*., 2009). However, amphibian surveys are sorely lacking for large areas in Brazil and a greater resolution is required at some of the already investigated locations (Silvano and Segalla, 2005). Therefore, the ecology of many Brazilian amphibians is still poorly known and few species are well characterized with respect to their temporal variation, spatial distribution, and natural history (Silvano and Segalla, 2005). Insufficient knowledge of amphibian ecology proves to be even more critical when we realize that freshwater environments, on which the majority of amphibians rely, are among the most threatened habitats in the world (Dudgeon *et al*., 2006). More reliable monitoring of amphibian assemblages and gathering information of temporal and spatial patterns of species associated with aquatic environments become is critical, particularly to provide conservation guidelines (Rees *et al*., 2014).

Field surveys enable researchers to assess species occurrence and are traditionally conducted through a myriad of methods (e.g., active surveys or traps placement). However, various difficulties might arise when fauna inventories are carried out with traditional

methods. One difficulty is, for example, the detection of small, rare, cryptic or less conspicuous species (MacKenzie *et al*., 2002; Silveira *et al*., 2010). Furthermore, some methods may be not effective for some species, habits or environments, such as acoustic survey for amphibian species that do not vocalize or pitfall traps for arboreal species (Rödel and Ernst, 2004). Moreover, species identification based on morphological characters is subject to researcher biases such as its taxonomic experience (Hopkins and Freckleton, 2002; Silveira *et al.*, 2010). Other difficulties may lie in the fact that the vast majority of methods are seasonally constrained and fail to survey species in certain seasons when individuals are inactive, or species identification in certain life cycle stages such as eggs, larval or juveniles (Ficetola *et al*., 2008). The combination of these factors affects wildlife surveys by allowing for false-positive (i.e., the species is detected where is not actually present) and especially false-negative results (i.e., the species is not detected when is in fact present), which blurs the true occurrence and distribution of species.

In the last 20 years, sequencing technologies progressed rapidly and the emergence of next-generation sequencing (NGS) allowed researchers to overcome costly and time-consuming steps. These advances in sequencing procedures have prompted the growth of molecular databases (e.g. NCBI: http://www.ncbi.nlm.nih.gov; EMBL, http://www.ebi.ac.uk/embl, BOLD, http://www.barcodinglife.org) containing genic and genomic sequences from several taxa making molecular data readily accessible to answer new questions (Valentini *et al*., 2009). The availability of molecular data allowed the exploration of innovative tools in fields such as molecular ecology, particularly with regard to the development DNA barcode (short stretch of a species-specific DNA sequence, capable to distinguish species) for species identification (Valentini *et al*., 2009).

A recent and promising technique for species identification makes use of what is known as environmental DNA (eDNA). Environmental DNA is the DNA recovered from

an environmental sample such as air, soil or water, with no evident sign of biological material (Ficetola *et al*., 2008). This DNA can then be sampled for sequencing by NGS and used in subsequent identification of the species that released it. Environmental DNA detection provides us an excellent way to determine species occurrences with the advantages of not depending on visual detection or removal of organisms, and not relying on taxonomic knowledge (Darling and Mahon, 2011; Deiner and Altermatt, 2014). On the other hand, eDNA analyses demand molecular biology and bioinformatics skills. Recovery of DNA from environmental samples is therefore a new approach for species survey with the potential to overcome many of the previously mentioned limitations of traditional methods and advance our knowledge of species diversity.

The use of eDNA does not mean replacing traditional methodologies, however. Even though sequencing of environmental samples has the potential to enhance our ability to detect species that are otherwise difficult to spot or collect, it is important to recognize that only by traditional field observations one can gather information on the species natural history, such as habitat use, and abundance. Overall, eDNA analysis and traditional methodologies provide baseline data, paramount for our understanding of the studied taxa and for taking the best informed conservation decisions.

STRUCTURE

In this study, we used data from a five-year survey obtained through traditional methodologies to describe the temporal and spatial occurrence and abundance of amphibians at the Atlantic Forest, Ubatuba, Brazil. Also based on the five-year survey, we investigate whether a single visit for eDNA sampling can be effective in detecting anuran communities in tropical streams. The dissertation is divided in two chapters. Specifically,

the first chapter describes the occurrence of three torrent frogs (*Cycloramphus boraceiensis, Hylodes asper* and *H. phyllodes*) in four streams, investigate the abundance of these species over time and characterize their microhabitat use. The second chapter explores the applicability of eDNA metabarcoding to characterize the amphibian community associated with streams and investigate whether eDNA detection coincides with the data obtained by traditional sampling.

eDNA OVERVIEW

In the second chapter we apply a recent and unfamiliar methodology to assess species presence in aquatic environments detecting eDNA. Therefore, we will briefly describe the advances in this methodology to review the state of the art on aquatic eDNA analysis:

Previous sampling of DNA from the environment

The first studies to extract genomic material from environmental samples explored the microbial diversity in soil and water (Schmidt *et al*., 1991; Stein *et al*., 1996; Handelsman *et al*., 1998). Schmidt *et al* (1991) were the first to amplify ribosomal RNA of picoplankton communities extracted from sea water samples. Their approach enabled microbiologists to access the genome of uncultivable microorganisms in the laboratory and showed that microbial diversity can be more complex than previously imagined. Molecular sequences of microbial populations taken directly from the environment were then designated as the "metagenome", and the study of genomes obtained thereby as "metagenomics" (Handelsman *et al*., 1998).

Species detection based on DNA obtained directly from the environment was then extended to macro organisms in paleoecology studies. By using soil samples, researchers

revealed the pattern of distribution of extinct mammals, birds and plants (Willerslev *et al*., 2003; Willerslev and Cooper, 2005). Subsequently, DNA extractions were also performed from cave sediment (Hofreiter *et al*., 2003) and ice-core (Willerslev *et al*., 2007). The current availability of high-throughput sequencing technology and the design of barcodes boosted the use of eDNA in ecological studies. While the goal of metagenomic and paleoecology studies has often been to characterize all genomes in the sample and the functional diversity of microbial communities, eDNA analyses in ecology has, at least initially, focused on one or a few species previously known. For instance, the first study to collect DNA from freshwater environments attempted to comprehend the distribution of the invasive bullfrog in France (Ficetola *et al*., 2008).

Early detection of invasive and endangered species

Nowadays, eDNA detection has been used extensively and successfully to monitor several exotic and endangered species (Darling and Mahon, 2011). In freshwater environments, examples include the detection of Asian carp, Chinook salmon, American bullfrogs, snakes (*Python bivittatus*), Bluegill fish, crustaceans (*Procambarus clarkii*) and gastropods (*Potamopyrgus antipodarum*) (Chapter 2 - Supplementary List I). Studies on dispersion of invasive aquatic species confirmed the effectiveness of eDNA analysis as a survey tool for early detection of populations at low densities - as low as 1 individual per 100 m^2 (Secondi *et al.* 2016) - and at any life stage (e.g. Dejean *et al*., 2012; Goldberg *et al*., 2013; Piaggio *et al*., 2013; Takahara *et al*., 2013). These studies support the eDNA detection as a good alternative for the survey of cryptic, elusive and at low density species. Also, these studies have proved eDNA methodology to be useful in occupancy model and species' range delimitation (e.g., Hunter *et al*., 2015).

Persistence of eDNA in aquatic environment

eDNA detection rely on the secretion of DNA by organisms and also on the rate of disintegration of these molecules (Takahara *et al*., 2013). In order to accurately determine the presence or absence of a species it is thus of paramount importance to know whether DNA molecules can remain in the environment and for how long prior to degradation (Dejean *et al.*, 2011). Knowing the persistence of DNA in the environment is essential when it comes to defining precisely the time limit of detection of an organism - i.e. what is the maximum interval between the presence of the organism and its DNA detection.

Studies investigating DNA degradation have determined that these molecules can be detected within 25 and 14 days in controlled and natural aquatic environments, respectively, and that the DNA detection rate was negatively correlated with time (Dejean *et al*., 2011). In a controlled laboratory experiment with *Pelobates fuscus* and *Triturus cristatus*, Thomsen *et al.* (2012b) found that immediately after the animals are removed from the aquarium, a fast and continuous reduction in DNA concentrations was observed, with DNA becoming undetectable within one to two weeks. These results led the authors to suggest that eDNA samples found in the environment are contemporary to the species presence and the donor species occurrence in the sampling locality can be safely inferred. However, these studies were conducted in aquariums or lentic environments in the absence of water flow. In lotic environments, on the other hand, eDNA concentrations from salamanders were shown fall within hours after removal of the individuals, with DNA molecules not being detected after only 24 h (Pilliod *et al*., 2013b).

Therefore, a short interval of persistence of detectable amounts of DNA allows access to presence or absence of the species in a fine time scale. This information is critical to limit the incidence of false-positive errors in cases where the molecule is detected but the

organism no longer occupies the site, and to ensure the DNA identified belongs to an organism recently present in that given environment (Dejean *et al.*, 2012).

Advantages of using eDNA

Monitoring species using eDNA detection offers a number of potential advantages over traditional methods (Darling and Mahon, 2011). An environmental sample for DNA extraction is considerably simple to obtain, performed in a non-invasive and non-disruptive manner, and does not require the capture and removal of organisms from the environment (Jerde *et al*., 2011).

Monitoring species can be challenging when applying conventional sampling methods, particularly if the initial starting place to look for the species is not known or if they occur at low abundances (Wilson and Wright, 2013). The high sensitivity of eDNA has been helpful to guide locations for traditional search of individuals. For instance, directed by the discovery of carp eDNA in a pool in Illinois, United States, Jerde *et al.* (2011) was able to find one silver carp after 93 person-days of electrofishing effort.

Compared to traditional survey methods, obtaining eDNA is also considerably faster and less costly (Rees *et al.*, 2014a). In the same study with the Asian carp held in Chicago (Illinois, USA), only 0.174 person-days were necessary to get a positive detection with eDNA detection (Jerde *et al.*, 2011).

The detection rate of eDNA was also compared to that of conventional methodologies in aquatic environment (such as snorkel, "kick-netting" and electrofishing). eDNA resulted in detection of a greater number of species and in more locations than conventional methods (Jerde *et al*., 2011; Pilliod *et al*., 2013b; Tréguier *et al*., 2014). For instance, Dejean *et al.* (2012) detected bullfrog eDNA in 38 lakes in France, and in only

seven using visual and acoustic survey. In other study for amphibian survey in Mediterranean ponds, Valentini *et al.* (2016) estimated that it was necessary at least four successive visits to the field using traditional methods to obtain the same detection probability achieved by a single sampling using eDNA metabarcoding approach. This difference suggests that traditional methods may underestimate the species' occurrence and distribution, at least in the aquatic environment.

Restrictions on the use of eDNA

Despite the aforementioned advantages, the eDNA detection is not devoid of constraints. Lodge *et al.* (2012) and Taberlet *et al.* (2012) pointed hurdles to be overcome so that eDNA detection becomes more reliable as an ecological tool. For example, the efficiency of this approach depends on the development of molecular techniques, such as primer specificity and on the absence of amplification inhibitors in the sample. Primers not so specific might result in false-positive results - particularly if any successful amplification is interpreted as a positive result without further investigation through sequencing (Wilcox *et al*., 2013).

Additionally, DNA release in the environment - as well its persistence and detection - can be influenced in varying degrees by biotic and abiotic factors. Little is known on the importance of the species natural history (e.g. activity, life stages habitat) for interspecific and intraspecific difference in DNA secretion, and on the influence of climatic factors in DNA detection. A review by Barnes *et al.* (2014) indicated that environmental factors should be considered in studies sampling eDNA. Enzyme present in water, for example, can decompose DNA and so can microorganism activity, temperature, ultraviolet radiation and acidity. These factors can act synergistically or antagonistically to preserve or facilitate DNA degradation (Barnes *et al*, 2014; Pilliod *et al.*, 2013). Understanding eDNA

degradation is crucial if we are to make accurate statements about the presence of a species at a given location, since failing to detect eDNA will not be necessarily due to the species absence.

In summary, the literature exploring eDNA as a possible survey tool for aquatic species is slightly recent but escalating, with many studies investigating different aspects of the feasibility of eDNA on species detection. This includes a better understanding of how eDNA can be applied for community survey. To fill gaps in our knowledge on eDNA applicability, we sampled eDNA from four streams in the Atlantic Forest with well-known species compositions to test whether the results of eDNA analyses were consistent with those of traditional methodologies.

GENERAL REFERENCES

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A, Chadderton, W. L., Lodge, D. M., 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environmental Science & Technology, 48, 3, 1819-1827.

Darling, J. A., Mahon, A. R., 2011. From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. Environmental Research, 111, 978-988.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., Miaud, C., 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS One, 6, e23398.

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., Miaud, C., 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. Journal of Applied Ecology, 49, 953-959.

Deiner, K., Altermatt, F., 2014. Transport distance of invertebrate environmental DNA in a natural river. PLoS One, 9, e88786.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z., Knowler, D., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J. and Sullivan, C. A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews, 81,163-182.

Ficetola, G. F., Miaud, C., Pompanon, F., Taberlet, P., 2008. Species detection using environmental DNA from water samples. Biology Letters, 4, 423-425.

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., Waits, L. P., 2013. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). Freshwater Science, 32, 792-800.

Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., Goodman, R. M., 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chemistry and Biology, 5, 245-249.

Hofreiter, M., Mead, J. I., Martin, P., Poinar, H. N., 2003. Molecular caving. Current Biology, 13, 693-695.

Hopkins, G. W., Freckleton, R. P., 2002. Declines in the numbers of amateur and professional taxonomists: implications for conservation. Animal Conservation, 5, 245-249.

Hunter, M. E., Oyler-McCance, S. J., Dorazio, R. M., Fike, J. A., Smith, B. J., Hunter, C. T., Reed, R. N., Hart, K. M. 2015. Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. PLoS One, 10, e0121655.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., Lodge, D. M., 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letter, 4, 150-157.

Piaggio, A. J., Engeman, R. M., Hopken, M. W., Humphrey, J. S., Keacher, K. L., Bruce, W. E., Avery, M. L. 2013. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. Molecular Ecology Resources, 14, 374-380.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., Waits, L. P., Richardson, J., 2013. Estimating occupancy and abundance of stream amphibians using eDNA from filtered water samples. Canadian Journal of Fisheries and Aquatic Science, 70, 1123-1130.

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J. L., Mahon, A. R., Pfrender, M. E. 2012. Conservation in a cup of water:

estimating biodiversity and population abundance from environmental DNA. Molecular Ecology, 21, 2555-2558.

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, J. A., Langtimm, C. A., 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology, 83, 2248-2255.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., Gough, K. C. 2014. The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. Journal of Applied Ecology, 51, 5, 1450-1459.

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., Hirota, M. M., 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation, 142, 1141-1153.

Rödel, M., Ernst, R. 2004. Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. Ecotropica, 10, 1-14.

SBH - Sociedade Brasileira de Herpetologia, 2014. Brazilian amphibians - List of species. Electronic database accessible at http://www.sbherpetologia.org.br.

Schmidt, T. M., DeLong, E. F., Pace, N. R., 1991. Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. Journal of Bacteriology, 173, 4371-4378.

Secondi, J., Dejean, T., Valentini, A., Miaud, C., Audebaud, B., 2016. Detection of a global aquatic invasive amphibian, *Xenopus laevis*, using environmental DNA. Amphibia-Reptilia, 37, 131-136.

Silvano, D. L., Segalla, M. V., 2005. Conservação de anfíbios no Brasil. Megadiversidade, 1, 79-86.

Silveira, L. F., Beisiegel, B. de M., Curcio, F. F., Vadujo, P. H., Dixo, M., Verdade, V. K., Mattox, G. M. T., Cunningham, P. T. M., 2010. Para que servem os inventários de fauna? Estudos Avançados, 24, 173-207.

Stein, J. L., Marsh, T. L., Wu, K. Y., Shizuya, H., DeLong, E. F., 1996. Characterization of uncultivated prokaryotes: isolation and analysis of a 40-kilobase-pair genome fragment from a planktonic marine archaeon. Journal of Bacteriology, 178, 591-599.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology, 21, 2045-2050.

Takahara, T., Minamoto, T., Doi, H., 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. PLoS One, 8, e56584.

Thomsen, P., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., Willerslev, E., 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One, 7, e41732.

Tréguier, A., Paillisson, J. M., Dejean, T., Valentini, A., Schlaepfer, M. A., Roussel, J. M., 2014. Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkia* in freshwater ponds. Journal of Applied Ecology, 51, 871-879.

Valentini, A., Pompanon, F., Taberlet, P., 2009. DNA barcoding for ecologists. Trends in Ecology and Evolution, 24, 110-117.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J. M., Peroux, T., Crivelli, A.J., Olivier, A., Acqueberge, M., Le Brun, M., Moller, P. R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular Ecology, 25, 929-942.

Wilcox, T. M, McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley A. R., Schwartz, M. K., 2013. Robust detection of rare species using environmental DNA: the importance of primer specificity. PloS one, 8, e59520.

Wilson, C., Wright, E. 2013. Using environmental DNA (eDNA) as a tool in risk-based decision-making. Ontario Ministry of Natural Resources, Aquatic Research and Development Section, Aquatic Research Series, 2013- 01.

Willerslev, E., Cappellini, E., Boomsma, W., Nielsen, R., Martin, B., Brand, T.B., Hofreiter, M., Bunce, M., Poinar, H. N., Dahl-jensen, D., Johnsen, S., Steffensen, J. P., Bennike, O., 2007. Ancient biomolecules from deep ice cores reveal a forested Southern Greenland. Science, 317, 111-114.

Willerslev, E., Cooper, A., 2005. Ancient DNA. Proceedings Biological Sciences / The Royal Society, 272, 3-16.

Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D. A., Cooper, A., 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science, 300, 791-795.

GENERAL CONCLUSION

Habitat use, abundance and occurrence data are crucial to the success of conservation initiatives and serve as material for various research areas. Our studies contribute to the understanding of Brazilian amphibians and encourage the use of eDNA metabarcoding to evaluate the diversity of amphibian communities in tropical streams.

The Atlantic Forest has an important diversity of microenvironments and harbors a particular richness in amphibian species which explore a variety of habitats. Some terrestrial species breed in streams and migrate to environments such as leaf litter or trees in other life stage. On the other hand, torrent frogs reside most of the time in lotic environments, as the case of the species studied, *Cycloramphus boraceiensis, Hylodes asper* and *H. phyllodes*, mainly found inhabiting wet rocks near the water. This behavior apparently contributed to a greater chance of eDNA detection from the water samples for these species. Environmental DNA from species with other habits was also successfully detected, nonetheless, in a lesser extent.

Our study also showed that traditional sampling and eDNA metabarcoding can be complementary. Through eDNA analysis it was possible to detect amphibian diversity in tropical streams in a non-invasively manner. Through visual survey, we gathered information regarding the use of habitat and species abundance. Thus, a combination of both methods will be potentially useful for future ecological studies. However, it is important to note that most of the traditional methods applied for amphibians meet the expected effectiveness for only a few species, habits or locations and cannot provide extensive information of the species ecology, like the species' microhabitat use (Rödel and Ernst, 2004). Therefore, the choice of the traditional method should reflect the objectives of the study and be one that provides a complete picture of the ecology of

amphibians, complementing the use of eDNA. In addition to difference in purposes, different survey methods implies in different survey effort. In this study it was necessary only a single visit for eDNA sampling to gather information on amphibian communities, which was obtained through a 5-year survey using traditional methods. Hence, as a survey methodology for species occurrence, eDNA metabarcoding is capable of optimizing considerable time effort.

Finally, through traditional survey we described abundance variation over a long-term study. Estimate the abundance or density of species accurately is not yet possible through eDNA analysis. This is because DNA secretion might vary between life stages or between species and might be not clearly related to the organism biomass. Klymus *et al*. (2015), for example, found that a single individual may secrete DNA in quantities varying from zero to hundreds of thousands of copies within a few weeks. Moreover, it is not known how activity, metabolism, seasonality and stress affect the eDNA production. Another impairment to abundance estimation is the PCR procedures or sequencing bias towards the more abundant DNA copies, which in turn may conceal the abundance estimation of less abundant species.

GENERAL REFERENCES

Klymus, K. E., Richter, C. A., Chapman, D. C., Paukert, C., 2015. Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. Biological Conservation, 183, 77-84.

Rödel, M., Ernst, R., 2004. Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. Ecotropica, 10, 1-14.

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