UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

“UTILIZAÇÃO DO MÉTODO ACÚSTICO PASSIVO PARA AVALIAR SONS DE PEIXES MARINHOS E CONTINENTAIS EM ECOSSISTEMAS TROPICAIS BRASILEIROS”

Alfredo Borie Mojica

Tese apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Doutor.

Prof. Dr. Paulo Travassos
Orientador

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Tese julgada adequada para obtenção do título de doutor em Recursos Pesqueiros e Aquicultura. Defendida e aprovada em 27/02/2018 pela seguinte Banca Examinadora.

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Dedicatória

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Resumo

A comunicação está omnipresente nos animais aquáticos, principalmente naqueles capazes de produzir diferentes tipos de sinais acústicos. Esta cacofonia, denominada paisagem acústica, vem permitindo avaliar a distribuição e comportamentos dos organismos sonoros, simplesmente registrando e avaliando os sons por eles produzidos utilizando o método acústico passivo. O objetivo deste trabalho foi usar metodologias inovadoras de pesquisa não invasiva, como a acústica passiva, na coleta e análise de dados acústicos em ambientes aquáticos continentais e marinhos, para fins de monitoramento, conservação e gestão de recursos vivos destes ambientes, principalmente peixes. No que se refere ao ambiente marinho, foram avaliadas as paisagens acústicas de duas áreas de recifes costeiros no litoral sul do Estado de Pernambuco, sendo uma em Porto de Galinhas, área já impactada pelo livre acesso para uso turístico e pesqueiro, e outra em Tamandaré, no interior da Área de Proteção Ambiental Costa dos Corais, na Zona de Preservação da Vida Marinha, utilizada apenas para pesquisa. Em ambiente de água doce, avaliaram-se a produção de sons de cinco espécies de importância comercial da ordem Characiformes no Rio Madeira, sul da Amazônia. Nos experimentos marinhos, Os resultados dos experimentos marinhos indicaram que os sons nas duas áreas de estudo ocorreram principalmente no final da tarde e à noite, onde foram detectados seis coros de peixes recorrentes, com distribuição da banda de frequência entre 200 e 2000 Hz, sem sobreposição e com diferentes características acústicas entre as espécies. Os coros apresentara alta energia em mar aberto, após a última linha de recife na área protegida de Tamandaré (~ 130 dB re1µPa^2 Hz^{-1}). No entanto, os coros de peixes apresentou baixos níveis de energia em Porto de Galinhas, com uma diferença de 30 dB re1µPa^2 Hz$^-$1. Também foram detectados sons característicos de invertebrados, com bandas de frequências dominantes de 2 e 3 kHz, além de ruídos produzidos por diferentes tipos de embarcações pesqueiras, lanchas recreativas e navios, que podem mascarra e interferir na comunicação dos peixes. O monitoramento da complexa paisagem acústica encontrada em Tamandaré durante o verão mostrou uma constante sobreposição temporal dos coros produzidos pelos peixes no final da tarde. A ocorrência dos coros esteve relacionada com as diferentes fases lunares, influenciando de forma distinta o tempo de início e de detecção, como variações principalmente durante a lua de quarto crescente. A influência lunar também ocorreu nos ruídos de embarcações detectados, com embarcações pesqueiras apresentando registros principalmente no início da manhã, nas luas gibosa crescente e minguante, com bandas de frequência <200 Hz e com elevados picos de energia facilmente distinguíveis. Em águas continentais, a acústica passiva permitiu avaliar sons de cinco characiformes de importância comercial (*Potamorhina latior*, *P. altamazonica*, *Psectrogaster amazonica*, *Semaprochilodus insignis* e *Prochilodus nigricans*). Os sons apresentaram diferenças entre gêneros e mesmo entre as espécies, mostrando a especificidade dos sons. Gravações subaquáticas em áreas de confluências de rios na bacia do Madeira durante o período reprodutivo nas águas altas (janeiro e fevereiro), indicaram que *P. latior* e *S. insignis* produziram sons semelhantes, utilizando o mesmo nicho ecológico no rio Guaporé. *P. latior* preferiu as confluências do rio e foi a principal espécie produtora de som durante o período de estudo, sendo encontrada na maioria dos locais avaliados. Foi observado que apenas os machos dessas espécies têm um músculo sônico extrínseco associado às primeiras costelas e fixado em uma aponeurose que rodeia a bexiga natatoria. Esses resultados podem ajudar a identificar e diferenciar sons subaquáticos, monitorando e avaliando áreas de ocorrência, indicando a utilização do método como ferramenta complementar para o monitoramento de populações de organismo aquáticos, principalmente espécies de peixes marinhos e de água doce.

Palavras-chave: Ecologia acústica**,** Paisagem acústica aquática, bioacústica, ruía antrofônico.

Abstract

Communication is omnipresent in aquatic animals, especially in those capable of producing different types of acoustic signals. This cacophony, called soundscape has allowed to evaluate both the distribution and behavior of organisms, simply recording and evaluating the sounds produced by them using the passive acoustic method. The objective of this work was to use innovative methods of noninvasive research, such as passive acoustics, in the sample and analysis of acoustic data in continental and marine aquatic environments, for monitoring, conservation and management of the living resources in these environments, mainly fish. In marine environment, the soundscape were evaluated of two coastal reef areas on the southern coast of the State of Pernambuco. Porto de Galinhas, an area already impacted by free access for tourism and fishing, and another in Tamandaré, within the Coastal Corals Protection Area, in the Marine Life Preservation Zone, used for research only. In a freshwater environment, the sounds production was appraise of five species of commercial importance of the order Characiformes in the Madeira River, southern Amazonia. The results of the marine experiments indicated that the sounds in both study areas occurred mainly in the late afternoon and at night, where six recurrent fish chorus were found, with a frequency band between 200 and 2000 Hz, without overlapping and with different acoustic characteristics between species. The chorus presented high energy in the open sea, after the last reef line in the Tamandaré protected area (~ 130 dB re1µPa2 Hz-1). However, fish coral presented low energy levels in Porto de Galinhas, with a difference of 30 dB re1µPa2 Hz-1. In addition, characteristic sounds of invertebrates were detected with a dominant frequencies band of 2 and 3 kHz, besides noise produced by different types of fishing vessels, recreational boats and ships, that can masked and interfere in the fish communication. The monitoring of the complex soundscape found in Tamandaré during the summer showed a constant temporal overlap of fish chorus produced in the late afternoon. The fish chorus occurrence were related to different lunar phases, influencing in a different way both the initial and detection time, with variation mainly during the first quarter moon. This also occurred in the noises of detected vessels, with fishing vessels reporting mostly early in the morning, during the crescent and waning gibbous moon, and frequency bands <200 Hz with high energy peaks of easily distinguishable. In continental waters, passive acoustics allowed the evaluation of five characiform sounds of commercial importance (*Potamorhina latior*, *P. altamazonica*, *Psectrogaster amazonica*, *Semaprochilodus insignis* and *Prochilodus nigricans*). The sounds presented differences between genus and even between species, showing the specificity of sounds. Underwater recordings in areas of river confluences in the Madeira basin during the reproductive period in the high waters (January and February) indicated that *P. latior* and *S. insignis* produced similar sounds, using the same ecologic niche in the Guaporé River. *P. latior* preferred the confluences of the river and was the main sound producing species during the study period, being found in most of the evaluated sites. It was observed that only the males of these species have an extrinsic sonic muscle associated with the first ribs and fixed in an aponeurosis that surrounds the lateral swimbladder. These results can help identify and differentiate underwater sounds by monitoring and evaluating areas of occurrence, indicating the use of the method as a complementary tool for the monitoring of aquatic organism populations, mainly marine and freshwater fish species.

Key words: Acoustic ecology, Aquatic soundscape, Bioacoustics, Anthrophonic noise

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1. Introdução

Diversos organismos aquáticos são capazes de emitir sons para a comunicação associados a diferentes tipos de comportamentos (TYACK, 1998). O conjunto destes sons permite definir a paisagem acústica de diversos ambientes, indicando seu estado de conservação e o grau de diversidade de espécies, além de ser útil para avaliar a ocorrência e distribuição dos organismos (PIJANOWSKI et al., 2011; FARINA e PIERETTI, 2012).

Para estudar a paisagem acústica e os sons que a compõem, recentemente vem sendo utilizado o método acústico passivo, que se baseia em ouvir os sons produzidos por organismos sonoros, possibilitando sua utilização dos sons como verdadeiros marcadores naturais das espécies, uma vez que são espécie-específicos. A acústica passiva também provê benefícios importantes para a investigação científica já que é uma ferramenta observacional não invasiva e não destrutiva, com uma capacidade de monitoramento remoto permanente ou de longa duração e fornece importantes informações sobre padrões diários e sazonais (ROUNTREE et al., 2006).

Estudos utilizando a acústica passiva vem permitindo avaliar a paisagem acústica em diversos ecossistemas aquáticos, principalmente marinhos de águas profundas (WALL et al., 2014) e costeiras de zonas temperadas (HARRIS et al., 2010, 2016; BUTLER et al., 2016) e tropicais, incluindo recifes de corais (STAATERMAN et al., 2013; BERTUCCI et al., 2015). Em ambientes marinhos predominam os sons biológicos (biofônicos) produzidos por diversas espécies de mamíferos aquáticos (JANIK et al., 2005), peixes (KAATZ, 2002; AMORIM et al., 2006), crustáceos (BOON et al., 2009), equinodermos (RADFORD et al., 2008) e moluscos (COQUEREAU et al., 2016), associados a diferentes comportamentos, como comunicação, predação, alimentação e reprodução. Dentre eles, os sons dos peixes (mais de 800 espécies conhecidas) está chamando a atenção devido a sua relação com diversos comportamentos, como reprodução (cortejo e desova), alimentação e proteção contra predadores (LADICH 2014; TRICAS e BOYD, 2014). Alguns peixes sonoros podem formar agregações e produzir grandes eventos sonoros do tipo coros (MOK e GILMORE, 1983).

Entretanto, investigar o comportamento dos peixes é especialmente difícil porque eles raramente podem ser vistos e contados. Neste caso, um dos maiores desafios para o estudo das populações de peixes é a capacidade de se coletar dados em diferentes escalas espaciais e temporais sem provocar interferências ou perturbações a destes animais.

Neste caso específico, o método acústico passivo vem sendo utilizado para monitorar atividades da desova de diversas espécies de importância comercial, principalmente da família Sciaenidae no Atlântico norte, como a atividade reprodutiva de uma agregação residente de *Cynoscion nebulosus* por longo período de tempo na costa oeste da Florida (WALTERS et al., 2009). Também foi possível mapear áreas de desova de *Cynoscion regalis, C. nebulous, Bairdiella chrysoura* e *Sciaenops ocellatus* em áreas costeiras da Carolina do Norte (LUCZKOVICH et al., 2008). Em águas temperadas do Atlântico sul, sabe-se que espécies da mesma família, como *Umbrina canosai* (TELLECHEA et al. 2017), *Cynoscion guatucupa* (TELLECHEA et al. 2012), *Pogonias cromis* (TELLECHEA et al. 2011a) e *Micorpogonias furnieri*, que emite sons associados ao comportamento de cortejo durante a época de desova de novembro a março, produzindo um som sazonal e diário característico de comportamento reprodutivo no estuário do Rio de la Plata o (TELLECHEA et al. 2011b).

A acústica passiva também tem sido muito útil para avaliar a produção de sons em espécies ameaçadas, como o bacalhau do Atlântico, onde foram identificados sítios de desova através dos sons produzidos pelos peixes durante o período reprodutivo, demonstrando ser a espécie fiel aos sítios de desova historicamente conhecidos. No mero (*Epinephelus itajara*), o uso desta técnica também tem sido útil para avaliar locais de reprodução, ajudando a implementar planos de manejo para a conservação de suas populações (ROWELL et al. 2012).

Em água doce da América do Sul, os primeiros trabalhos foram realizados com peixes da Amazônia, como é o caso da produção de sons de piranhas em cativeiro (MILLOT et al., 2011). JUNK, et al. (1997) relatam que os machos do jaraqui (*Semaprochilodus insignis*) produzem altos sons altos no início da época de reprodução, através de um músculo ao redor da bexiga do banho natatória, que por contração rítmica, causa a vibração da bexiga. Apesar da bacia amazônica ser o maior centro de biodiversidade de peixes de água doce do mundo (JUNK et al., 2007), representando provavelmente mais de 3500 espécies que habitam uma densa rede de rios conectados a extensas áreas de planícies de inundação (JUNK et al., 1997), apenas foram avaliados os sons de uma pequena parcela da ictiofauna. Alguns destes sons são simples relatos de pescadores, que afirmam estar vinculados à formação de grandes cardumes e a processos migratórios durante a reprodução. Acredita-se que áreas de confluências sejam habitats essenciais durante o período reprodutivo destas espécies (GOULDING, 1980).

No Brasil, a acústica passiva vem sendo utilizada recentemente, com gravações da pescada-branca em água doce, onde BORIE et al., (2014), avaliaram o dimorfismo sexual e padrões temporais dos sons produzidos no ambiente natural e de cultivo na bacia amazônica. Mais recentemente, SMITH et al. (2017) caracterizaram sons reprodutivos em três espécies de peixes curimbatás (Prochilodontidae), permitindo avaliar e controlar o comportamento de desova nessas espécies. Por outro lado, no ambiente marinho, SANCHES e PADOVESE (2016) iniciaram análises da paisagem acústica em áreas costeira do sudeste, encontrando diferentes tipos de coros. Na ilha oceânica de Trindade, BITTENCOURT et al. (2016) observaram que a paisagem acústica encontrada no verão foi predominantemente composta por coros de peixe, diminuindo no inverno, período no qual houve maior detecção de sons de baleias.

Nos peixes, os sons podem ser produzidos por diversos mecanismos, entre eles, e o mais comum, é o musculo sonoro (intrínseco e extrínseco) associado ou não com a bexiga natatória (LADICH e FINE, 2006), como também sons estridulatórios das nadadeiras peitorais, no caso dos bagres (FINE et al., 2011), ou dos ossos do crânio, como no cavalo marinho (OLIVEIRA et al., 2014), além da estridulação dos dentes faringeanos ainda pouco estudados, observado em alguns Perciformes.

Os sons produzidos por contração de um par de músculos sonoros, de forma geral, estão tipicamente presentes nos machos e ocasionalmente em ambos os sexos de ciaenídeos (CHAO, 1978). Em algumas espécies, o músculo sonoro se desenvolve junto com a maturação dos testículos (HILL et al., 1987) e triplica em massa durante o período reprodutivo (CONNAUGHTON et al., 2002, LAGARDÈRE e MARIANI, 2006). Em espécies do Atlântico sul, como a pescada-amarela (*C. acoupa*), pescada (*C. leiarchus*), pescada-go (*Macrodon ancylodon*) e boca-mole (*Larimus breviceps*), foi observado um par de músculos sonoros (observação pessoal), associados com a bexiga natatória, comumente encontrados em espécies da família Sciaenidae e possivelmente responsável pela produção de sons nestas espécies. No caso de *Micropogonias furnieri*, o músculo sonoro não mostra diferenças entre macho e fêmea (DEVINCENTI et al. 2012). Na Amazônia, as gônadas dos machos de *Plagioscion squamosissimus* se desenvolvem em sincronia com o músculo sonoro, emitindo sons mais longos quando madura durante a seca, onde se agregam para o cortejo/desova (BORIE et al., 2014). Levar-nos a supor, que provavelmente espécies de ciaenídeos marinhos também tenham tais características.

Recentemente, diversos estudos avaliaram a interferência de sons antropogênicos sobre a comunicação dos organismo aquáticos. No ambiente marinho, são diversas as formas de emissão de sinais acústicos gerados pelos seres humanos, sendo as principais delas as embarcações, de diferentes tipos e tamanhos, e também as plataformas de petróleo.

O tráfego de embarcações em áreas costeiras pode produzir ruídos capazes de influenciar de forma negativa o comportamento dos organismos marinhos. Recentemente, o número de estudos que avaliam a paisagem acústica vem comprovando que a poluição sonora no ambiente marinho pode afetar o comportamento de diversos táxons (WILLIAMS et al., 2015; KAPLAN et al., 2016; PINE et al., 2016), interferindo na comunicação dos peixes (SIMPSON et al., 2016) durante o período reprodutivo ou na comunicação de mamíferos aquáticos para fins de orientação espacial (BLAIR et al., 2017). Os estudos também sugerem uma variedade de impactos biológicos e ecológicos em crustáceos, que vão do aumento da locomoção e estresse, comportamento anti-predador reduzido e mais lento, a mudanças na alimentação e no comportamento intraespecífico (TIDAU e BRIFFA, 2016). Desta forma, identificando os sons gerados por organismos marinhos e possíveis interferências dos sons antrópicos na comunicação entre os indivíduos.

Neste sentido, pouco ou quase nada sabemos a respeito da paisagem acústica e os sons emitidos por diversas fontes que a compõem nos diferentes ecossistemas aquáticos tropicais brasileiros. Assim, faz-se necessário conhecer e avaliar a paisagem acústica em ambientes altamente produtivos, como as áreas de recifes costeiros e importantes rios da bacia amazônica, com espécies de importância comercial, principalmente peixes emissores de sons, nos quais a acústica passiva pode ser aplicada para o monitoramento das populações e do habitat.

2. Referencias

AMORIM, M.P. Diversity of sound production in fish. **Communication in fishes**, v. 1, p. 71-104, 2006.

BERTUCCI, F. et al. Temporal and spatial comparisons of underwater sound signatures of different reef habitats in Moorea Island, French Polynesia. **PLoS ONE**, v. 10, n. 9, p. 1-12, 2015. ISSN 10.1371/journal.pone.0135733.

BITTENCOURT, L. et al. Underwater noise pollution in a coastal tropical environment. **Marine Pollution Bulletin**, v. 83, n. 1, p. 331-336, 2014. ISSN 10.1016/j.marpolbul.2014.04.026.

BITTENCOURT, L. et al. Acoustic habitat of an oceanic archipelago in the Southwestern Atlantic. **Deep-Sea Research Part I: Oceanographic Research Papers**, v. 115, p. 103-111, 2016. ISSN 10.1016/j.dsr.2016.06.001.

BLAIR, H. B. et al. Evidence for ship noise impacts on humpback whale foraging behaviour. **Biology Letters**, v. 12, n. 8, p. 20160005, 2016. ISSN 10.1098/rsbl.2016.0005.

BOON, P. Y.; YEO, D. C. J.; TODD, P. A. Sound production and reception in mangrove crabs *Perisesarma* spp. (Brachyura: Sesarmidae). **Aquatic Biology**, v. 5, n. 2, p. 107-116, 2009. ISSN 10.3354/ab00136.

BORIE, A. et al. Spatiotemporal variability and sound characterization in silver croaker *Plagioscion squamosissimus* (Sciaenidae) in the Central Amazon. **PLoS ONE**, v. 9, n. 8, 2014. ISSN 10.1371/journal.pone.0099326.

BUTLER, J.; STANLEY, J. A.; BUTLER, M. J. Underwater soundscapes in near-shore tropical habitats and the effects of environmental degradation and habitat restoration. **Journal of Experimental Marine Biology and Ecology**, v. 479, p. 89-96, 2016. ISSN 10.1016/j.jembe.2016.03.006.

CONNAUGHTON, M. A.; FINE, M. L.; TAYLOR, M. H. Review. Weakfish sonic muscle: influence of size, temperature and season. **Journal of Experimental Biology**, v. 205, n. Pt 15, p. 2183-2188, 2002. ISSN 10.1111/J.1749-6632.1908.TB55101.X.

COQUEREAU, L. et al. Marine soundscape shaped by fishing activity. **Royal Society Open Science**, v. 4, n. 1, p. 160606, 2017. ISSN 10.1098/rsos.160606.

COUNTY, R. Sound Production in, v. 2000, n. 2, p. 567-571, 2000.

FARINA, A.; PIERETTI, N. The soundscape ecology: A new frontier of landscape research and its application to islands and coastal systems. **Journal of Marine and Island Cultures**, v. 1, n. 1, p. 21-26, 2012. ISSN 10.1016/j.imic.2012.04.002.

FINE, M. L. et al. A primer on functional morphology and behavioural ecology of the pectoral spine of the channel catfish. **American Fisheries Society Symposium**, v. 77, n. Collin 2003, p. 745-753, 2011.

GOULDING, Michael. **The fishes and the forest: explorations in Amazonian natural history**. Univ of California Press, 1980.

HARRIS, S. A.; SHEARS, N. T.; RADFORD, C. A. Ecoacoustic indices as proxies for biodiversity on temperate reefs. **Methods in Ecology and Evolution**, v. 7, n. 6, p. 713-724,

2016. ISSN 10.1111/2041-210X.12527.

HILL, G. L.; FINE, M. L.; MUSICK, J. A. Ontogeny of the Sexually Dimorphic Sonic Muscle in Three Sciaenid Species. **Copeia**, v. 1987, n. 3, p. 708, 5 ago. 1987. ISSN 10.2307/1445663.

JANIK, VINCENT M. Underwater acoustic communication networks in marine mammals. **Animal communication networks**, p. 390-415, 2005.

JUNK, Wolfgang J. General aspects of floodplain ecology with special reference to Amazonian floodplains. In: **The Central Amazon Floodplain**. Springer, Berlin, Heidelberg, 1997. p. 3-20.

KAATZ, I. M. Multiple sound-producing mechanisms in teleost fishes and hypotheses regarding their behavioural significance. **Bioacoustics**, v. 12, n. 2-3, p. 230-233, jan. 2002. ISSN 10.1080/09524622.2002.9753705.

KAPLAN, M. B. et al. Temporal and spatial variability in vessel noise on tropical coral reefs, v. 005002, p. 005002, 2016. ISSN 10.1121/2.0000250.

LADICH, F. Fish bioacoustics. **Current Opinion in Neurobiology**, v. 28, p. 121-127, 2014. ISSN arXiv:1011.1669v3.

LADICH, F.; FINE, M. L. Sound-Generating Mechanisms in Fishes: A Unique Diversity in Vertebrates. **Communication in Fishes**, n. January, p. 3-43, 2006. ISSN 978-1578083282.

LAGARDÈRE, J. P.; MARIANI, A. Spawning sounds in meagre Argyrosomus regius recorded in the Gironde estuary, France. **Journal of Fish Biology**, v. 69, n. 6, p. 1697-1708, 1 dez. 2006. ISSN 10.1111/j.1095-8649.2006.01237.x.

LUCZKOVICH, J. J. et al. Identifying Sciaenid Critical Spawning Habitats by the Use of Passive Acoustics. **Transactions of the American Fisheries Society**, v. 137, n. 2, p. 576-605, 2008. ISSN 10.1577/T05-290.1.

LUCZKOVICH, J. J.; MANN, D. A.; ROUNTREE, R. A. Passive Acoustics as a Tool in Fisheries Science. **Transactions of the American Fisheries Society**, v. 137, n. 2, p. 533-541, 2008. ISSN 10.1577/T06-258.1.

MILLOT, S.; VANDEWALLE, P.; PARMENTIER, E. Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. **Journal of Experimental Biology**, v. 214, n. 21, p. 3613-3618, 2011. ISSN 10.1242/jeb.061218.

MOK, Hin-Kiu; GILMORE, R. Grant. Analysis of sound production in estuarine aggregations of *Pogonias cromis*, *Bairdiella chrysoura*, and *Cynoscion nebulosus* (Sciaenidae). **Bulletin of the Institute of Zoology, Academia Sinica**, 1983.

OLIVEIRA, T. P. R. et al. Sounds produced by the longsnout seahorse: A study of their structure and functions. **Journal of Zoology**, v. 294, n. 2, p. 114-121, 2014. ISSN 10.1111/jzo.12160.

PIJANOWSKI, B. C. et al. Soundscape Ecology: The Science of Sound in the Landscape. **BioScience**, v. 61, n. 3, p. 203-216, 2011. ISSN 10.1525/bio.2011.61.3.6.

PINE, M. K. et al. The potential for vessel noise to mask biologically important sounds within ecologically significant embayments. **Ocean and Coastal Management**, v. 127, p. 63-73, 2016. ISSN 10.1016/j.ocecoaman.2016.04.007.

PRELIMINAR, E. et al. Preliminary Study of the Sonic Muscle of *Micropogonias furnieri* (Actinopterygii , Sciaenidae): Morphology and Histochemistry. **Int. J. Morphol**, v. 30, n. 4, p. 1442-1448, 2012.

RADFORD, C. et al. Resonating sea urchin skeletons create coastal choruses. **Marine Ecology Progress Series**, v. 362, p. 37-43, 2008. ISSN 10.3354/meps07444.

ROUNTREE, R. A. et al. Listening to fish: acoustics to fisheries science. **Fisheries**, v. 31, n. 9, p. 433-446, 1 set. 2006. ISSN 10.1577/1548-8446(2006)31[433:LTF]2.0.CO;2.

ROWELL, T. J. et al. Sound production as an indicator of red hind density at a spawning aggregation. **Marine Ecology Progress Series**, v. 462, p. 241-250, 2012. ISSN 10.3354/meps09839.

SÁNCHEZ-GENDRIZ, I.; PADOVESE, L. R. Underwater soundscape of marine protected areas in the south Brazilian coast. **Marine Pollution Bulletin**, v. 105, n. 1, p. 65-72, 2016. ISSN 10.1016/j.marpolbul.2016.02.055.

SIMPSON, S. D. et al. Anthropogenic noise increases fish mortality by predation. **Nature**

Communications, v. 7, p. 1-7, 2016. ISSN 10.1038/ncomms10544.

SMITH, M. E. et al. Mating calls of three prochilodontid fish species from Brazil. **Environmental Biology of Fishes**, n. December, p. 1-13, 2017. ISSN 10.1007/s10641-017-0701-3.

STAATERMAN, E. et al. Soundscapes from a Tropical Eastern Pacific reef and a Caribbean Sea reef. **Coral Reefs**, v. 32, n. 2, p. 553-557, 2013. ISSN 10.1007/s00338-012-1007-8.

TELLECHEA, J. S. et al. Calls of the black drum (*Pogonias cromis*: Sciaenidae): Geographical differences in sound production between northern and southern hemisphere populations. **Journal of Experimental Zoology Part A: Ecological Genetics and Physiology**, v. 315 A, n. 1, p. 48-55, 2011. ISSN 10.1002/jez.651.

TELLECHEA, J. S.; BOUVIER, D.; NORBIS, W. Spawning sounds in whitemouth croaker (Sciaenidae): Seasonal and daily cycles. **Bioacoustics**, v. 20, n. 2, p. 159-168, 2011. ISSN 10.1080/09524622.2011.9753641.

TELLECHEA, J. S.; FINE, M. L.; NORBIS, W. Passive acoustic monitoring, development of disturbance calls and differentiation of disturbance and advertisement calls in the Argentine croaker *Umbrina canosai* (Sciaenidae). **Journal of Fish Biology**, v. 90, n. 4, p. 1631-1643, 2017. ISSN 10.1111/jfb.13257.

TELLECHEA, J. S.; NORBIS, W. Sexual dimorphism in sound production and call characteristics in the striped weakfish *Cynoscion guatucupa*. **Zoological Studies**, v. 51, n. October, p. 946-955, 2012.

TIDAU, S.; BRIFFA, M. Review on behavioral impacts of aquatic noise on crustaceans. **Proceedings of Meetings on Acoustics**, Vol. 27, 2016. p. 010028.

TRICAS, T. C.; BOYLE, K. S. Acoustic behaviors in Hawaiian coral reef fish communities. **Marine Ecology Progress Series**, v. 511, p. 1-16, 2014. ISSN 10.3354/meps10930.

TYACK, P. L. Acoustic communication under the sea. In: **Animal acoustic communication**. Springer, Berlin, Heidelberg, 1998. p. 163-220.

WALL, Carrie C. et al. An exploration for deep-sea fish sounds off Vancouver Island from the NEPTUNE Canada ocean observing system. **Deep Sea Research Part I:**

Oceanographic Research Papers, v. 83, p. 57-64, 2014.

WALTERS, S. et al. Using a Passive Acoustic Survey to Identify Spotted Seatrout Spawning Sites and Associated Habitat in Tampa Bay, Florida. **Transactions of the American Fisheries Society**, v. 138, n. 1, p. 88-98, 2009. ISSN 10.1577/T07-106.1.

3. Objetivos

3.1 Objetivo geral

- ✓ Estudar a paisagem sonora de ambientes aquáticos tropicais brasileiros, identificando os padrões sonoros de organismos que neles habitam, através da acústica passiva.

3.2 Objetivos específicos

- ✓ Caracterizar a paisagem sonora e os sinais acústicos de origem biológica (peixes e crustáceos) detectados em duas áreas de recifes costeiros do litoral sul de Pernambuco (Porto de Galinhas e Tamandaré).

- ✓ Avaliar a distribuição espacial e temporal das fontes emissoras de sons, identificando possíveis formações de agregações nestas áreas de recifes costeiros do litoral sul de Pernambuco.

- ✓ Avaliar e caracterizar os sinais acústicos de origem antropogênica, principalmente de embarcações que trafegam pela Zona de Preservação da Vida Marinha em Tamandaré.

- ✓ Avaliar a paisagem acústica em áreas de confluência de rios da bacia do rio Madeira (RO) onde acredita-se ocorrerem agregações reprodutivas de espécies migradoras de Characiformes.

- ✓ Caracterizar e avaliar os padrões acústicos de perturbação e os mecanismos sonoros de espécies de peixes migradores da ordem Characiformes em rios da Bacia do Rio Madeira.

4. Artigo Científico I

SOUNDSCAPE AND BIOPHONIC SIGNATURES IN PROTECTED AND UNPROTECTED AREAS OF COSTAL REEF IN NORTHEAST BRAZIL

Abstract

In marine environment is possible to detect and record different sound events, that allow to infer on behavioral patterns and distribution of several species of crustaceans, fish and mammals. In order to evaluate the occurrence of acoustic signals in two tropical coastal reef areas located in Tamandaré and Porto de Galinhas, recorders were made between January and April of 2016, using two "sonobuoys" equipped with recorder and hydrophone. The recordings were started during the sunset (~12 hours of recording per day). Results point to a complex soundscape dominated by biological sounds audibly distinguished, produced by crustaceans and fish. Fish chorus produced different dominant frequency, distributed between 200 to 1000 Hz. The chorus occurred in a similar time during the sunset (~17:30) except the Chorus I, detected after midnight in both areas with similar acoustic energy. The chorus presented high-energy callings after the last reef line in the protected area of Tamandaré (~130 dB re1µPa^2 Hz^{-1}), in the open sea. However, fish chorus presented low-energy levels in Porto de Galinhas, with a difference of 30 dB re1µPa^2 Hz^{-1} being difficult to detect the sunset chorus. These results indicate the importance of the protected area for fish populations, and the usefulness of the passive acoustic method to monitoring the biodiversity of possible commercial fish sounds in Brazilian tropical costal reef.

Key words: Bioacoustics, Marine cacophony, Fish sounds.

Introduction

Monitoring changes in environments and their inhabitants is a critical management need and a considerable technological challenge. In this sense, the marine soundscape can be an effective means of assessing biological and anthropogenic activity in places where continuous monitoring by traditional research methods is impractical. Listening the ambient has allowed us to study the sound production and interaction of sounds from specific sources in different environments.

The sound sources that compose the soundscape in the marine environment may be of biological origin, such as sounds produced by various aquatic animals (mammals, fish and invertebrates), anthropogenic sounds (especially vessels) and geological sounds (wind, waves and rain). The soundscape provides a means to understand biotic and abiotic relationships in many different environments (PIJANOWSKI et al., 2011). The soundscape is a reliable indicator of habitat type and potentially transmit habitat quality information (LILLIS et al., 2014). Applied to any ecosystem, it provides valuable information on natural acoustic signals that occur in several ecosystems (STAATERMAN et al., 2013).

In general, each type of aquatic environment has characteristic acoustic signals. In this sense, listening the marine soundscape using passive acoustics as a complementary tool can allow the inference of social interactions and obtain information about the behaviour and distribution of sound sources.

Marine soundscapes are composed of many different biotic components (biophonic), including, fish (AMORIM et al., 2006), shrimps (LAMMERS and MUNGER., 2016), bivalves (COQUEREAU et al., 2016; LILIS et al., 2016), crabs (BOON et al., 2009), lobster (BUSCAINO et al., 2011), sea urchins (RADFORD et al. 2008), and several marine mammals. All these sounds are produced in a variety of behavioural context, which can influence important life history strategies, such as reproduction and aggression, and potentially drive the population dynamics of certain habitats, such as rocky and coral reefs.

In the case of fish, the sound is species-specific, allowing researchers to make inferences about the spatial and temporal distribution and behaviors (ROUNTREE et al., 2006). In addition, there are a relationship between sounds and several behaviors (TRICAS and BOYLE, 2014), like courtship and spawning aggregation (MOK et al., 2009; HERNANDEZ et al., 2013; LOWERRE-BARBIER et al., 2013; SCHÄRER et al., 2014; WILSON et al., 2014;), aggression and territorialism (PICCULIN et al., 2006; MILLOT et al., 2011; COLLEYE and PARMENTIER, 2012; PEREIRA et al., 2014; WALL et al., 2014), and during predation and feeding (AMORIM et al., 2004; FINE et al., 2011). The fish sounds aggregation during courtship, spawning or feeding in schools, producing massive fish chorus events (MCCAULEY, 2012; PARSON et al., 2016, 2017).

One of these behaviors, like fish aggregation is getting the attention since involves commercial importance sonorous species. Many of these fish chorus have been studied in the North Atlantic and Caribbean, especially members of the families Sciaenidae, Gadidae, Ictaluridae, Batrachoididae, Haemulidae, Lutjanidae and Serranidae. (LUCZKOVICH et al., 2008).

Although efforts have been made studies in the region of the South Atlantic are scarce and limited to the southwest Brazil (SÁNCHEZ-GENDRIZ and PADOVESE, 2015; 2016), it is little known about fish choruses in tropical Atlantic coastal areas. Therefore, the aims of this work was to investigate the soundscapes of protected and unprotected coastal reef areas in a tropical Brazilian ecosystem. More specifically, the present study was designed to detect and assess fish chorusing, thus providing a baseline on which future long-term soundscape monitoring programs can be based to conservations actions.

Material and methods

Study area

The study was carried out in two coastal reefs, located at Tamandaré and Porto de Galinhas beaches, southern Pernambuco State of northeastern Brazil (Figure 1). These areas are part of the north-eastern coral reef system characterized by reef lines parallel to the coast (RODRÍGUES-RAMÍREZ *et al*. 2008). The areas consists of elongated and discontinuous reefs, with the major axis parallel to the coastline. The individual dimensions of these reefs vary from less than 1 km in length to 4 km in the reefs closer to the beach (DOMINGUEZ et al., 1990). The first reef line is near to the beach, the second reef line is between the first line and the outer reef (third line), which is exposed to the sea (MAIDA and FERREIRA, 2006).

The two areas are popular tourist destinations, but Porto de Galinhas, located closer to Recife (60 km), is one of the most visited beaches in Brazil. Almost unrestricted tourism infers direct sources of environmental impact through trampling, waste and artificial feeding offered to fish by tourists (BARRADAS et al., 2010, 2012; OLIVEIRA et al., 2010). Although the two areas are inserted in the same reef type system the management regimes are different. The first area (Porto de Galinhas) is not under any type of protection, while the second (Tamandaré) is part of the largest Brazilian coastal conservation unit, the “Costa dos Corais” Marine Protected Area (CCMPA), created in 1997. Located in the CCMPA, it is inserted the Marine Life Preservation Zone (MLPZ), closed to fishing and visitation since 1999 (FERREIRA and MAIDA, 2006). The fish community within Tamandaré is diverse and composed of estuarine and reef associated species as well as pelagic species (FERREIRA and CAVA, 2001; MERIGOT *et al*., 2016)

Soundscape and fish chorus recordings

A sonobuoy was developed for the recordings in costal reef. The sonobuoy was and equipped with manufacturer-calibrated omnidirectional hydrophone (H2A, Aquarian Audio, Anacortes, WA, USA, useful range of <10 Hz to >100 KHz, sensitivity of -180 dB re: 1 V / µPa, flat frequency response: ±4 dB in the range: 20 Hz to 4.5 kHz), connected to a Panasonic RR-XS450 digital recorder (16-bit WAV format and sampling rate of 44 kHz). The sonobuoy was built with low-cost materials, using a 20 mm diameter and 2.5 m long PVC pipe. A weight was fixed at the lower end of the pipe and in its middle a buoy was installed for the flotation, and a PVC box of 1000 cm^3 located in the upper end (1.5 m out of the water) housed the digital recorder (Figure 2).

The sonobuoy were installed in three sites within the Marine Life Preservation Zone (MLPZ 1, 2 and 3) near the reef called "*Ilha da Barra*" in the bay of Tamandé (Figure 1), during non-consecutive days of January 2016. Recordings started at the end of the morning, with a duration of 20, 23 and 13 hours in each site respectively.

After the prior analyses, two sonobuoys similar to previous ones (with a Zoom H1 digital recorder, Zoom, New York, USA) were used to evaluate simultaneously the acoustic signals in two stations in Tamandaré (MLPZ 3 and MLPZ 4) and two in Porto de Galinhas (PGA 1 and PGA 2) between February and April of 2016. Recordings were performed simultaneously in both sites of each location and began during the sunset and ended at dawn. During the summer, the sunset takes place at approximately 5:30 and sunrise at 5:20, with almost the same duration of day and night.

The sonobuoys were launched and attached to an anchor buoy in areas with depths between 6-8 m near to the reef and 14-16 m beyond the last reef line, leaving the hydrophone cable submerged 6 m from the surface. In Tamandaré the buoys were installed equidistant from each other at about 1200 m and at a distance of 1.4 and 2 km of the beach line of Tamandaré bay (Figure 1). Recordings in Porto de Galinhas were sampled in similar areas located before the last reef line (PGA 1) and beyond the last reef line (PGA 2) at about 800 m between each other, and 600 m and 1200 m from the coast, respectively.

Fish calls analyses

Combined recordings of 286 hours of were analysed, being 180 h from Tamandaré and 106 h from Porto de Galinhas. The files were downloaded to a portable computer where a pre-evaluation was done using Audacity® (v.2.2) recording and editing software to exclude any anthropogenic noise during sonobuoy installation. Audacity® was also used to select parts of the recordings for individual analyses of the signal.

In order to evaluate the frequency band distribution over time and the energy of the coastal reef soundscape, spectrograms and power spectra were created using the PAMGuide toolbox (MERCHANT et al., 2015) in the MATLAB 2016. The PAMGuide was used to calculate the relative power spectral density (PSD, dB re 1 µPa2 / Hz). The spectrograms and power spectra were initially plotted with a frequency band between 50 and 10000 Hz (pre-analyses), and was used a band between 50 and 5000 Hz for the soundscape analysis. The Mean Square Root (RMS) levels of the PSD values were calculated for all days of recording at each point.

In order to perform the individual analysis of each acoustic signature ("calls"), Raven Pro 1.4 (Cornell Laboratory of Ornithology) software was used. Five minutes sections of each sound were used for individual characterization analysis of each call (acoustic unit) type. The sections were chosen according to quality and intensity, being selected sections where there was no signal overlapping (when possible). The selected parts were firstly filtered between the limits of the frequency bands observed for each acoustic signal in the spectrograms. The individual characterization of each signal was performed using the following acoustic parameters: 1. Number of pulses/call (n), 2. Call duration (time between first and last pulse, ms), 3. Pulse rate (n pulses/second), 4. Pulse period (time between the peaks of second and third pulses), 5. Low-frequency limit (Hz), 6. High-frequency limits (Hz), 7. Central frequency (Hz), 8. Dominant frequency (Hz) (the description of the last two parameters can be obtained in Charif et al. (2010). The acoustic parameters were measured using oscillograms and spectrograms, using a resolution (DFT) of 1024 and overlapping of 99%. The acoustic parameters of each sound signal were compared using non-parametric Kruskal-Wallis multiple comparison tests between the different groups for each variable ($P<0.05$) in the STATISTICA 7 software (Dell Inc.).

Results

Soundscape and fish chorus recordings

The recordings showed a soundscape composed by a biophony of snapping shrimps and different fish sounds around the "*Ilha da Barra*" reef in Tamandaré (Figure 3). The frequency of these biophony was less than 4 kHz, and the acoustic signals occurred partitioned by time. The snapping sounds presented frequencies between 2 and 3 kHz, and occurred at higher densities sound during sunset and dawn. Fish chorus presented frequencies between 200 and 1800 Hz.

Around the "*Ilha da Barra*" reef (MPLZ 1 and 2) fish choruses were also detected with different acoustic characteristics. Chorus I was a tonal signal with harmonics in the frequency band 400 – 2000 Hz, while Chorus II slightly overlapped with Chorus I and had a frequency band of 1600 – 1800 Hz with no harmonics (Figure 3A). These sounds were emitted after midnight and during late-night. Chorus III had a frequency band of 200 to 800 Hz and occurred with high energy levels just before the sunset and after midnight. In this area, the snapping frequency predominated, while Chorus I, II and III presented lower acoustics energy levels (Figure 3B). A little further away from the "*Ilha da Barra*" (MLPZ 3), and despite a shorter recording time, three other types of fish choruses were detected, "Chorus IV" with a frequency band of 800 – 3500 Hz, "Chorus V" (150 – 900 Hz) and "Chorus VI" (80 – 300 Hz). These signals occurred at the beginning of the night with temporal overlap. The snapping and "Chorus III" sounds were not evident in MLPZ 3 (Figure 3C). The Power Spectral Density (PSD) analyses indicated higher acoustic levels in MLPZ 3, as well as Chorus I peaks of a dominant frequency of ~900 Hz, but with a wide frequency band overlapping and masking the other signals (Figure 4).

The number of occurrence during the recordings showed a Chorus I predominance that occurred more often than any other chorus observed in Tamandaré and Porto de Galinhas (Figure 5). The costal soundscape of Tamandaré (MLPZ 3 and MLPZ 4) and Porto de Galinhas (PGA 1 and PGA 2) were composed by fish chorus producing sounds during the sunset and late night. Furthermore, the fish choruses previously founded were better detected in the furthest site in Tamandaré (MLPZ 4). Snapping sounds and Chorus III were less detectable from sites MLPZ 3 and 4 in Tamandaré bay (Figure 1), most likely due to the distance of the site from the reefs.

In Porto de Galinhas was possible to detect low energy levels of "Chorus V" and "Chorus VI", as well a sound produced like a rapping (denominated "Rap") in the beginning of the night (Figure 5 C and D). The "Rap" sound presented similar characteristics of sound type "Snap", with a dominant frequency ~2000 and 2400 Hz respectively (Table 1), but it was audibly different and found also near Ilha da Barra reef in Tamandaré (Figure 6). This rapping sound was detected only in Porto de Galinhas, in areas near the second reef line (PGA 1).

The spectral analyses were separated in two schedules due to the wide frequency band of the “Chorus I” (overlapping the other sounds), before and after midnight (Figure 6). The MLPZ 4 presented higher acoustic levels for the “Chorus I”, “Chorus V” and “Chorus VI” (detected before sunset), “Chorus II” And “Chorus IV” (Figure 7A). The "Chorus I" chorus (detected after midnight) showed similar acoustic energy at the four stations sampled (Figure 7B).

Fish calls analyses

In general, the calls were complex, and were formed by sets of pulse trains, presenting different individual characteristics (acoustic signatures). The calls were composed of 3 or more pulses (Figure 9 and Table 1), ranging from 3 to 39 depending on the type of call. Calls with 3 pulses were found in Chorus II, III and VI . The calls of “Chorus I” presented the highest number of pulses per call (mean of 25.1) followed by “Chorus V” (mean of 19.3 pulses per call) (Table 2).

The pulses contain simple or several or simple cycles, as it occurs in “Chorus V” (Figure 9 A), “Chorus III” (Figure 9E) and “Chorus II” (Figure 7. F), or paired, as in the case of "Chorus I" (Figure 9B), "Chorus IV" (Figure 9C) and “Chorus VI” (Figure 9D). The "Chorus II" sound presented a couple of pulses emitted prior to the call and had the highest number of pulses per minute when compared to the other sounds, with an average of 25 pulses per call (Table 2). The number of pulses has a positive relationship with the duration of each individual sound (Figure 10A), increasing the duration as the number of pulses increases. This is directly related to both pulse rate and pulse period (Figure 9A). The pulse rate showed that the call was higher in "Chorus V" and "Chorus I", emitting about 60 and 122 pulses per second respectively and a shorter pulse period (~9 ms) for the case of “Chorus I” calls (Table 2), evidencing a very fast and efficient sound mechanism.

There was a correlation between dominant frequency and pulse period, and between dominant frequency and pulse rate for the "Chorus I", "Chorus V", "Chorus VI" and "Chorus III" sounds, and dominant frequency increased as both the pulse period decreased (Figure 9B.) and pulse rate increased (Figure 9C.). This was not observed for the "Chorus IV" and "Chorus II" calls. The "Chorus IV" sound, unlike the other four choruses, had a duration of approximately 1 second, with less pulses.

Dominant frequencies were 204, 570, 925 and 1838 Hz for Chorus VI, Chorus V, Chorus I and Chorus IV, respectively. Although there is a frequency overlap between

"Chorus IV" and "Chorus II" sounds, and their values did not differ significantly between them, it may be related to the pulse rate, since this value also did not show differences. Nevertheless, these sounds have a different frequency band (low and high), pulse period, number of pulses and, consequently, call duration (Table 2). To the same extent that "Chorus V" and "Chorus VI" sounds have presented frequencies close to each other, all acoustic parameters indicate a significant difference between both sounds ($p<0.05$). Even though the dominant frequency values between "Chorus VI" and "Chorus III" do not show differences, they do not occur in the same location ($p>0.05$).

Comparing individual calls of Chorus I and *Larimus breviceps* (sound-producing fish commonly found in the present study area), collected and recorded by Fish and Mowbray (1970) (available at www.fishbase.org), it was possible to observe a similarity of both pulses, composed of two oscillations of greater magnitude , and mainly with the harmonic distribution of the frequency bands, occurring with peak intervals every 100 Hz (Figure 10)

Discussion

Marine soundscape will depend on the different physical, biological and geological factors that compose the marine environments. In reefs, biological factors include biomass, density and diversity of fish with frequencies <1 kHz, as well as benthic community (> 1 kHz) in addition to depth and sea state (KENNEDY et al., 2010). In the present work it was possible to detect different acoustic signals with distinguishable sound characteristics. These sounds present different frequency bands (between ~200 and 4000 Hz) emitted by aggregations of several species of fish, and probably crustaceans.

The snapping sounds founded in Tamandaré coastal reefs areas (MLPZ 1, 2 and 3) emitted a frequency band between 1 - 4 kHz and a dominant frequency between ~ 2 and 3 kHz. This sound occurred as a constant cacophony along the day, with highest energy at night, with frequencies reaching up to 22050 Hz (our sampling limit). The "Snap" type signal presented similar characteristics to those found in some marine invertebrates, mainly the *Alpheus* genera produces snapping sounds with a wide frequency band between ~ 1 and 15 kHz (SCHMITZ, 2002; COQUEREAU et al., 2016). Snapping shrimp (family Alpheidae) produce the major component of reef noise at frequencies above 2 kHz, with higher intensities at the beginning and end of the night in Hawaiian reef (LAMMERS and MUNGER, 2016).

Another important component of the cacophony found in this work may be the collaboration of sounds emitted by lobsters. Species of the genus *Palunirus* sp. can emit stridulatory sounds with frequencies between 2 to 5.5 kHz (MULLIGAN and FISCHER, 1977), and dominant frequency between 3.7 and 5.2 kHz (LATHA et al., 2005). Kikuchi et al. (2015) suggest that the frequency of stridulant sounds possibly reflects the activity and presence of commercially important lobsters of the same genus. Recently, the feeding and stress sounds of *Panulirus argus* and *P. laevicauda* have been evaluated, showing a sound production in *P. argus* (BORIE et al., In prep) emitted by the movement of the antenna, as previously observed by Patek (2002).

The “Rap” type sound found as an aggregation in Porto de Galinhas during the sunset has similar characteristics to Brachyura: Ocypodidae, with some species having frequencies of up to 2 kHz (HORCH, 1975). In addition, they present long and dominant frequencies between 1.1 and 3.2 kHz (CLAYTON, 2001) and can transmit frequencies of 5 kHz during feeding in species of the genus *Maja* sp. (COQUEREAU et al., 2016). The fiddler and ghost crabs possess a genetic tendency to use acoustic display (beyond visual) as both calling and/or courtship signals. In this case, for nocturnally active females, acoustic calling by males is clearly better (LOWRY, 1980). The rapping and snapping sounds were also detected with similar acoustic characteristics and time in the intermediate estuarine portion of the Maracaipe River (BORIE *et al., in prep*), with similar acoustic characteristics (Annex II), indicating different habitats. Species of the Majidae family, together with Alpheidae and Palinuridae can be commonly found in our study area, mainly in Porto de Galinhas (GIRALDES et al., 2015) as well as in other areas of the Pernambuco coast (COELHO et al., 2006, 2007; BARRETO et al., 1993), contributing positively to the cacophony of crustaceans found in this study.

The six chorus types detected and analyzed individually have characteristics of sounds produced by fish, being mainly detected during sunset and late night and audibly distinguishable from each other. The "Chorus III" was found only near to the “Ilha da barra” reef (MLPZ 1 and 2), and is commonly heard during diurnal diving activities in the study area (authors pers. observ.). This Chorus presented a dominant frequency mean of 414 Hz, similar to reproductive sounds found in the coral reef damselfish (Pomacentridae) (MANN and LOBEL., 1997; MARUSKA et al., 2007; PARMENTIER et al., 2009, 2016). The Chorus III is also similar to Batrachoididae fish, with dominant frequencies between 300 and 500 Hz (AMORIN et al., 2006; RICE and BASS, 2009), producing sounds during the day (RICE et al., 2001).

The "Chorus IV", "Chorus II", "Chorus V" and "Chorus VI" presented temporal and slightly low and high limits frequency overlap during the sunset (~17:30). The frequency bands of the choirs were varied, being broad in the "Chorus I" and "Chorus IV" and narrow in "Chorus II" and "Chorus VI". In this sense, some of the choruses may present different frequency band distribution, and could show spatial and temporal overlapping (PEARSON et al., 2016a), which would show that in the absence of visual sense, sound communication is more important (RUPPÉ et al., 2015). The lack of time and chorus frequency partitioning in hours of darkness illustrates the complexity of monitoring different communities of sound-producing fish (PEARSON et al., 2016b) in Australia..

Similar chorus were founded in fish chorus detected in coastal marine protected areas in southeaster sub-tropical regions of Brazil (SÁNCHEZ-GENDRIZ and PADOVESE, 2016), although they were emitted in different times during the day. The Chorus frequency was similar to "Chorus I" harmonic signals with a frequency band between 400 to 1400 Hz, as well as the "Chorus V" (frequency between 400 and 800 Hz), and the "Chorus II" sound, as a narrow frequency band between 1500 and 1800 Hz

The "Chorus I" had similar spectral characteristics and was also detected twice a day, with no temporal acoustic competition between them, but rather a temporal overlap such as the "Chorus V" observed during the early morning hours (SÁNCHEZ-GENDRIZ and PADOVESE, 2017). This could indicate that the fish species emitting this type of signals can have a wide distribution in the Brazilian coast, being able to occur in both tropical and temperate waters, and that the time difference found can be related to the migratory patterns of these species.

Thus, in this study we present evidence that the acoustic energy of the "Chorus IV", "Chorus II", "Chorus V" and "Chorus VI" in Tamandaré were higher in the offshore, just beyond the last coastal reef line. It has been seen that several habitats showed significantly different energies for most of frequencies and a decreasing sound energy was observed from the reef to the coast, showing that closely related habitats separated by 1 km may differ significantly in their spectral composition and that these signatures can be typical and conserved along the coast (BERTUCCI et al., 2015). The imperceptive or low acoustic energy of crustaceans observed in MLPZ 4 and PGA 2 may be related to the distance from the reef. In this sense, Kaplan and Mooney (2016) said that the sound of the reef is of low intensity and may not reach distances > 1.5 km.

The detection of fish chorus in this area may be associated with the type of substrate that occurs at these sites. In coastal reefs located on the southern coast of Pernambuco it is possible to find muddy substrate from coastal rivers contribution (KEMPF, 1967) present in the study area, where sediments accumulate (mainly loam) in a depression after the last reef line. The muddy patches areas present an important commercial interest, since in these areas shrimps and fish, mainly from the Sciaenidae family, are caught by artisanal fishery, the last ones as bycatch in trawling, where several species such, as *Larimus*, *Stellifer*, *Isopisthus*, *Paralonchurus*, *Ophioscion*, *Cynoscion*, *Macrodon*, *Menticirrhus*, *Micropogonias*, and *Nebris* are found (JÚNIOR et al., 2105). Some of these species can also be found in Brazilian temperate waters (SCHMIDT and DIAS, 2012), and with genera widely distributed throughout the western Atlantic.

One of the most interesting and known aspects about the Scieanidae family is sound production, whose species are commonly called "croakers". The sounds are emitted by several species of this family, mainly during the night in the reproductive period, possibly to aggregate the shoal (LAGARDÈRE and MARIANI, 2006; LUCZKOVICH et al., 2008; MOK et al., 2009). Several studies related to Sciaenid sounds were developed in the Northwest Atlantic, where field studies using the "passive acoustic" method allowed monitoring the spawning activity of a resident aggregation of *Cynoscion nebulosus* over a long period of time (WALTERS et al., 2005). It was also possible to map critical spawning habitats areas of *Cynoscion regalis*, *Bairdiella chrysoura*, and *Sciaenops ocellatus* (LUCZKOVICH et al., 2008). In the Southwest Atlantic, *Micropogonias furnieri* produces a characteristic seasonal and daily sound of courtship/spawning behaviour in the Rio de la Plata estuary from November to March (TELLECHEA et al., 2011). Larvae of Sciaenidae were one of the most abundant in areas near our study site (BEZERRA-JUNIOR et al., 2011), which may indicate that the sounds found could be species of the family Sciaenidae during reproductive process.

The results of this work allow to infer that "Chorus I", "Chorus V" and "Chorus VI" sounds have similar acoustic characteristics to those found in the west Atlantic, where the acoustic record of *Larimus braviceps* (FISH and MOWBRAY, 1970, and their audio available at www.fishbase.com), a widely distributed specie with harmonious "hornlike" sounds and frequencies between 500 and 1000 Hz (RAMCHARITAR et al., 2006), which is commonly found in the study area, it was observed a pair of sonic muscle associated with the swimbladder (personal observations). Similarly, *Micropogonias furnieri*, a species of commercial importance and commonly captured by artisanal fishermen, which during the reproductive period can emit disturbance sounds with frequencies of ~300 Hz (TELLECHEA et al., 2010).

Several species of the genus *Cynoscion*, with widly distributed throughout the west Atlantic, are known to emit sounds during the reproductive period, with species emitting a wide range of dominant frequencies of ~ 347 to 1046 Hz (SPRAGUE et al., 2000), mainly in northwest Atlantic, and *C. gutupaca* with a dominant frequency of 450 Hz in southwest Atlantic (TELLECHEA and NORBIS., 2012). These differences in frequencies could be caused by differences in swimbladder size, which is correlated with fish size (CONNAUGHTON et al., 2000). Therefore, along the western Atlantic it is possible to find much of the acoustic records of Sciaenidae. The close relationship between the choruses I, V and VI may also be related to the sound mechanism used. Sounds production in those fish occurs through a pair of sonic muscles, commonly found in males (CHAO, 1978).

These results indicate a complex soundscape, mainly composed by biological sounds Produced by crustaceans and six fish chorus in the tropical Brazilian coast, that can be easily acoustically distinguished. This points to the usefulness of the passive acoustic method in long term monitoring of commercially important sound-producing species in areas where they are located after the last reef line, in places with the occurrence of loam substrates, which may be considered as essential habitats for these species. Greater efforts are still needed to identify the different sound sources that compose the soundscape in this area of study, as well as other coastal environmental along Brazilian coast.

References

AMORIM, M. C. P. Diversity of sound production in fish. **Communication in fishes**, v.1, p.71-104, 2006.

AMORIM, M. C. P.; STRATOUDAKIS, Y.; HAWKINS, A. D. Sound production during competitive feeding in the grey gurnard. **Journal of fish biology**, v.65, 182-194, 2004.

BARRADAS, J. I.; AMARAL, F.D.; HERNÁNDEZ, M. I.; FLORES-MONTES, M. J.; STEINER, A. Q. Tourism impact on reef flats in Porto de Galinhas beach, Pernambuco, Brazil. **Arquivos de Ciências do Mar**, v.45, p.81-88, 2012.

BARRADAS, J. I.; AMARAL, F. D.; HERNÁNDEZ, M. I. M.; MONTES, M. D. J. F.; STEINER, A. Q. Spatial distribution of benthic macroorganisms on reef flats at Porto de Galinhas Beach (northeastern Brazil), with special focus on corals and calcified hydroids. **Biotemas**, v.23, p.61-67, 2010.

BARRETO, A.; COELHO, P.; RAMOS-PORTO, M.; TORRES, M. Distribuição geográfica dos Brachyura (Crustacea, Decapoda) coletados na plataforma continental do Norte e Nordeste do Brasil. **Revista Brasileira de Zoologia**, v.10, p.641-656, 1993.

BERTUCCI, F.; PARMENTIER, E.; BERTEN, L.; BROOKER, R. M.; LECCHINI, D. Temporal and spatial comparisons of underwater sound signatures of different reef habitats in Moorea Island, French Polynesia. **PloS one**, v.10, p.e0135733, 2015.

BEZERRA, J. L.; JUNIOR, X.; NEUMANN-LEITÃO, S. Diversidade de larvas de peixes das áreas internas e externas do porto de Suape (Pernambuco-Brazil). **Tropical Oceanography**, v.39, p.1-13, 2011.

BITTENCOURT, L.; BARBOSA, M.; SECCHI, E.; LAILSON-BRITO, J.; AZEVEDO, A. Acoustic habitat of an oceanic archipelago in the Southwestern Atlantic. **Deep Sea Research Part I: Oceanographic Research Papers**, v.115, p.103-111, 2016.

BOARD, O. S.; COUNCIL, N. R. Marine Mammals and Low-Frequency Sound: Progress Since 1994. Washington: DC, The National Academies Press, 2000, 160p.

BOHL, E. Diel pattern of pelagic distribution and feeding in planktivorous fish. **Oecologia**, v.44, 368-375, 1979.

BOHNENSTIEHL, D. R.; LILLIS, A.; EGGLESTON, D. B. The curious acoustic behavior of estuarine snapping shrimp: temporal patterns of snapping shrimp sound in sub-tidal oyster reef habitat. **PloS one**, v.11, p.e0143691, 2016.

BOON, P.; YEO, D.; TODD, P. Sound production and reception in mangrove crabs *Perisesarma* spp. (Brachyura: Sesarmidae). **Aquatic Biology**, v.5, p.107-116, 2009.

BURKE, N. C. Nocturnal foraging habitats of French and bluestriped grunts, *Haemulon flavolineatum* and H. sciurus, at Tobacco Caye, Belize. **Environmental Biology of Fishes**, v.42, p.365-374, 1995.

BUSCAINO, G.; FILICIOTTO, F.; GRISTINA, M.; BELLANTE, A.; BUFFA, G.; DI STEFANO, V.; MAZZOLA, S. Acoustic behaviour of the European spiny lobster *Palinurus elephas*. **Marine Ecology Progress Series**, v.441, p.177-184, 2011.

CHAO, L. N. A bassis for classifying Western Atlantic Sciaenidae (Teleostei: Perciformes).

NOAA Technical Report, v.415, p.1-64, 1978.

CHARIF, R.A.; WAACK, A.M.; STRICKMAN, L.M. Raven Pro 1.4 User's Manual. Cornell Lab of Ornithology, Ithaca: NY, 2010, 379p.

CHAVES, L.; PEREIRA, P.; FEITOSA, J. Coral reef fish association with macroalgal beds on a tropical reef system in North-eastern Brazil. **Marine and Freshwater Research**, v.64, p.1101-1111, 2013.

COELHO, P. A.; ALMEIDA, A.; BEZERRA, L.; SOUZA-FILHO, J. An updated checklist of decapod crustaceans (infraorders Astacidea, Thalassinidea, Polychelida, Palinura, and Anomura) from the northern and northeastern Brazilian coast. **Zootaxa**, v.1519, p.1-16, 2007.

COLLEYE, O.; PARMENTIER, E. Overview on the diversity of sounds produced by clownfishes (Pomacentridae): importance of acoustic signals in their peculiar way of life. **PloS one**, v.7, p.e49179, 2012.

COQUEREAU, L.; GRALL, J.; CHAUVAUD, L.; GERVAISE, C.; CLAVIER, J.; JOLIVET, A.; DI IORIO, L. Sound production and associated behaviours of benthic invertebrates from a coastal habitat in the north-east Atlantic. **Marine Biology**, v.163, p.1-13, 2016.

FARINA, A.; PIERETTI, N. The soundscape ecology: A new frontier of landscape research and its application to islands and coastal systems. **Journal of Marine and Island Cultures**, v.1, p.21-26, 2012.

FERREIRA, B. P.; MAIDA, M. 2006. Monitoramento dos recifes de coral do Brasil. Brasília-DF, MMA, Secretaria de Biodiversidade e Florestas, 2006, 120 p.

FINE, M. L.; SISMOUR, E. N.; NEWTON, S. H.; BOSHER, B. T.; SULLIVAN, A. D.; MIANO, J. P.; MICHALETZ, T. A primer on functional morphology and behavioral ecology of the pectoral spine of the channel catfish. **American Fisheries Society Symposium**, v.77, p.745–753, 2011.

GIRALDES, B. W.; COELHO FILHO, P. A.; SMYTH, D. M. Decapod assemblages in subtidal and intertidal zones—Importance of scuba diving as a survey technique in tropical reefs, Brazil. **Global Ecology and Conservation**, v.3, p.163-175, 2015.

HERNANDEZ, K. M.; RISCH, D.; CHOLEWIAK, D. M.; DEAN, M. J.; HATCH, L. T.; HOFFMAN, W. S.; VAN PARIJS, S. M. Acoustic monitoring of Atlantic cod (*Gadus morhua*) in Massachusetts Bay: implications for management and conservation. **ICES Journal of Marine Science**, v.70, p.628–635, 2013.

HOBSON, E. S. Diurnal-nocturnal activity of some inshore fishes in the Gulf of California. **Copeia**, v.3, p.291-302, 1965.

HOBSON, E. S. Feeding patterns among tropical reef fishes. **American Scientist**, v.63, p.382-392, 1975.

HORCH, K. The acoustic behavior of the ghost crab *Ocypode cordimana* Latreille, 1818 (Decapoda, Brachyura). **Crustaceana**, v.29, p.193-205, 1975.

KAPLAN, M. B.; MOONEY, T. A. Coral reef soundscapes may not be detectable far from the reef. **Scientific reports**, v.6, p.31862, 2016.

KEMPF, M. A plataforma continental de Pernambuco (Brasil): nota prelirninar sobre a natureza do fundo. **Trab. Oceanog. Univ. Fed. Pernambuco**, v.11, p.111-24, 1970.

KENNEDY, E.; HOLDERIED, M.; MAIR, J.; GUZMAN, H.; SIMPSON, S. Spatial patterns in reef-generated noise relate to habitats and communities: evidence from a Panamanian case study. **Journal of Experimental Marine Biology and Ecology**, v.395, p.85-92, 2010.

KIKUCHI, M.; AKAMATSU, T.; TAKASE, T. Passive acoustic monitoring of Japanese spiny lobster stridulating sounds. **Fisheries Science**, v.81, p.229-234, 2015.

LABOREL, J. Les peuplements de madréporaires des côtes tropicales du Brésil. **Annales de l'Université d'Abidjan**, v.2, p.1-260, 1969.

LADICH, F. Fish bioacoustics. **Current opinion in neurobiology**, v.28, p.121-127, 2014.

LAGARDERE, J.; MARIANI, A. Spawning sounds in meagre *Argyrosomus regius* recorded in the Gironde estuary, France. **Journal of fish biology**, v.69, p.1697-1708, 2006.

LAMMERS, M. O.; MUNGER, L. M. From Shrimp to Whales: Biological Applications of Passive Acoustic Monitoring on a Remote Pacific Coral Reef. In: AU, W.; LAMMERS, M. Listening in the Ocean. Modern Acoustics and Signal Processing. New York: NY. Springer, 2016, p.61-81.

LATHA, G.; SENTHILVADIVU, S.; VENKATESAN, R.; RAJENDRAN, V. Sound of shallow and deep water lobsters: Measurements, analysis, and characterization (L). **The Journal of the Acoustical Society of America**, v.117, p.2720-2723, 2005.

LILLIS, A.; EGGLESTON, D. B.; BOHNENSTIEHL, D. R. Estuarine soundscapes: distinct acoustic characteristics of oyster reefs compared to soft-bottom habitats. **Marine Ecology Progress Series**, v.505, p.1-17, 2014.

LOBEL, P. S.; KAATZ, I. M.; RICE, A. N. Acoustical behavior of coral reef fishes. In: COLE, K. Reproduction and sexuality in marine fishes: patterns and processes. Berkeley: University of California Press, 2010, p.307-386.

LOWERRE-BARBIERI, S. K.; WALTERS, S.; BICKFORD, J.; COOPER, W.; MULLER, R. Site fidelity and reproductive timing at a spotted seatrout spawning aggregation site: individual versus population scale behavior. **Marine Ecology Progress Series**, v.481, p.181-197, 2013.

LUCZKOVICH, J. J.; MANN, D. A.; ROUNTREE, R. A. Passive acoustics as a tool in fisheries science. **Transactions of the American Fisheries Society**, v.137, p.533-541, 2008.

MAIDA, M.; FERREIRA, B, P. Coral reefs of Brazil: an overwiew. **Proc. 8th International Coral Reef Symposium,** v.1, p.263-274, 1997.

MANN, D. A. Remote sensing of fish using passive acoustic monitoring. **Acoustics today**, v.8, p.8-15, 2012.

MANN, D. A.; LOCASCIO, J. V.; COLEMAN, F. C.; KOENIG, C. C. Goliath grouper *Epinephelus itajara* sound production and movement patterns on aggregation sites. **Endangered Species Research**, v.7, p.229-236, 2009.

MCCAULEY, R. D. Fish choruses from the Kimberley, seasonal and lunar links as determined by long term sea noise monitoring. Proceedings of Acoustics 2012 Fremantle, Western Australia, November, 21-23, 2010.

MCCAULEY, R. D.; CATO, D. H. Patterns of fish calling in a nearshore environment in the

Great Barrier Reef. **Philosophical Transactions of the Royal Society of London B: Biological Sciences**, v.355, p.1289-1293, 2000.

MERCHANT, N. D.; FRISTRUP, K. M.; JOHNSON, M. P.; TYACK, P. L.; WITT, M. J.; BLONDEL, P.; PARKS, S. E. Measuring acoustic habitats. **Methods in Ecology and Evolution**, v.6, p.257-265, 2015.

MILLOT, S.; VANDEWALLE, P.; PARMENTIER, E. Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. **Journal of Experimental Biology**, v.214, p.3613-3618, 2011.

MOK, H.K.; YU, H.Y.; UENG, J.P.; WEI, R.C. Characterization of sounds of the blackspotted croaker *Protonibea diacanthus* (Sciaenidae) and localization of its spawning sites in estuarine coastal waters of Taiwan. **Zoological Studies**, v.48, p.325-333, 2009.

MULLIGAN, B.; FISCHER, R. Sounds and behavior of the spiny lobster *Panulirus argus* (Latreille, 1804) (Decapoda, Palinuridae). **Crustaceana**, v.32, p.185-199, 1977.

NAGELKERKEN, I.; DORENBOSCH, M.; VERBERK, W.; DE LA MORINIÈRE, E. C.; VAN DER-VELDE, G. Day-night shifts of fishes between shallow-water biotopes of a Caribbean bay, with emphasis on the nocturnal feeding of Haemulidae and Lutjanidae. **Marine Ecology Progress Series**, v.194, p.55-64, 2000.

PARMENTIER, E.; VANDEWALLE, P.; BRIÉ, C.; DINRATHS, L.; LECCHINI, D. Comparative study on sound production in different Holocentridae species. **Frontiers in zoology**, v.8, p.1-12, 2011.

PARSONS, M. J.; SALGADO-KENT, C. P.; MARLEY, S.A.; GAVRILOV, A. N.; MCCAULEY, R. D. Characterizing diversity and variation in fish choruses in Darwin Harbour. **ICES Journal of Marine Science: Journal du Conseil**, v.73, p.2058-2074, 2016.

PARSONS, M. J.; SALGADO-KENT, C. P.; RECALDE-SALAS, A.; MCCAULEY, R. D. Fish choruses off Port Hedland, Western Australia. **Bioacoustics**, v. 26, p.135-152., 2017.

PEREIRA, P. H. C.; MORAES, R. L.; SANTOS, M. V. B.; LIPPI, D. L.; FEITOSA, J. L. L.; PEDROSA, M. The influence of multiple factors upon reef fish abundance and species richness in a tropical coral complex. **Ichthyological Research**, v.61, p.375-384, 2014.

PEREIRA, R.; RISMONDO, S.; CAIANO, M.; PEDROSO, S. S.; FONSECA, P. J.; AMORIM, M. C. P. The role of agonistic sounds in male nest defence in the painted goby *Pomatoschistus pictus*. **Ethology**, v.120, p.53-63, 2014.

PICCIULIN, M.; SEBASTIANUTTO, L.; COSTANTINI, M.; ROCCA, M.; FERRERO, E. A. Aggressive territorial ethogram of the red-mouthed goby, *Gobius cruentatus* (Gmelin, 1789). **Electronic Journal of Ichthyology**, v.2, p.8419, 2006.

PIJANOWSKI, B. C.; VILLANUEVA-RIVERA, L.J.; DUMYAHN, S. L.; FARINA, A.; KRAUSE, B. L.; NAPOLETANO, B. M.; PIERETTI, N. Soundscape ecology: the science of sound in the landscape. **BioScience**, v.61, p.203-216, 2011.

RADFORD, C.; JEFFS, A.; TINDLE, C.; MONTGOMERY, J. C. Resonating sea urchin skeletons create coastal choruses. **Marine Ecology Progress Series**, v.362, p.37-43, 2008.

RADFORD, C.; STANLEY, J.; TINDLE, C.; MONTGOMERY, J.; JEFFS, A. Localised coastal habitats have distinct underwater sound signatures. **Marine Ecology Progress Series**, v.401, p.21-29, 2010.

RAMCHARITAR, J.; GANNON, D. P.; POPPER, A. N. Bioacoustics of fishes of the family Sciaenidae (croakers and drums). **Transactions of the American Fisheries Society**, v.135, p.1409-1431, 2006.

RICE, A. N.; BASS, A. H. Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. **Journal of Experimental Biology**, v.212, p.1377-1391, 2009.

ROUNTREE, R. A.; GILMORE, R. G.; GOUDEY, C. A.; HAWKINS, A. D.; LUCZKOVICH, J. J.; MANN, D. A. Listening to fish: applications of passive acoustics to fisheries science. **Fisheries**, v.31, p.433-446, 2006.

RUPPÉ, L.; CLÉMENT, G.; HERREL, A.; BALLESTA, L.; DÉCAMPS, T.; KÉVER, L.; PARMENTIER, E. Environmental constraints drive the partitioning of the soundscape in fishes. **Proceedings of the National Academy of Sciences**, v.112, p.6092-6097, 2015.

SÁNCHEZ-GENDRIZ, I.; PADOVESE, L. Underwater soundscape of marine protected areas in the south Brazilian coast. **Marine pollution bulletin**, v.105, p.65-72, 2016.

SÁNCHEZ-GENDRIZ, I.; PADOVESE, L. Temporal and spectral patterns of fish choruses in two protected areas in southern Atlantic. **Ecological Informatics**, v.38, p.31-38, 2017.

SCHÄRER, M. T.; NEMETH, M. I.; ROWELL, T. J.; APPELDOORN, R. S. Sounds associated with the reproductive behavior of the black grouper (*Mycteroperca bonaci*). **Marine Biology**, v.161, p.141-147, 2014.

SCHMITZ, B. Sound production in Crustacea with special reference to the Alpheidae. In: Wiese, K. The crustacean nervous system. Springer, Berlin, 2002. p. 536-547.

SILVA-JÚNIOR, C. A. B.; VIANA, A. P.; FRÉDOU, F. L.; FRÉDOU, T. Aspects of the reproductive biology and characterization of Sciaenidae captured as bycatch in the prawn trawling in the northeastern Brazil. **Acta Scientiarum. Biological Sciences**, v.37, p.1, 2015.

SOUZA, C.; BATISTA, V.; FABRE, N. N. Caracterização da pesca no extremo sul da Área de Proteção Ambiental Costa dos Corais, Alagoas, Brasil. **Boletim do Instituto de Pesca**, v.38, p.155-169, 2012.

STAATERMAN, E.; RICE, A.; MANN, D.; PARIS, C. Soundscapes from a Tropical Eastern Pacific reef and a Caribbean Sea reef. **Coral Reefs**, v.32, p.553-557, 2013.

TELLECHEA, J. S.; MARTINEZ, C.; FINE, M. L.; NORBIS, W. Sound production in the whitemouth croaker and relationship between fish size and disturbance call characteristics. **Environmental Biology of Fishes**, v.89, p.163-172, 2010.

TELLECHEA, J. S.; NORBIS, W. Sexual dimorphism in sound production and call characteristics in the striped weakfish *Cynoscion guatucupa*. **Zoological Studies**, v.51, p.946-955, 2012.

TORRES, F. T. P.; OLIVEIRA MACHADO, P. J. Introdução à climatologia. São Paulo: Cengage Learning, 2011, 280 p.

TRICAS, T. C.; BOYLE, K. S. Acoustic behaviors in Hawaiian coral reef fish communities. **Marine Ecology Progress Series**, v.511, p.1-16, 2014.

VASCONCELOS, R. O.; CARRIÇO, R.; RAMOS, A.; MODESTO, T.; FONSECA, P. J.; AMORIM, M. C. P. Vocal behavior predicts reproductive success in a teleost fish. **Behavioral Ecology**, v.23, p. 375-383, 2011.

WALL, C.; SIMARD, P.; LINDEMUTH, M.; LEMBKE, C.; NAAR, D.; HU, C.; MANN, D. Temporal and spatial mapping of red grouper *Epinephelus morio* sound production. **Journal of fish biology**, v.85, p.1470-1488, 2014.

WALTERS, S.; LOWERRE-BARBIERI, S.; BICKFORD, J.; CRABTREE, L.; MANN, D. Preliminary results on seasonal and diel periodicities of a resident *Cynoscion nebulosus* spawning aggregation in Tampa Bay, Florida. **Proceedings of the Gulf and Caribbean Fisheries Institute**, v.58, p.295–299, 2007.

WILSON, L.; BURROWS, M.; HASTIE, G.; WILSON, B. Temporal variation and characterization of grunt sounds produced by Atlantic cod *Gadus morhua* and pollack *Pollachius pollachius* during the spawning season. **Journal of fish biology**, v.84, p.1014-1030, 2014.

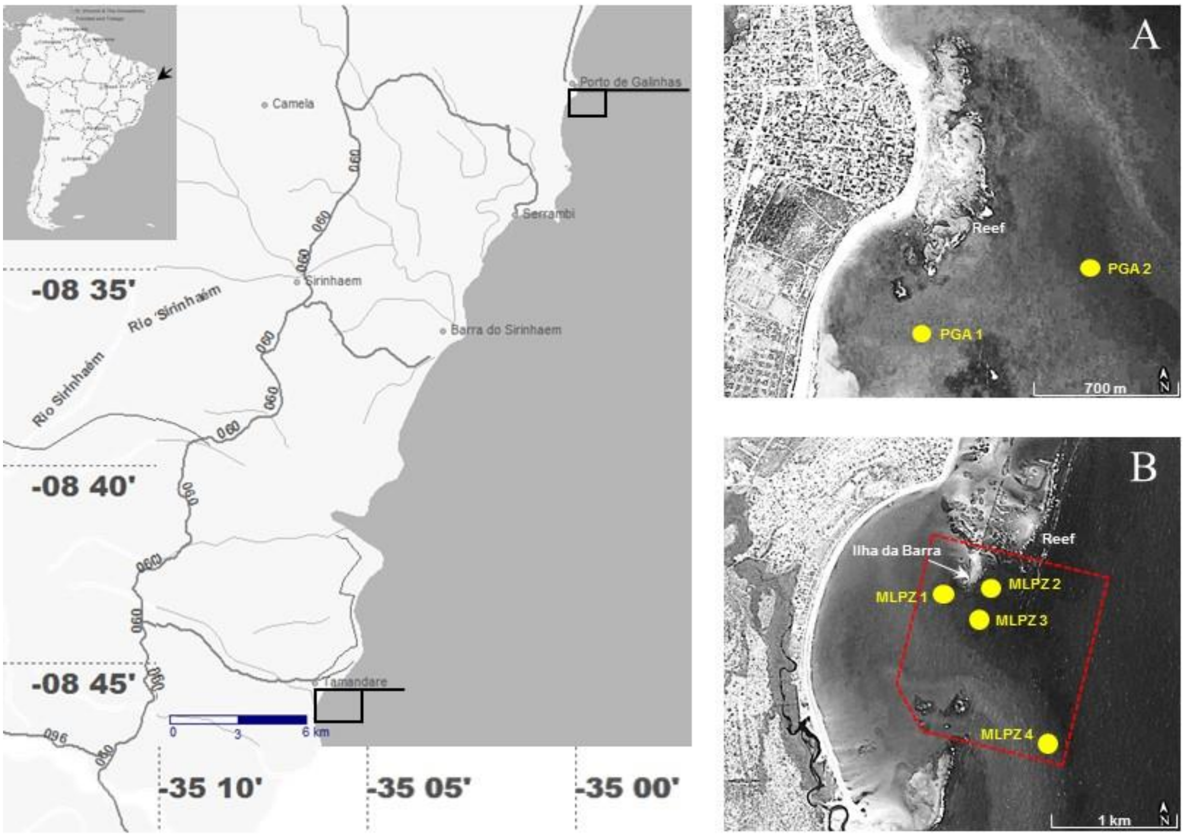

Figure 1. Map of study areas and location of “sonobuoy” deployed in Porto de Galinhas (A) and Tamandaré (B), and reef proximity in both locations. The limits of Marine Life Preservation Zone in Tamandaré (MLPZ) is represent by the red dashed line.

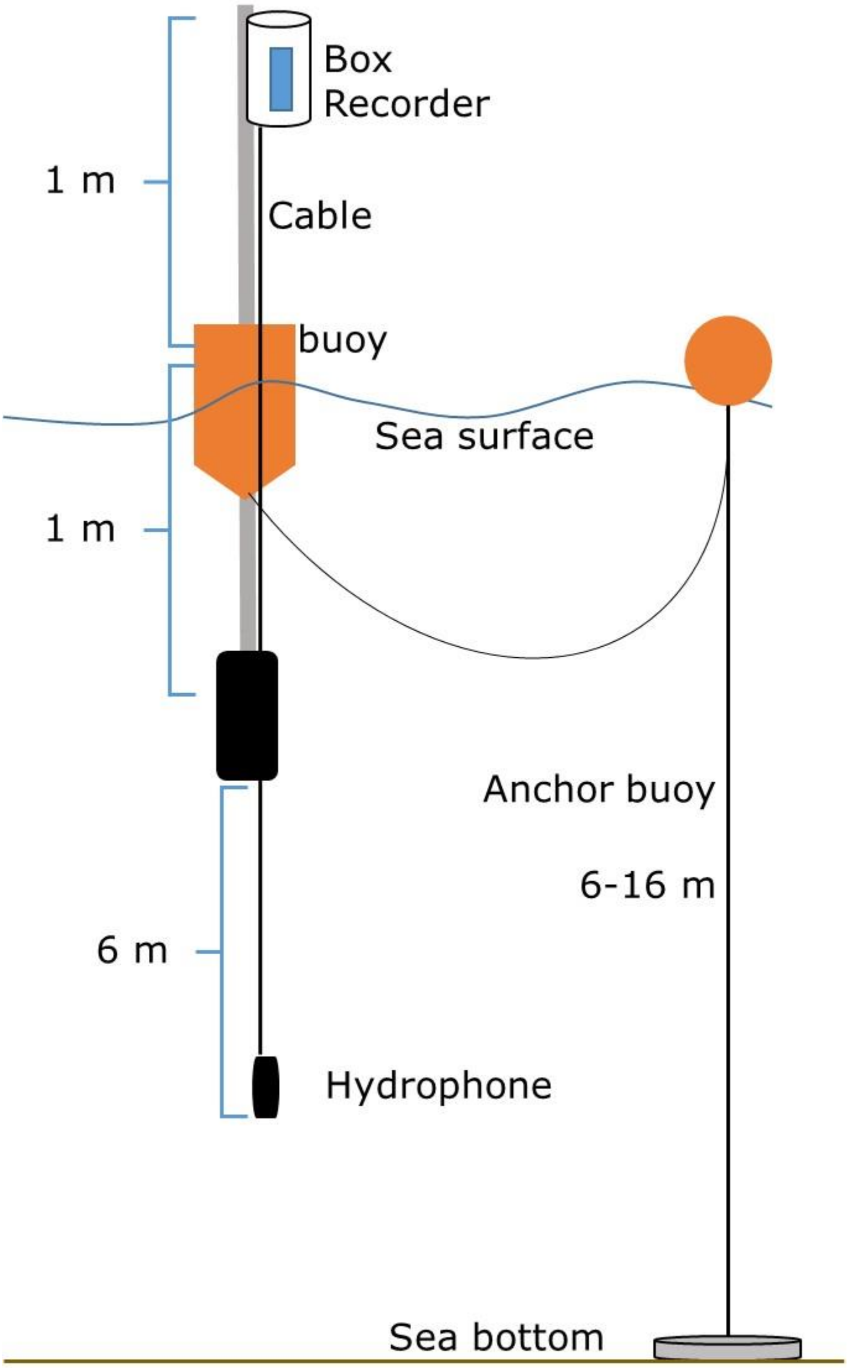

Figure 2. Sonobuoy configuration deployed in Tamandaré and Porto de Galinhas

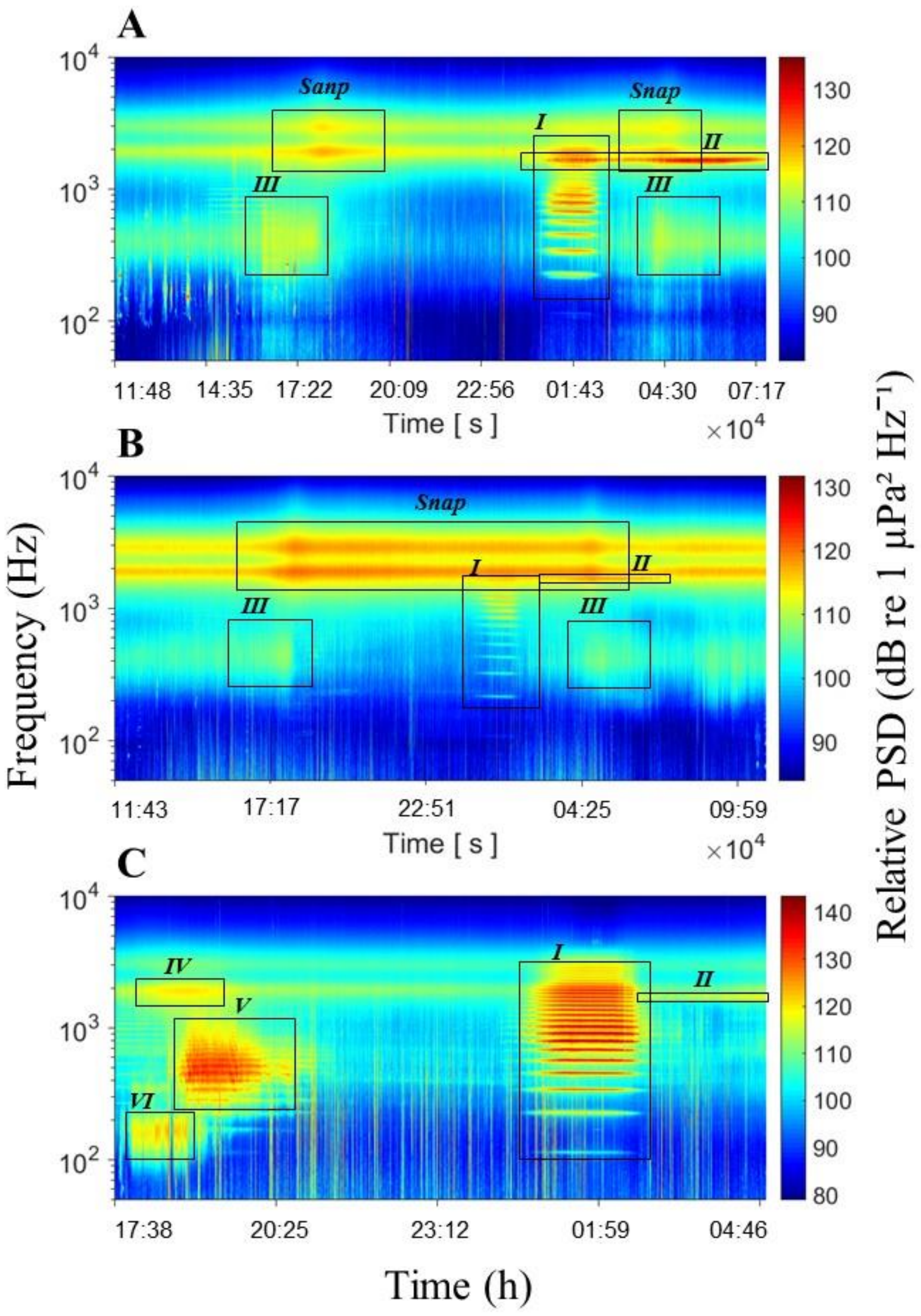

Figure 3. Spectrograms (sonograms) showing the time variation of the frequency and the power spectral density (PSD) of recording near to “Ilha da barra” reef in Tamandaré. A. MLPZ 1 (Jan, 24); B. MLPZ 2 (Jan, 26) and C. MLPZ 3 (Feb, 04), highlighting the main acoustics signals. “Snap” corresponding to crustacean sounds and “Chorus I”, “Chorus II”, “Chorus III”, “Chorus IV”, “Chorus V” and “Chorus VI”considered as fish sounds.

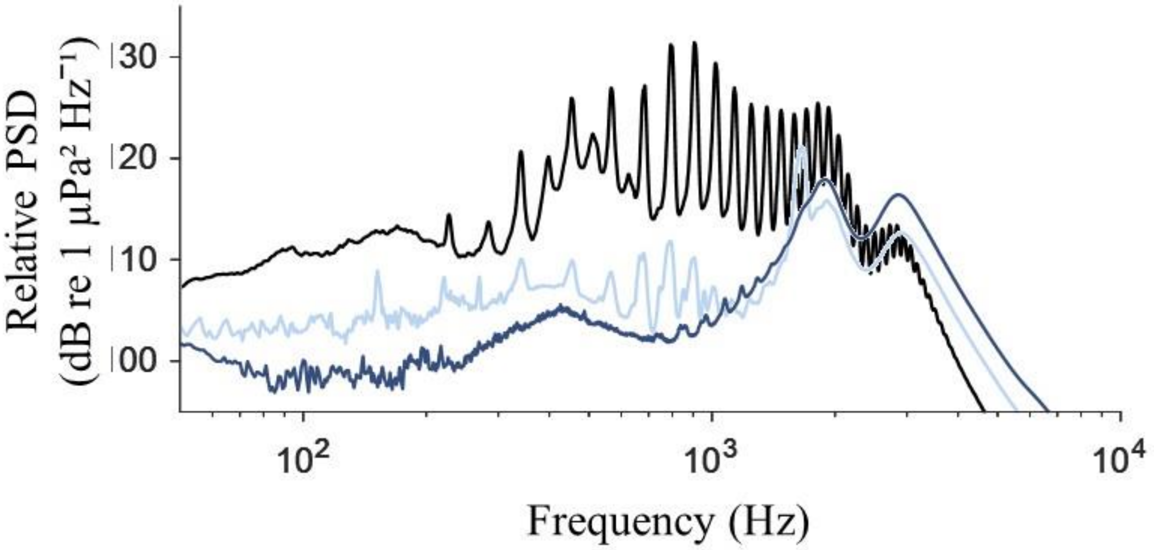

Figure 4. Average power spectrum (spectra) averaged emphasizing the frequency (log) components that occur continually in the recordings around the “Ilha da barra” reef. MLPZ 1 Jan, 24 (grey line), MLPZ 2 Jan, 26 (blue line) and C MLPZ 3 Feb, 04 at night (black line). The peaks of dominant frequency correspond to “Chorus II” (~1700 Hz, grey line), “Chorus IV” (~1800 Hz, blue line) and “Chorus I” (~900 Hz, black line), with broad frequency band masking the other signals.

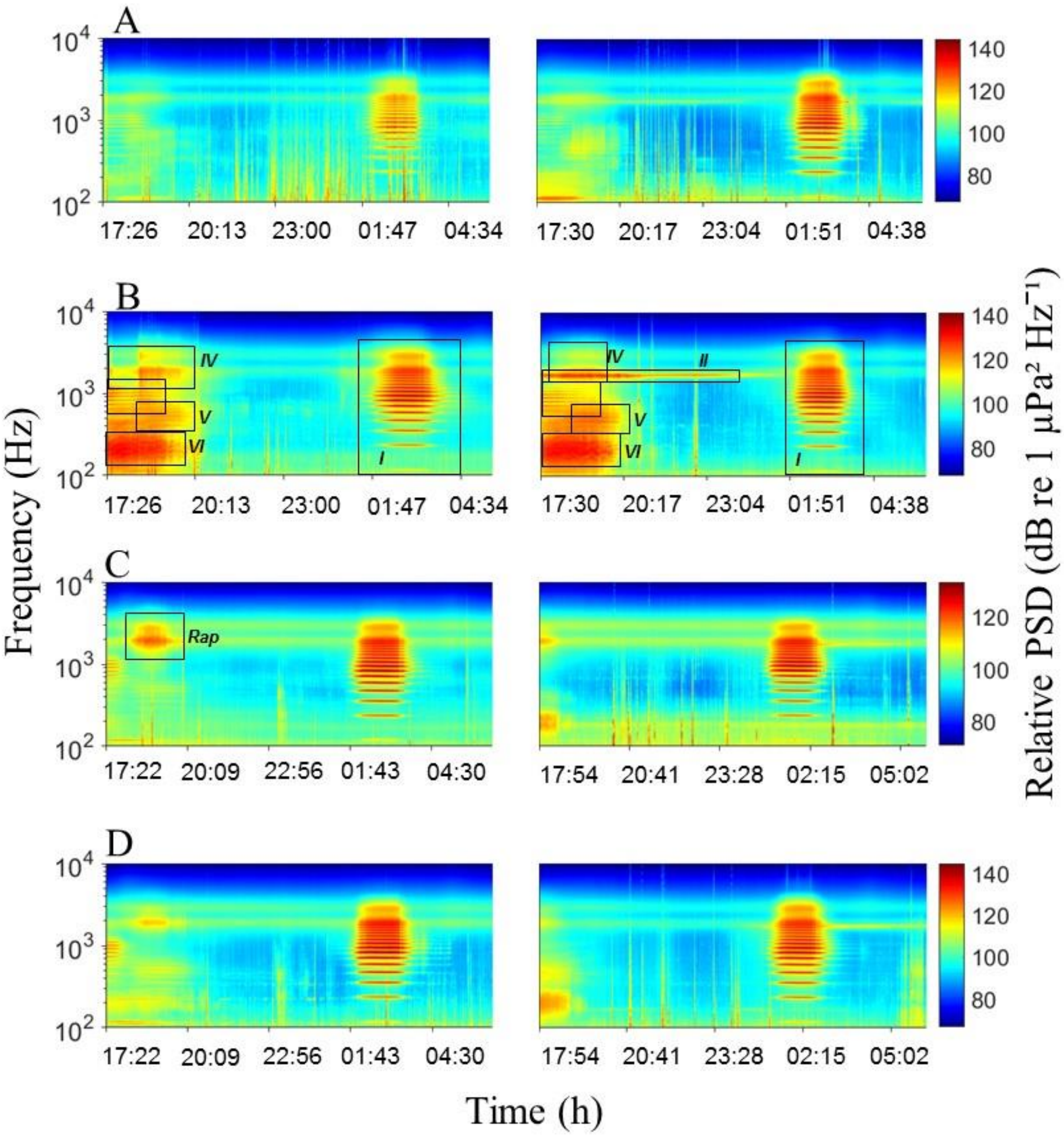

Figure 5. Spectrograms showing the time variation of the frequency and the power spectral density (PSD) of recordings between ~17:30 to 05:30 in Tamandaré and Porto de Galinhas in March. A. MLPZ 3; B. MLPZ 4; C. PGA 1 and D. PGA 2, highlighting the mainly acoustics signals. “Chorus I”, “Chorus II”, “Chorus IV”, “Chorus V” and “Chorus VI” were considered as fish sounds (overlapping during the sunset), and “Rap” corresponds to crustacean sounds.

Table 1. Acoustic parameters of crustacean sounds pulses detected in Porto de Galinhas

Sound type	Dominant frequency (Hz)	Low frequency (Hz)	High frequency (Hz)	Pulse duration (ms)
Snap (n=80)	2413 ± 564 (1637 - 3618)	1771 ± 416 (1093 - 2876)	3192 ± 579 (1997 - 4057)	2.3 ± 1.2 (1.0 - 7.0)
Rap (n=120)	2071 ± 319 (1723 - 3531)	1532 ± 276 (978 - 2760)	3000 ± 450 (2118 - 4727)	3.4 ± 2.1 (1.0 -17.0)

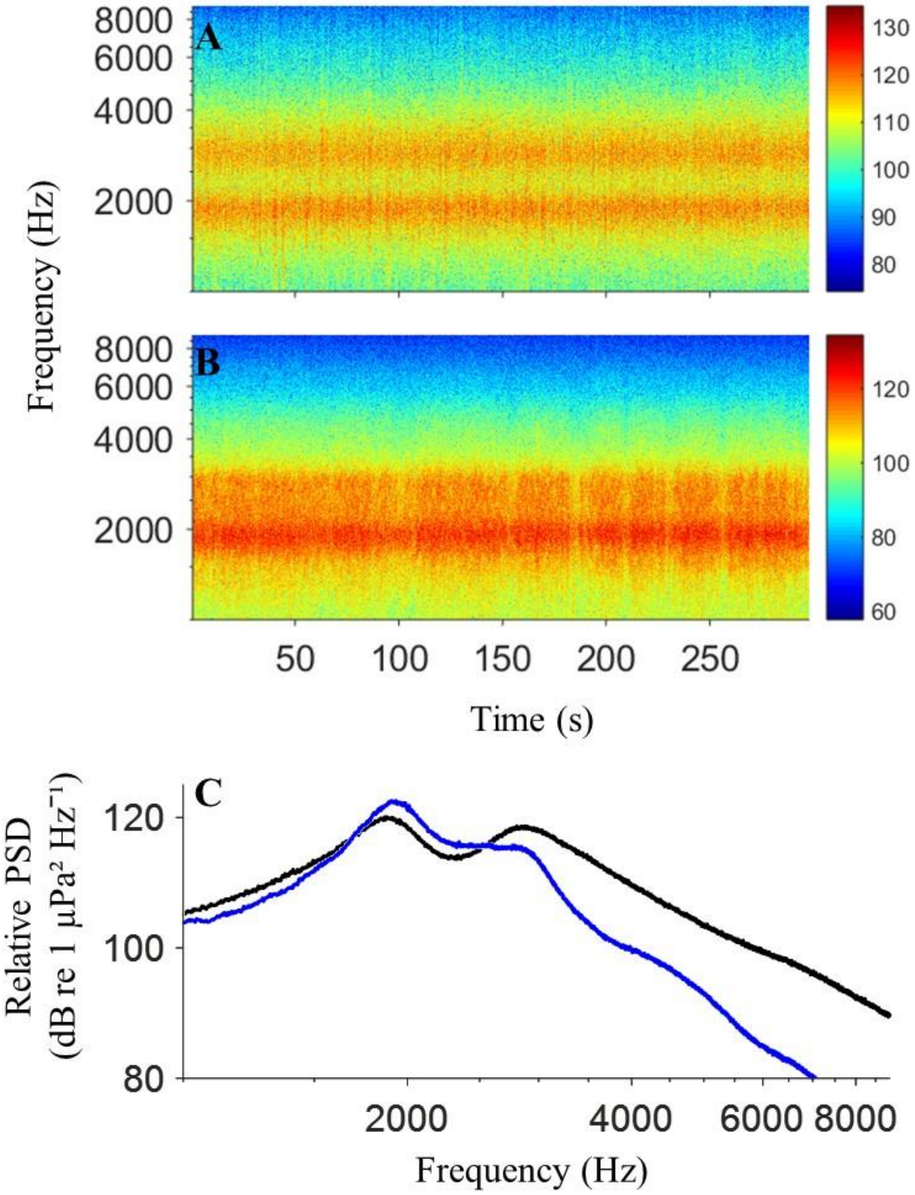

Figure 6. Spectrograms of crustacean sounds A. “Snap” and B. “Rap” (recorded in Porto de Galinhas) and C. power spectrum of “Snap” (black line) and “Rap” (blue line) frequency.

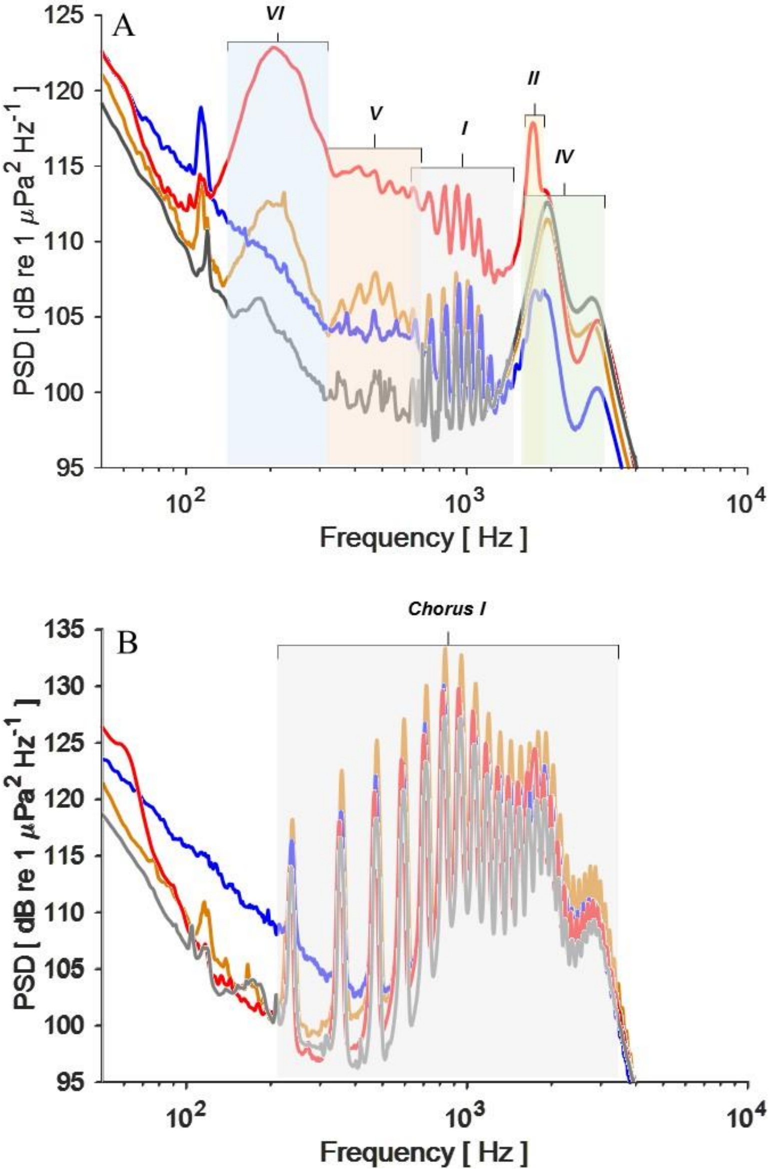

Figure 7. Power spectrum of averag RMS emphasizing the frequency (log) components that occurs continually in the recordings during A. night (between ~17:30 to 00:00 h) and B. after midnight (between ~00:00 to 05:00 h), showing different peaks of fish chorus dominant frequency in MLPZ 3 (blue lline), MLPZ 4 (red line), PGA 1 (grey line) and PGA 2 (orange line).

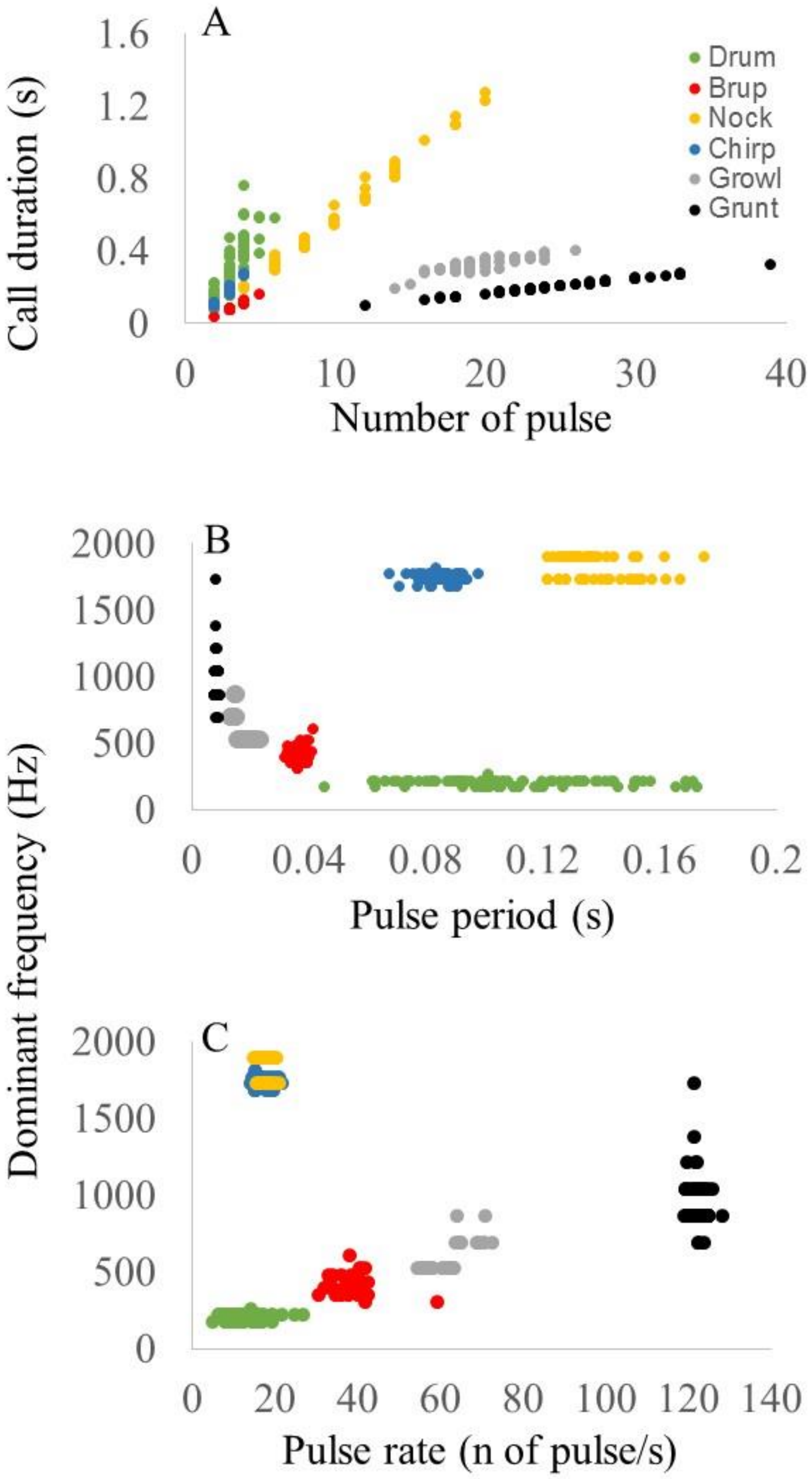

Figure 8. Relationship of acoustic parameters A. call duration (ms) and number of pulse, B. the dominant frequency (Hz) decrease when pulse period (s) increase and C. Increasing positively both the dominant Frequency (Hz) and pulse rate (n of pulse/s). The acoustic relation between “Grunt”, “Growl”, “Drum” and Brup” could indicate similar acoustic mechanism.

Table 2. Acoustic parameters of the different fish chorus found in Tamandaré, Mean ± Standard Deviation (Minimum - Maximum). Different letters in the same line represent significant differences by Kruskal-Wallis non-parametric test: H (5, N = 430).

ACOUSTIC PARAMETERS	**Chorus I (n=84)**	**Chorus II (n=90)**	**Chorus III (n=30)**	**Chorus IV (n=72)**	**Chorus V (n=64)**	**Chorus VI (n=90)**
N of pulses/ call	25.1 ± 4.6c (12.0 - 39.0)	2.7 ± 0.5a (2.0 - 4.0)	3.4 ± 0.6ab (2.0 - 5.0)	9.7 ± 3.8d (4.0 - 20.0)	19.3 ± 2.3c (14.0 - 26.0)	3.1 ± 0.9ab (2.0 - 6.0)
Call duration(ms)	205.1 ± 38.4a (97.4 - 320.5)	163.3 ± 43.2c (90.8 - 276.2)	90.2 ± 25.9e (33.7 - 162.8)	556.6 ± 253.2b (189.8 - 1273.0)	322.5 ± 35.2d (192.5 - 400.6)	283.9 ± 138.9a (73.7 - 758.9)
Pulse rate	122.4 ± 1.8c (119.3 - 128.5)	17.1 ± 1.9a (14.5 - 22.0)	38.3 ± 3.8b (5.3 - 27.2)	17.8 ± 1.3a (14.9 - 21.1)	59.9 ± 4.5b (54.7 - 72.7)	12.3 ± 3.8d (5.3 - 27.2)
Pulse period (ms)	8.7 ± 0.4b (7.7 - 9.9)	84.2 ± 4.9d (68.1 - 98.2)	37.1 ± 2.4a (31.9 - 41.6)	136.6 ± 11.1c (122.0 - 175.7)	19.1 ± 2.6a (13.5 - 24.0)	111.6 ± 34.3e (45.5 - 210.9)
Low frequency (Hz)	375.5 ± 124.2b (120.4 - 658.4)	1623.0 ± 36.5d (1554.6 - 1712.6)	207.8 ± 44.5ab (134.7 - 373.6)	859.8 ± 229.3c (372.5 - 1478.9)	169.9 ± 62.6a (105.1 - 354.3)	83.2 ± 13.5e (54.5 - 112.7)
High frequency (Hz)	2089.9 ± 329.3a (1114.6 - 2964.9)	1863.3 ± 35.6a (1775.7 - 1927.2)	816.5 ± 97.6bc (569.6 - 973.9)	3668.0 ± 725.9.6d (2252.1 - 5577.7)	924.5 ± 98.0b (729.8 - 1178.8)	332.0 ± 25.8c (281.2 - 421.5)
Central frequency (Hz)	1050.0 ± 150.4d (689.1 - 1550.4)	1735.6 ± 25.4a (1679.6 - 1765.7)	433.5 ± 55.3bc (344.5 - 559.9)	1839.9 ± 80.9a (1722.7 - 1894.9)	554.5 ± 78.1b (516.8 - 861.3)	214.8 ± 7.9c (172.3 - 258.4)
Dominant frequency (Hz)	924.9 ± 143.5d (689.1 - 1722.7)	1736.5 ± 27.2a (1679.6 - 1808.8)	414.9 ± 72.0bc (301.5 - 602.9)	1837.5 ± 86.7a (1722.7 - 2067.2)	554.5 ± 83.9b (516.8 - 861.3)	204.3 ± 19.9c (172.3 - 258.4)

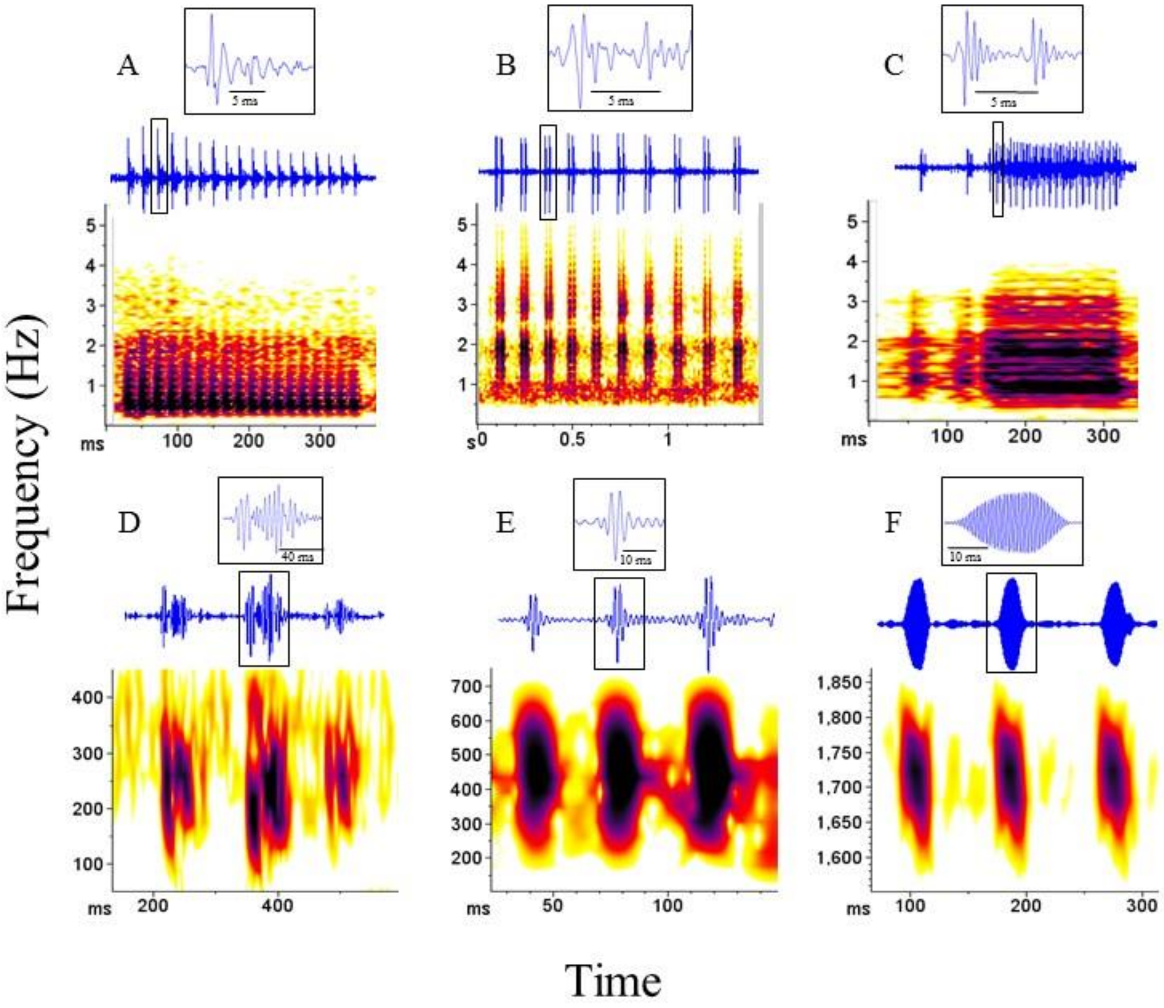

Figure 9. Oscillograms (waveforms), spectrograms of individual calls (below) and respective pulse magnified (quadrants), presenting a train of pulses in A. “Chorus V”, B. “Chorus IV” (paired), C. “Chorus I” (paired), D. “Chorus VI”, E. “Chorus III” and F. “Chorus II”.

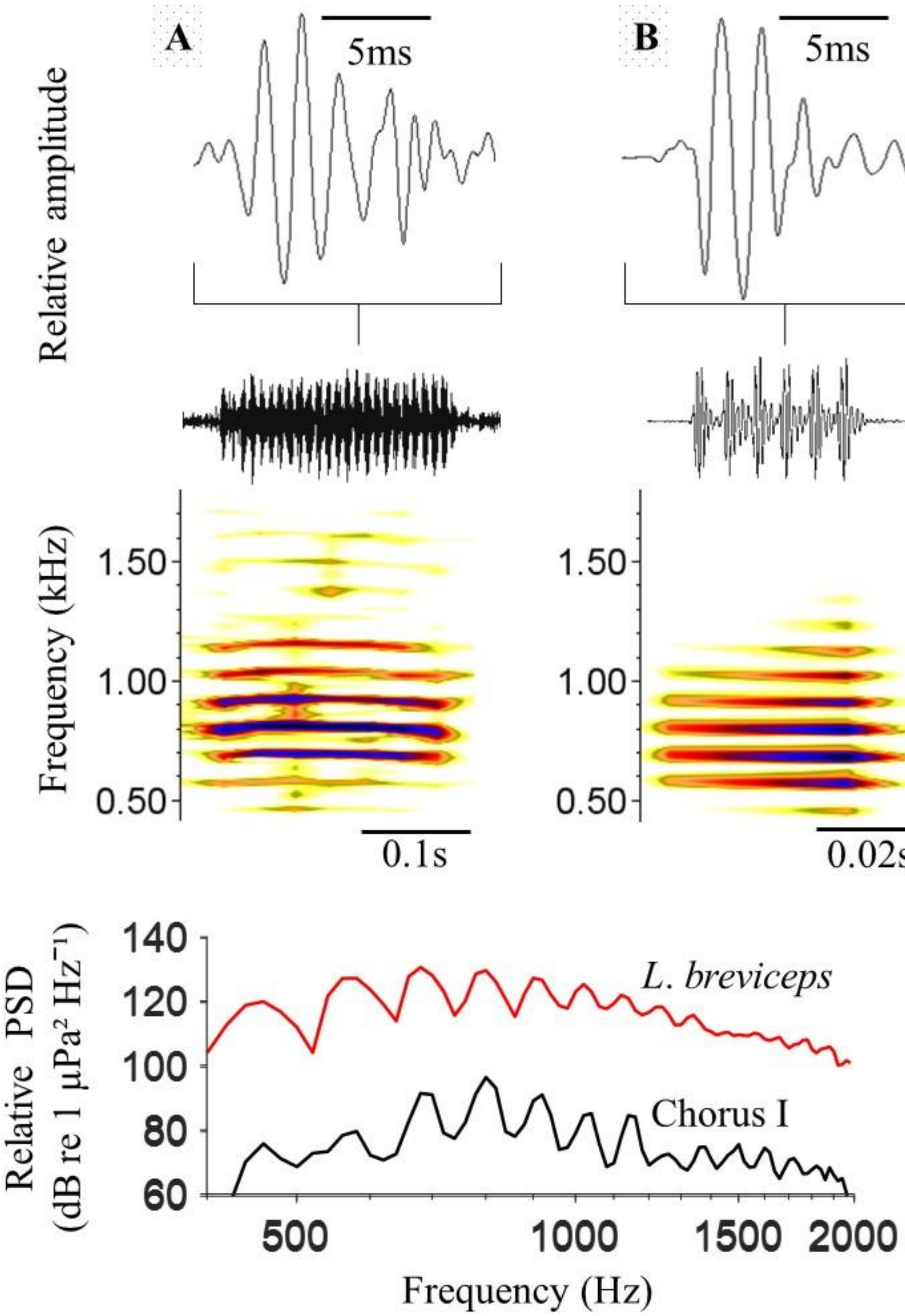

Figure 10. Individual call waveform, spectrogram and power spectrum of A. Chorus I and B. *Larimus breviceps* sound file recorder under stress by Fish and Mowbray 1970, available in http://www.fishbase.org/physiology/FishSoundsSummary.php?autoctr=149.

5. Artigo científico II

EFFECTS OF SEASONAL VARIATION AND LUNAR CYCLES ON PATTERNS OF FISH CHORUSES AND ANTHROPOGENIC NOISES IN A MARINE PROTECTED COSTAL REEF

Abstract

A Passive Acoustic Monitoring (PAM) was implement to evaluate temporal acoustic patterns in a protected area of coastal reefs located in Tamandaré beach (100 km south of Recife, Pernambuco, Brazil) using an autonomous underwater recorder that allowed continuous recordings during summer and winter periods. The sounds detected in the marine environment indicated the presence of eight fish chorus types, two of them only detected in the winter period. The sounds detected in the marine environment indicated the presence of eight fish chorus types producing energy of 70 to 110 dB re 1 $\mu Pa^2\ Hz^{-1}$, two of them only detected in the winter period. The chorus were detected in different parts of day, presenting a daily pattern, with differences in the initial time of detection ($P<0.05$). The chorus III, IV, V and VI overlapping occurring mainly after sunset (17:30h), while Chorus I and II were detected after midnight. In general, the time detection of chorus was 1h 30m. The Chorus I produce a wide frequency band (300 to 4000 Hz), masking the other chorus frequency. All chorus were also influenced by the changes of the lunar phase, presenting major differences during the 1st quarter moon. Vessels noise were detected primarily in the early morning and at night. Vessels had low dominant frequency values, with higher peaks below 120 Hz, and different peaks that can reach 7000 Hz. The vessels produced noises with energy of 90 dB re 1 $\mu Pa^2\ Hz^{-1}$, distributed in a wide frequency band, enough to mask the chorus, although characterized by short peaks (> 10 minutes of detection). Fish chorus and vessels noise had been detected using passive acoustic tool, indicating the need to implement short- and long-term monitoring and management plans.

Keywords: Fish bioacoustics, Reef sounds, Anthropophony

Introduction

Monitoring marine environments and their populations organisms is not an easy task. New technologies have allowed researchers to evaluate these populations using different signals, like sounds that species are able to produce. These sounds can be emitted in different contexts for communication and serve as natural markers of fish species (LUCZKOVICH et al., 2008). The passive acoustic tool allows researchers to identify and monitor fish populations just by listening to them (ROUNTREE et al., 2006).

There are more than 800 known sonorous fish species (KAATZ et al., 2002) in the world, and their sounds are specie-specific, which means that each species can generate its own unique sound, like an acoustic signature. Fish may produce sounds related to several behaviors, like predation avoidance, territorialism, reproductive process, or during feeding activity (TRICAS and BOYLE, 2014). Generally, fish sounds are composed of a simple pulse or pulse train, generating a frequency less than 2 kHz (LADICH, 1997; WEBB et al., 2014). The sound mechanism in fish is related with a pair of sonic muscle vibration or the stridulatory movements of pharyngeal teeth or pectoral fins (LADICH, 2014).

The "passive acoustic" method has been used to monitor the spawning activity of several species of commercialy important fish, mainly of the family Sciaenidae in the North Atlantic, allowing to evaluate the reproductive activity of a resident aggregation of *Cynoscion nebulosus* over a long period of time (WALTERS et al., 2009). It was also possible to map spawning areas of *Cynoscion regalis*, *C. nebulous*, *Bairdiella chrysoura* and *Sciaenops ocellatus* in North Carolina (LUCZKOVICH et al., 1998). In temperate waters of the South Atlantic, it is known that *Micropogonias furnieri* emits sounds associated with courtship / spawning behavior during the spawning season from November to March, in the Rio de la Plata Estuary (TELLECHEA et al., 2011).

Passive acoustics has also been very useful for evaluating the production of sounds by endangered species such as Atlantic cod, whose spawning sites were identified through the sounds produced by fishes during the reproductive period, being "faithful" to the historically known spawning sites (ZEMECKIS et al., 2014). This technique has also been useful for evaluating the breeding sites of the giant grouper (*Epinephelus itajara*), helping to implement management plans for these populations (ROWELL et al., 2012).

Different factors interfere in the reproductive behavior of fish. These factors can be anthropogenic, such as vessel traffic, interfering with fish communication (SIMPSON et al., 2016); physical-chemical factors (mainly temperature and salinity); and astronomical factors, such as the phases of the moon and day and night.

The different phases of the moon can affect the behavior of several marine organisms. Findings in subtropical waters indicate that the lunar cycle influence spawning behavior and mortality in fish (HERNÁNDES-LEÓN 2008), affecting fisheries (LESSA et al., 2004; LOWRY et al., 2007; ORTEGA-GARCIA et al., 2008; POISSON et al., 2010; SETYADJI, 2016). The lunar phases are mainly related to reproductive behavior and spawning in most fish species (SPONAUGLE and PINKARD, 2004; TAKEMURA et al., 2004; MAN et al., 2009), including those in captivity (MONTIE et al., 2017). Several studies have also indicated that in most cases fish chorus occurs mainly at night. Previous results in the same area of the present study allowed to evaluate the presence of six types of chorus, enabling to infer a recurrent sound production (BORIE et al., *in prep*).

The importance of knowing the times of fish chorus occurrence becomes fundamental to develop conservation and management plans for the marine organisms that occur in coastal areas. In this sense, this work deals with the temporal and lunar variation of fish chorus and anthropogenic sounds produced by vessels in a marine protected area, where biological acoustic signals is mainly emitted by fish aggregation.

Material and methods

Study area

The study was develop in a coastal reef located in Tamandaré beach, which is part of the largest Brazilian coastal conservation unit, the “Costa dos Corais” Marine Protected Area (CCMPA), created in 1997. Located in the CCMPA, it is inserted the Marine Life Preservation Zone (MLPZ), closed to fishing and visitation since 1999 (FERREIRA and MAIDA, 2006).

The local ichthyofauna is diverse and composed of estuarine and reef associated species, as well as pelagic ones (FERREIRA and CAVA, 2001; MERIGOT et al., 2016). The reefs of this protected marine area have relatively high biodiversity represented by 26 fish species (FERREIRA and MAIDA, 2006). Tamandaré coral reef system is characterized by reef lines parallel to the coast. Beyond the last reef line, which is exposed to the sea (Maida and Ferreira, 2006), is possible to find muddy substrate, where sediments accumulate (mainly loam), from coastal rivers contribution (KEMPF, 1967). In the study area, from south to north are the Mamucabas, Formoso and Sirinhaém Rivers. This coastal habitat has support an important artisanal fishing activity in the CCMPA, characterized by gillnet and line fishing boats, identifying 99 species of fish, divided into three categories: reefs, estuarine and epipelagic (RANGELY et al., 2011).

In addition, it is possible to observe the traffic of several types of vessels, like fishing, recreational and ships. In the case of fishing vessels, fisherman use the bay of Tamandaré, where the only exit to open sea is made by crossing the protected zone, in order to get to the fishing area. Boats are also used for tourism, to visit adjacent areas, and both uses are estipulated by the Management Plan of CCMPA. Fishing vessels in Tamandaré have a length between 8 and 12 meters, a semi-open cabin in the stern and internal engines with 30 to 60 HP (Lira et al. 2010).

Acoustic survey

Underwater recordings were made using a stationary hydrophone system, the SoundTrap 300 (Ocean Instruments, New Zealand) an autonomous acoustic recorder consisted of a calibrated omnidirectional cylindrical hydrophone (sensitivity −186 dB re 1 μPa). The SoundTrap 300 working frequency range of 20 Hz - 60 kHz ± 3dB. The recordings sample rate was 24 kHz in this study.

Two SoundTraps (STs) allowed continuous recordings of 24 hrs/day for 3 months (December 10, 2016 to March 10, 2017), during the summer in the southern hemisphere, and between June 12 and 27 (winter). Due to the ST's autonomy being ~ 13 days, the equipment was replaced for another one within that period. The SoundTrap was installed in the Tamandaré bay at a ~13 meters deep, anchored and floating 2.5 m below the sea floor (Figure 12). Replacement was done through scuba diving, and after the equipment was removed, the data was downloaded to a laptop and the battery recharged for the next exchange.

Fish chorus and anthrophonic analyses

After recovering the sound files of each SoundTrap, the files were downloaded to a portable computer for a pre-evaluation using the acoustic software Audacity® (v.2.2), and spectrograms which were created to manually to evaluate temporal patterns of each fish chorus and anthropogenic event that could be heard and to select parts of the recordings for individual analyses of the signal.

Raven Pro 1.4 (Cornell Laboratory of Ornithology) was used to perform these individual analyses of each chorus. The temporal characterization of each chorus signal was performed using parameters like, initial and final time (HH:MM); total detection time; low- and high-frequency limits (Hz); and dominant frequency (Hz) [the description of the last two parameters can be obtained in CHARIF et al. (2010)]. The acoustic parameters were measured using oscillograms and spectrograms, with a resolution (DFT) of 1024 and overlapping of 99%. Vessel noise temporal analyses was perform using time detection (HH:MM), and the same frequency parameters used for fish chorus.

No visual census and sampling were performed for validation and identification of the sound-producing species that occur at the recording site. So, the acoustic characteristics were analyzed and compared with other works as a basis for inferring about fish family and species.

Spectrograms and power spectra were created using the PAMGuide toolbox (MERCHANT et al., 2015) in the MATLAB 2016 to evaluate the frequency band distribution over time and the energy of the coastal reef. PAMGuide was used to calculate the Power Spectral Density (PSD, dB re 1 μPa^2 / Hz) per 1 Hz, time-average data and file in stages of 300 seconds, with calibration type “end-to-end” and -173 dB system sensitive. The spectrograms were produced using 1024 point Hanning window (50% overlap), plotted on a logarithmic frequency scale between 50 and 5000 Hz. The Mean Square Root (RMS) levels of the PSD values were calculated for all days. To evaluate temporal and lunar patterns, a Matlab script was developed to detect a PSD greater than 70 dB re 1 μPa2 / Hz in the individual time bands and frequency band for each previously identified chorus.

Lunar phases were determined by dividing lunar month (29.5 days) into eight equal parts, i.e., new moon, waxing crescent, first quarter, waxing gibbous, full moon, waning gibbous, last quarter and waning crescent. The temporal acoustic parameters, the relation to the lunar cycle and the frequencies (Hz) emitted by the chorus were compared using non-parametric statistics through the Kruskal Wallis multiple comparison and Tukey HSD post hoc tests between the different groups for each variable in the STATISTICA 7 program (Dell Inc.).

Results

Acoustic patterns were detected in six chorus types related to species found using the same habitat, with different frequencies band and temporal variation of occurrence, influenced by the lunar cycle. The choruses also presented temporal overlap in the beginning of the night. Anthrophonic noise was often detected, and it was possible to identify noise from different vessels.

Fish Chorus

A total de six fish chorus were detected in the marine protected zone of Tamandaré, presenting typical fish characteristics, being produced by trains of pulses and frequencies up to 3000 Hz. The Chorus I, III, V, VI seem like drumming sounds, commonly found in several fish species, manly Sciaenidae. Some of the choruses detected were more recurrent than the others; in this case, Chorus I and V occurred in all recordings and were detected at 89 days of sampling. Following by Chorus VI with 82 occurrences and Chorus II with 71, and Chorus III and IV detected in 57 and 58 times of the total days (Figure 13).

In general, choruses were detected during different parts of the day, with significant differences ($p < 0.05$) at the initial time of detection. The Chorus I started after midnight, on average at 01:20, followed in sequence by Chorus II, starting on average at 04:06 h. During the afternoon there was also the chorus succession from 3:27 pm with Chorus III, followed by Chorus VI (4:57 pm). Chorus IV and V, which began after sunset, at 5:37 p.m. and 6:41 p.m., respectively (Table 3), during which time the four chorus produced temporal overlap (Figure 14). Chorus detection time ranged from 1 h and 33 min (Chorus IV) to 2 h and 23 min (Chorus III). Chorus I, IV and V were similar ($p > 0.05$), and significantly different to Chorus II, III and VI (Table 3).

The choruses presented different frequency bands. The Chorus I presented a wide band between 80, and 3861 Hz and does not mask the frequency of the other Chorus due to differences in their time of occurrence. The Chorus II produced a narrow and high frequency band (1600-1800 Hz). The shoals presented a similar frequency band pattern over time, with overlap in the high and low limits in Chorus III, IV and V. The Chorus VI presented lower values, with a mean frequency band between 80 and 311 Hz (Table 3). (Table 3).

The choruses presented significant differences in the mean values of energy (KW-H(5,88) = 60.4911, p = 0.0001; F(5,82) = 31.2765, p = 0.0000). Chorus I, III, IV and IV showed higher values of energy during summer, with Chorus II being more evident in winter >80 dB re 1 $\mu Pa^2 Hz^{-1}$ (Figure 15 B). Chorus III and VI had temporal energy variation ($P<0.05$) of 20 dB re 1 $\mu Pa^2 Hz^{-1}$ in a short time interval (Figure 15 A).

Lunar patterns influenced the initial and detection time of each chorus, with significant differences in detection time between moon phases in Chorus III ($p<0.05$), with longer chorus during the first quarter lunar phase (Figure 16 C), diminished until the full moon. Although they did not have significant differences, Chorus II (Figure 16 B) and IV showed higher detection time on the same lunar phase, and lower on the new moon (Figure 16 D). The inverse pattern was found in Chorus I and VI, with lower detection time in the first quarter , increasing to the waning gibbous in Chorus I, and last quarter in VI (Figure 16 F), showing significant differences from the other phases of the moon ($p<0.05$). In Chorus V the detection time increased rapidly until the waning crescent, gradually decreasing. The first quarter moon also showed to have an influence on the start time of detection. In Chorus II and III the beginning was anticipated with respect to the average, delaying it in the waxing crescent and full moon, respectively. In Chorus IV and V there was also a delay in the first quarter moon ($p<0.05$). Chorus I slowed its start on the new moon and anticipated the time on the last quarter lunar phase (Figure 17).

Although the chorus detected emitted temporal variations related with the lunar cycle during the summer, in winter this did not appear evident. A constant pattern in the same lunar period (during 15 days from the full moon) was detected, being difficult to identify each chorus, although they seem to occur in most part of the day (Figure 18 A). Nevertheless, the recordings generated in winter showed two different acoustic signals produced by fish that were not detected in summer. These signals occurred with narrow frequency bands close to each other, being Chorus VII and VIII, with a frequency between 100 and 110 Hz, and 140 and 160 Hz respectively (Figure 18 B).

In general, the fish chorus presented high energy levels during the summer, mainly Chorus I and IV, with a difference of 15 and 10 dB re 1 $\mu Pa^2 Hz^{-1}$ in the dominant frequency, respectively (Figure 19). Low frequency geological acoustic signals (<100 Hz), like waves and wind, were detected during this period.

Vessel noise

It was possible to detect and evaluate the occurrence of 289 anthropogenic acoustic signals, such as fishing vessels (n = 196), recreational motorboats (n = 72) and ships (n = 21). In general, the vessels had low dominant frequency values, with higher peaks below 120 Hz, and different peaks that can reach 7000 Hz (Table 4). The vessels produced noises with energy > 80 dB re 1 $\mu Pa^2 Hz^{-1}$ (Figure 20), distributed in a wide frequency band, enough to mask all the chorus, although characterized by short peaks (> 10 minutes of detection).

These vessels were classified by the types of signal produced and were audibly distinguishable. Fishing vessels were detected almost every day during the study, with peaks occurring in the middle of January and the end of February. This pattern was also observed in the boats, with the highest number of detections during holidays and weekends (Figure 21A). The number of fishing vessel detections was higher in the early morning (~ 06: 00h), with more than 40 detections, decreasing until the late afternoon (~ 17: 45h), with a slight increase in the early evening. The time of occurrence of boats and ships followed a daytime pattern, with higher peak in the morning. On the other hand, the boats had greater detections in the period between 09:00 and 14:00 h, a pattern similar to that found in ships (Figure 21 B).

A relationship between the number of fishing vessels and the phases of the moon occurs in a variable way. From the new moon the number of vessels increased, reaching a maximum number of fishing boats (n = 37) in the phases before and after the full moon (waxing and waning gibbous) (Figure 22A). The new and last quarter moon phases were the ones with the lowest occurrences (n = 14). The daily number of fishing vessels detected for each moon was higher on the last quarter moon, with more than three boats being identified on the days during that lunar period (Figure 22B). This number was lower in the waxing crescent moon phase (P <0.05).

Discussion

The fish choruses found in this study presented similar characteristics to those detected in coastal marine protected areas in temperate regions of southeaster Brazil, with five chorus types detected in this region (SÁNCHEZ-GENDRIZ and PADOVESE, 2016), with similar frequencies when compared with "Chorus I" harmonic signals and a frequency band between 400 to 1400 Hz, as well as the "Chorus V", with a frequency between 400 and 800 Hz, and the "Chorus II" sound, as a narrow frequency band between 1500 and 1800 Hz. Although these acoustic signals presented similar characteristics, they emitted in different times from those found in the present study. The "Chorus I" had similar spectral characteristics and was also detected twice a day, with no temporal acoustic competition between them, but rather a temporal overlap such as the "Chorus V" observed during the early morning hours (SÁNCHEZ-GENDRIZ and PADOVESE, 2017). This could indicate that the species of fish emitting this type of signals can have a wide distribution in the

Brazilian coast, being able to occur in both tropical and temperate waters, and that the time difference found can be related to the migratory patterns of these species.

The frequency bands of the choruses were varied, being broad in the "Chorus I" and "Chorus IV" and narrow in "Chorus II" and "Chorus VI". In this sense, it has been seen that some of chorus may present several combinations of frequency band distribution, while others exhibit predominant spatial and temporal overlapping (PARSON et al., 2016b). The acoustic signals of some fish show that there is no overlapping at the frequency level at night, which would show that in the absence of visual sense, sound communication is more important (RUPPÉ et al., 2015). The lack of time and chorus frequency partitioning in hours of darkness illustrates the complexity of monitoring different communities of sound-producing fish species (PARSON et al., 2016a). The wide frequency range emitted by Chorus I (average between 119 and 2981 Hz) can indicate an evolutionary adaptation, changing the occurrence timing (emitting sounds in the middle of the night) thus avoiding a complete overlap in the frequency emitted by the other chorus detected.

On the other hand, spectral and temporal patterns of our "Chorus I" and "Chorus VI" were similar to the spectrum and timing of "Drumming" chorus (so-called and associated with fish of the family Sciaenidae), and "Chorus I" found by McCauley (2000, 2012) in Australian reefs during the late-night. The associated species of the family Terapondidae (sound type "trumpets"), although not occuring in the Atlantic, may have a similar sound mechanism of “Chorus I” fishes, as they exhibit spectral peaks related to the swimbladder resonance frequency with peaks separated by the rate of muscle contraction extending to higher frequencies (>1000 Hz).

The acoustic pattern found in this study was also similar to the Sciaenidae *M. furnieri* activity increasing in the early morning between 07:00 to 10:00 a.m. and in the evening between 17:00 p.m. to 23:00 h, in a stream of Rio la Plata Estuary (TELLECHEA et al., 2011). Comparable daily temporal patterns have been found in species of the North Atlantic Sciaenidae, such as *Bairdiella chrysoura*, emitting sounds practically all night, and species *Sciaenops ocellatus*, *Cynoscion nebulosus* and *C. regalis*, whose sounds are mainly produced in the early hours of the night, after sunset (LUCZKOVICH et al., 2008).

The influence of the lunar cycle showed that greatest variation occurs mainly during the first quarter moon. In this sense, more calling was detected in *Cynoscion nebulosus* on the first quarter compared to calling on the new moon and on the third quarter compared to the full moon (MONCZAK et al., 2017). On the other hand, the greatest amount of spawning in *Atractoscion nobilis* occurred from the new moon until four days after the new moon (AALBERS, 2008). The “Chorus II” and “Chorus IV” also showed spectral and temporal similarity, and were associated with the chorus of planktivorous fish during feeding (with frequencies between 600 and 1500 Hz), strongly influenced by moon phase, with a longer period of maximum chorus level beginning 2-3 days after a full moon and staying high until just after the new moon, increasing at the next full moon (McCAULEY et al., 2012). On the other hand, Parson et al. (2016) detected a lower frequency chorus showing a progressive decline from new to full moon while a higher frequency chorus peaked between the full moon and new moon phases.

The occurrence of fish chorus during the night on the Brazilian coast and founded in this work may be associated with nocturnal migrations of some fish species of Holocentridae and Sciaenidae (HOBSON, 1965). During the day, these species remain in sheltered areas and at night they move to feeding grounds, where the major prey of nocturnal fishes are crustaceaans (HOBSON, 1975). For some of them, there already exists an acoustic record associated with different behaviours (PARMENTIER et al., 2011; RAMCHARITAR et al., 2006).

The Chorus VII detected during the winter has already been found in a lower estuarine portion of the Maracaipe River (BORIE et al., *in prep*), with similar acoustic characteristics (Annex II), which could indicate seasonal and different habitat uses. This can be related to the pluviometric level in the Tamandaré region, since during the summer (between December and March) it accumulated up to 156 mm, increasing with the beginning of the rains in April (305.2 mm), reaching up to 1,231 mm accumulated in June (APAC, 2018) a period when salinity and transparency decreasing in these areas (FERREIRA et al., 2001) by the contribution of Formoso River to the north and the Mamucabas River to the south, and probably allowing to enlarge the habitat of some species.

The acoustic characteristics of the six chorus types evaluated in this work can indicate different sound mechanisms. It has been seen that fundamental frequency is inversely related to fish size that use a sonic muscle. In this case, species producing Choruses I, III, V and VI could present such musculature based on sound characteristics. Larger fish emit a low fundamental frequency, while smaller fish produce a higher frequency. Thus, it is possible to say that the largest frequencies found in this work were emitted by small fish, in the case of Chorus I, with similarities found with *Larimus breviceps* (BORIE et al., *in prep.*), On average 20 cm in total length (TISHER and SANTOS, 2003). An observation in weakfish that is consistent with this model is that larger fish, which likely have longer contraction duration cycles because of their longer muscle fibers, produce lower frequency sounds (CONNAUGHTON et al., 2002). Larger fish are able to vibrate the swim bladder with more energy (BOYLE and TRICAS, 2014). Dominant frequency correlates with pulse duration (reflecting muscle twitch duration), and the inverse of the period of the second cycle of acoustic energy approximates the recorded frequency (CONNAUGHTON et al., 2002). This hypothesis would not apply to Chorus II and IV.

The fish chorus detected in this work can be masked by the noises produced by different types of vessels, even for a short time period, since the study area was mainly used as a passage area by the fishery and recreational motorboats. A higher number of boats was detected in the middle of January and February, mainly at the end of the month with higher peaks coinciding with weekends and holidays (carnival). Ships were heard sporadically during the study, but they are commonly observed at distances >10 Km, mainly departing from the port of Suape (www.marinetraffic.com/Brazil_port: SUAPE), one of the busiest in Brazil. Ships producing low levels of acoustic energy and fishing boats occur more frequently in the morning, probably those returning from fishing, and recreational boats, in the daytime, being more related to tourist activity.

The regional fishing vessels have a length of 8 to 12 meters, with a semi-open cabin in the stern and internal engines with a power of 30 to 60 HP (Lira et al., 2010), they could also be motor wooden rafts (SOUZA et al., 2012), and also, the type of fuel and size propeller used could be related to the different frequencies found in the fundamental frequencies of boat sounds, similar to vessels anthrophonic detected in Brazilian coastal areas (BITTENCOURT et al., 2014). The fishing vessels noise detected in this study occur in greater numbers near to the full moon, being related to the line fishing landings of the state of Pernambuco, which were significantly larger on the full moon. Contrasting, the seine fishing activity occurs mainly during the new moon. The different schedules found in the Tamandaré region may indicate different fishing efforts, once the fishing time of the motorized boats of Pernambuco vary between 0.5 and 17 days, with an average of 4 days (LESSA et al., 2004).

The occurrence of different biological and anthropogenic acoustic signals observed in tropical coastal reef area in Tamandaré demonstrate a complex soundscape in this ecosystem. Fish chorus and vessels noise have been detected using passive acoustic tool, indicating the need to implement short- and long-term monitoring plans, evaluating temporal and lunar occurrence patterns.

The acoustic patterns presented during the study period may indicate the presences

and condition of different sound-producing fish populations detected in the "Costa dos Corais" Environmental Protection Area (APACC). These results contribute to the development of monitoring programs for biological activities of fish and fishing vessels in the long term. The results showed a complex temporal variation of the choruses, implying the need of greater effort to monitor these aggregations and pattern changes. The fish chorus and the anthrophonic noises of different vessels detected can be monitored with the passive acoustic tool, contributing to the development of a Passive Acoustic Monitoring (PAM) program.

References

BITTENCOURT, L. et al. Underwater noise pollution in a coastal tropical environment. **Marine Pollution Bulletin**, v. 83, n. 1, p. 331-336, 2014. ISSN 10.1016/j.marpolbul.2014.04.026.

FERREIRA, B. P. et al. **Interações entre a pesca artesanal e o turismo em Tamandaré, APA Costa dos Corais**. II Congresso sobre Planejamento e Gestão das Zonas Costeiras dos Países de Expressão Portuguesa IX Congresso da Associação Brasileira de Estudos do Quaternário II Congresso do Quaternário dos Países de Línguas Ibéricas. [S.l.]: [s.n.]. 2003. p. 1-3.

HERNÁNDEZ-LEÓN, S. Natural variability of fisheries and lunar illumination: a hypothesis. **Fish and Fisheries**, v. 9, n. 2, p. 138-154, 1 jun. 2008. ISSN 10.1111/j.1467-2979.2008.00272.x.

HOBSON, Edmund S. Feeding patterns among tropical reef fishes. **Am Sci**, v. 63, n. 4, p. 382-392, 1975.

KAATZ, I. M. Multiple sound-producing mechanisms in teleost fishes and hypotheses regarding their behavioural significance. **Bioacoustics**, v. 12, n. 2-3, p. 230-233, jan. 2002. ISSN 10.1080/09524622.2002.9753705.

KEMPF, Marc. A Plataforma Continental de Pernambuco (Brasil): Nota Preliminar sobre a Natureza do Fundo. **Tropical Oceanography-ISSN: 1679-3013**, v. 9, n. 1, 1967.

LADICH, F. Agonistic behaviour and significance of sounds in vocalizing fish. **Marine and Freshwater Behaviour and Physiology**, v. 29, n. 1-4, p. 87-108, 1997. ISSN

10.1080/10236249709379002.

LADICH, F. Fish bioacoustics. **Current Opinion in Neurobiology**, v. 28, p. 121-127, 2014. ISSN arXiv:1011.1669v3.

LESSA, R. P.; JR., J. L. B.; NÓBREGA, M. F. D. Dinâmica das Frotas Pesqueiras da Região Nordeste do Brasil: Análise das principais pescarias, p. 126, 2004.

LIRA, Luiz et al. Diagnóstico socioeconômico da pesca artesanal do litoral de Pernambuco. vol. 4. **Recife Instituto Oceanário de Pernambuco: Departamento de Pesca e Aqüicultura da UFRPE**, 2010.

LOWRY, M.; WILLIAMS, D.; METTI, Y. Lunar landings-Relationship between lunar phase and catch rates for an Australian gamefish-tournament fishery. **Fisheries Research**, v. 88, n. 1-3, p. 15-23, 2007. ISSN 10.1016/j.fishres.2007.07.011.

LUCZKOVICH, J. J. et al. Identifying Sciaenid Critical Spawning Habitats by the Use of Passive Acoustics. **Transactions of the American Fisheries Society**, v. 137, n. 2, p. 576-605, 2008. ISSN 10.1577/T05-290.1.

MANN, D. A. et al. Goliath grouper *Epinephelus itajara* sound production and movement patterns on aggregation sites. **Endangered Species Research**, v. 7, n. 3, p. 229-236, 2009. ISSN 10.3354/esr00109.

ORTEGA-GARCIA, S. et al. The relative importance of lunar phase and environmental conditions on striped marlin (*Tetrapturus audax*) catches in sport fishing. **Fisheries Research**, v. 93, n. 1-2, p. 190-194, 2008. ISSN 10.1016/j.fishres.2008.04.005.

PARMENTIER, E. et al. Comparative study on sound production in different Holocentridae species. **Frontiers in Zoology**, v. 8, n. 1, p. 12, 2011. ISSN 10.1186/1742-9994-8-12.

PARSONS, M. J. G. et al. **Characterizing diversity and variation in fish choruses in Darwin Harbour**. ICES Journal of Marine Science. [S.l.]: Oxford University Press. 2016. p. 2058-2074.

PARSONS, M. J. G. et al. Fish choruses off Port Hedland, Western Australia. **Bioacoustics**, v. 26, n. 2, p. 135-152, 2017. ISSN 10.1080/09524622.2016.1227940.

POISSON, F. et al. Effects of lunar cycle and fishing operations on longline-caught pelagic fish: Fishing performance, capture time, and survival of fish. **Fishery Bulletin**, v. 108, n. 3, p. 268-281, 2010. ISSN 00900656 (ISSN).

RAMCHARITAR, J.; GANNON, D. P.; POPPER, A. N. Bioacoustics of Fishes of the Family Sciaenidae (Croakers and Drums). **Transactions of the American Fisheries Society**, v. 135, n. 5, p. 1409-1431, 2006. ISSN 10.1577/T05-207.1.

RANGELY, J. et al. ESTRATÉGIAS DE PESCA ARTESANAL NO LITORAL MARINHO ALAGOANO (BRASIL)*, v. 36, n. 4, p. 263-275, 2010.

ROUNTREE, Rodney A. et al. Listening to fish: applications of passive acoustics to fisheries science. **Fisheries**, v. 31, n. 9, p. 433-446, 2006.

RUPPÉ, L. et al. Environmental constraints drive the partitioning of the soundscape in fishes. **Proceedings of the National Academy of Sciences**, v. 112, n. 19, p. 6092-6097, 2015. ISSN 10.1073/pnas.1424667112.

SÁNCHEZ-GENDRIZ, I.; PADOVESE, L. R. Underwater soundscape of marine protected areas in the south Brazilian coast. **Marine Pollution Bulletin**, v. 105, n. 1, p. 65-72, 15 abr. 2016. ISSN 10.1016/j.marpolbul.2016.02.055.

SÁNCHEZ-GENDRIZ, I.; PADOVESE, L. R. Temporal and spectral patterns of fish choruses in two protected areas in southern Atlantic. **Ecological Informatics**, v. 38, p. 31-38, 1 mar. 2017. ISSN 10.1016/j.ecoinf.2017.01.003.

SETYADJI, B. The Effect of Moon Phase on The Catch of Bigeye Tuna (Thunnus obesus) in Eastern Indian Ocean, n. October, 2016. ISSN 10.14710/ik.ijms.21.3.101-106.

SIMPSON, S. D. et al. Anthropogenic noise increases fish mortality by predation. **Nature Communications**, v. 7, p. 1-7, 2016. ISSN 10.1038/ncomms10544.

SPONAUGLE, S.; PINKARD, D. Lunar cyclic population replenishment of a coral reef fish: Shifting patterns following oceanic events. **Marine Ecology Progress Series**, v. 267, p. 267-280, 2004. ISSN 10.3354/meps267267.

TAKEMURA, A. et al. Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes, p. 317-328, 2004.

TELLECHEA, J. S.; BOUVIER, D.; NORBIS, W. Spawning sounds in whitemouth croaker (sciaenidae): Seasonal and daily cycles. **Bioacoustics**, v. 20, n. 2, p. 159-168, 2011. ISSN 10.1080/09524622.2011.9753641.

TISCHER, Mário; SANTOS, Maria do Carmo Ferrão. Composição e diversidade da ictiofauna acompanhante de peneídeos no litoral sul de Pernambuco. **Arquivos de Ciências do Mar**, v. 36, n. 1-2, p. 105-118, 2003.

TOWER, R. W. The production of sound in the drumfishes, the sea-robin and the toadfish. **Annals of the New York Academy of Sciences**, v. 18, n. 1, p. 149-180, 1908. ISSN 10.1111/j.1749-6632.1908.tb55101.x.

TRICAS, T. C.; BOYLE, K. S. Acoustic behaviors in Hawaiian coral reef fish communities. **Marine Ecology Progress Series**, v. 511, p. 1-16, 2014. ISSN 10.3354/meps10930.

WALTERS, S. et al. Using a Passive Acoustic Survey to Identify Spotted Seatrout Spawning Sites and Associated Habitat in Tampa Bay, Florida. **Transactions of the American Fisheries Society**, v. 138, n. 1, p. 88-98, 2009. ISSN 10.1577/T07-106.1.

WEBB, J. F. Introduction to Fish Bioacoustics. **Fish Bioacoustics**, New York, NY, n. 1963, p. 1-15, 2008. ISSN 10.1007/978-0-387-73029-5_1.

ZEMECKIS, D. R. et al. Spawning site fidelity by Atlantic cod (Gadus morhua) in the Gulf of Maine**:** Implications for population structure and rebuilding. **ICES Journal of Marine Science**. [S.l.]: Oxford University Press. 2014. p. 1356-1365.

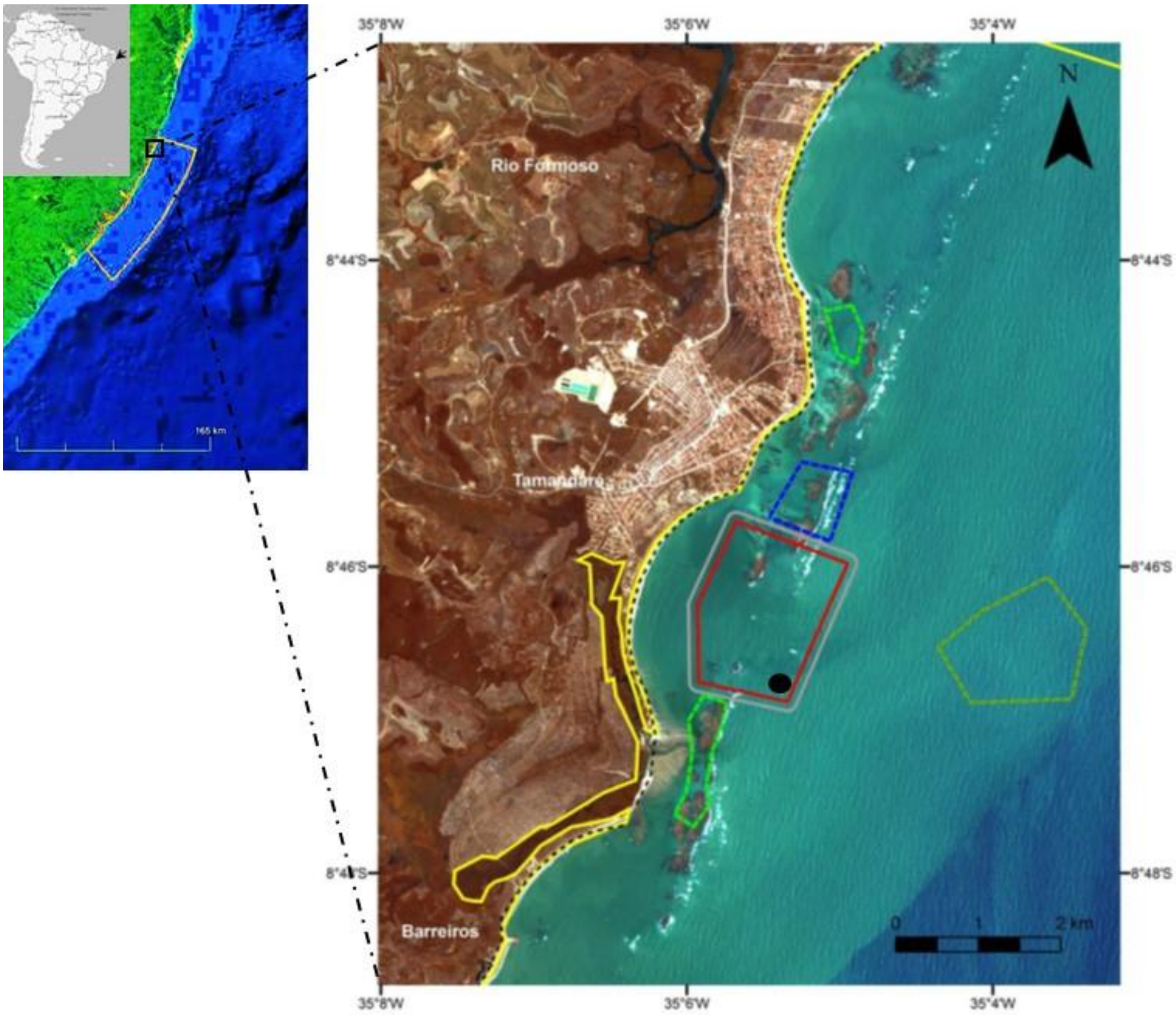

Figure 11. Map of study the area and location of SoundTrap deployed in Tamandaré, showing the “Costa dos Corais” Protection Area (yellow line in the medium map), limits of the Marine Life Preservation Zone in Tamandaré (red line), the acoustics recording sites in black circle. Beryline represent the conservation area, the yellow line sustainable use area, the blue line visitation zone, and the green line the fishing area (detailed map elaborated by APA Costa dos Corais / ICMBio).

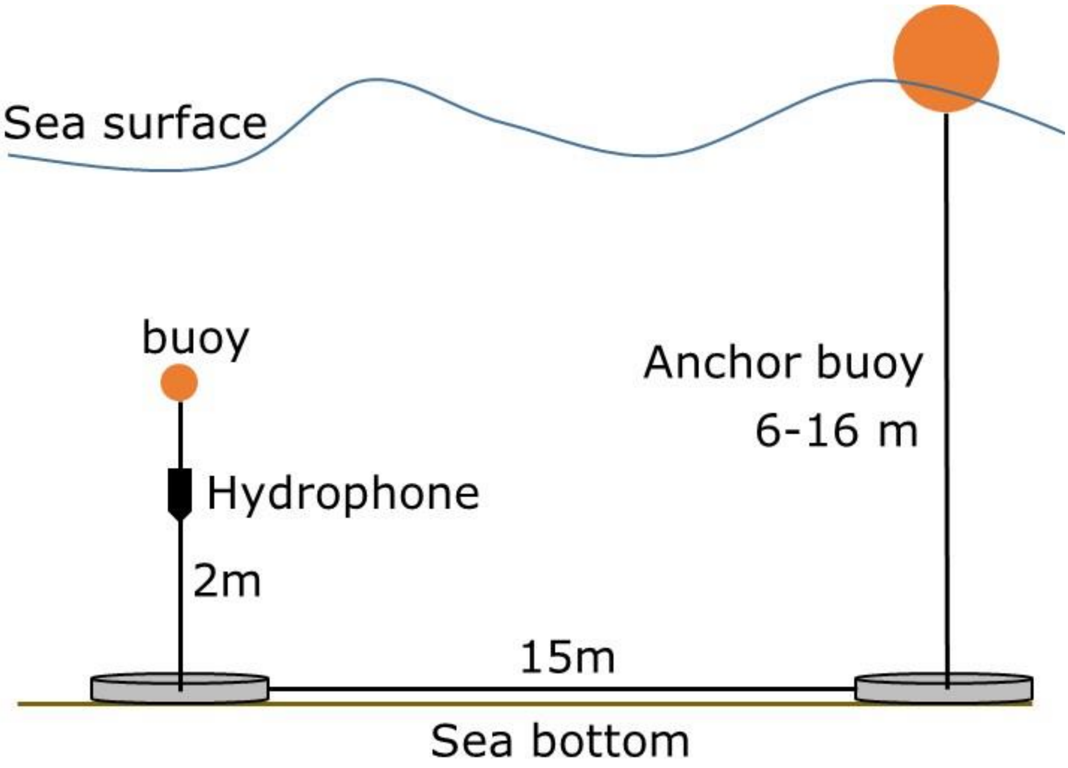

Figure 12. Autonomous acoustic underwater recorder configuration module.

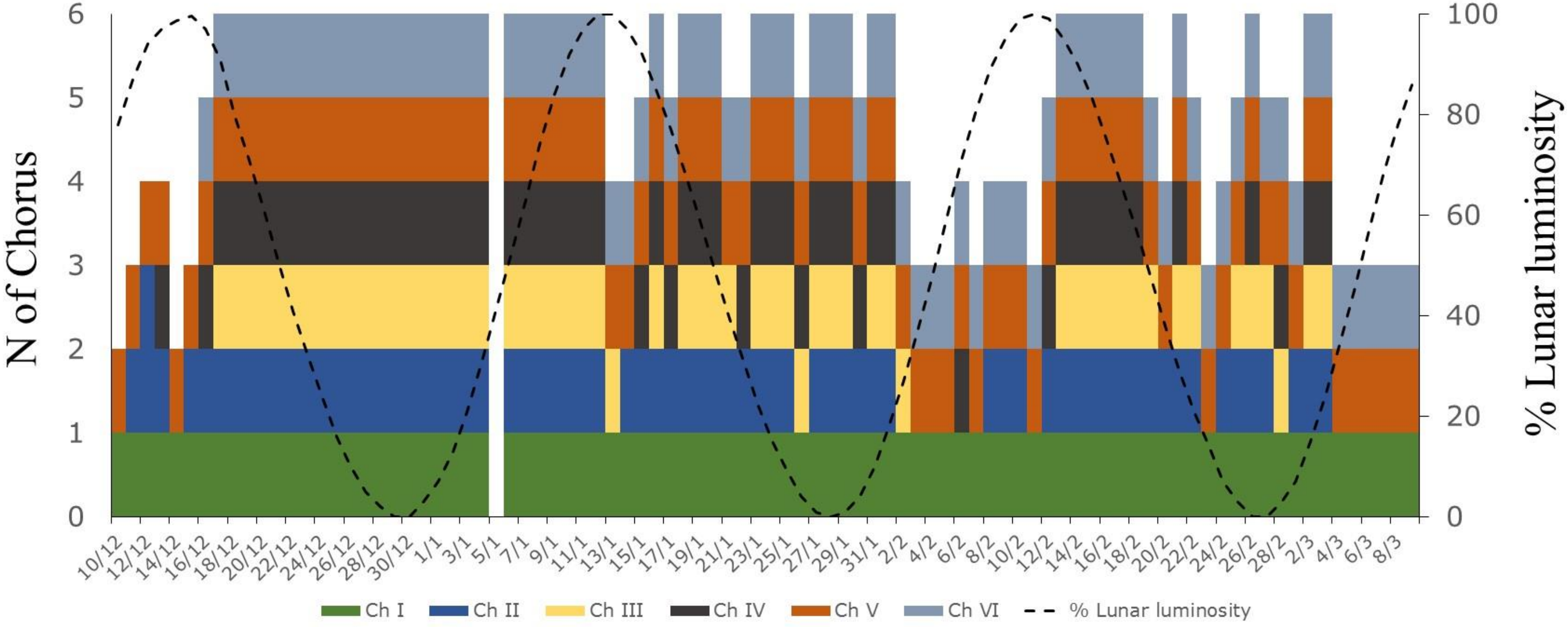

Figure 13. Daily patterns of different fish chorus detected in the Marine Life Protection Zone in Tamandaré during the summer. % of lunar luminosity (dotted dash line).

Table 3. Chorus temporal detections and frequency limits parameters detected. Mean ± SD (min-max)

Chorus type	**Initial time HH:MM**	**Final time HH:MM**	**Detection total HH:MM**	**Low frequency limit (Hz)**	**High frequency limit (Hz)**
I (n=89)	1:20 ± 0:30 [a] (00:08-2:16)	3:07 ± 0:23 [a] (1:52-3:45)	1:47 ± 0:21 [a] (1:04-2:37)	119 ± 29 [a] (52-222)	2981 ± 245 (2413-3424)
II (n=71)	4:06 ± 0:39 [b] (1:56-6:40)	6:22 ± 0:26 [b] (4:29-8:31)	2:15 ± 0:26 [b] (0:38-3:34)	1510 ± 51 [b] (1367-1597)	1845 ± 44 (1767-1948)
III (n=57)	15:27 ± 0:43 [c] (14:04-17:07)	17:51 ± 0:15 [c] (16:34-18:38)	2:23 ± 0:44 [b] (0:37-3:45)	578 ± 669 [c] (301-4021)	1529 ± 198 (1129-1910)
IV (n=58)	17:37 ± 0:31 [d] (16:15-19:42)	19:11 ± 0:29 [d] (15:54-20:33)	1:33 ± 0:20 [a] (0:45-2:37)	929 ± 180 [d] (494-1171)	3861 ± 530 (2285-4911)
V (n=89)	18:41 ± 0:28 [e] (17:22-20:33)	20:28 ± 0:30 [e] (19:13-22:30)	1:47 ± 0:23 [a] (0:49-2:43)	300 ± 82 [e] (56-464)	813 ± 119 (663-1067)
VI (n=82)	16:57 ± 0:33 [f] (15:08-18:31)	19:07 ± 0:26 [d] (17:43-20:19)	2:09 ± 0:21 [b] (1:06-3:02)	80 ± 13 [e] (56-103)	311 ± 19 (279-354)

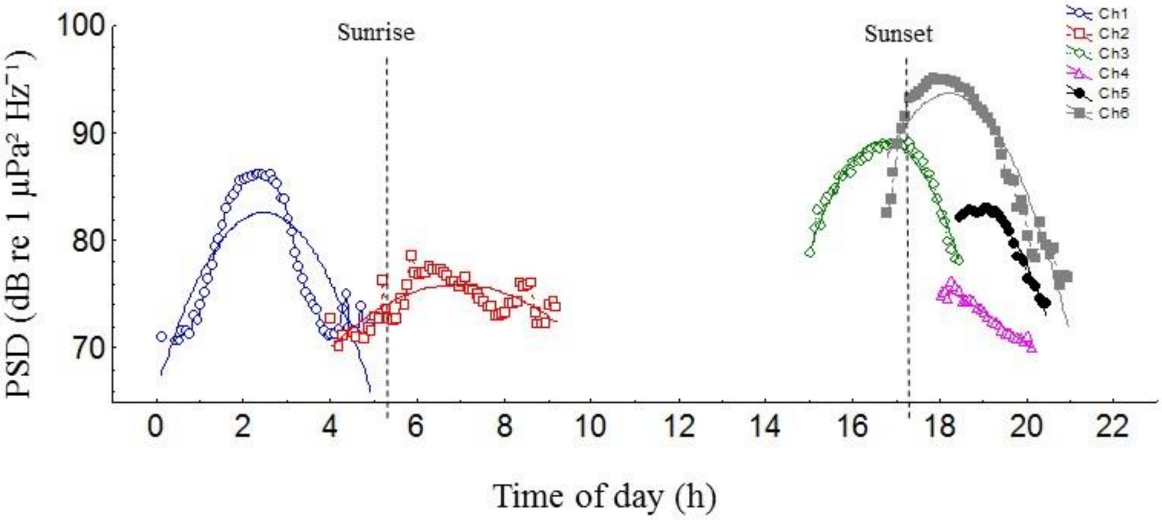

Figure 14. Daily pattern distribution of fish chorus related to the acoustic energy (dB re 1 μPa^2 Hz^{-1}) detected in the Marine Life Preservation Zone of Tamandaré during the summer. Dotted lines indicate local time at sunrise (5:15) and sunset (5:30 pm).

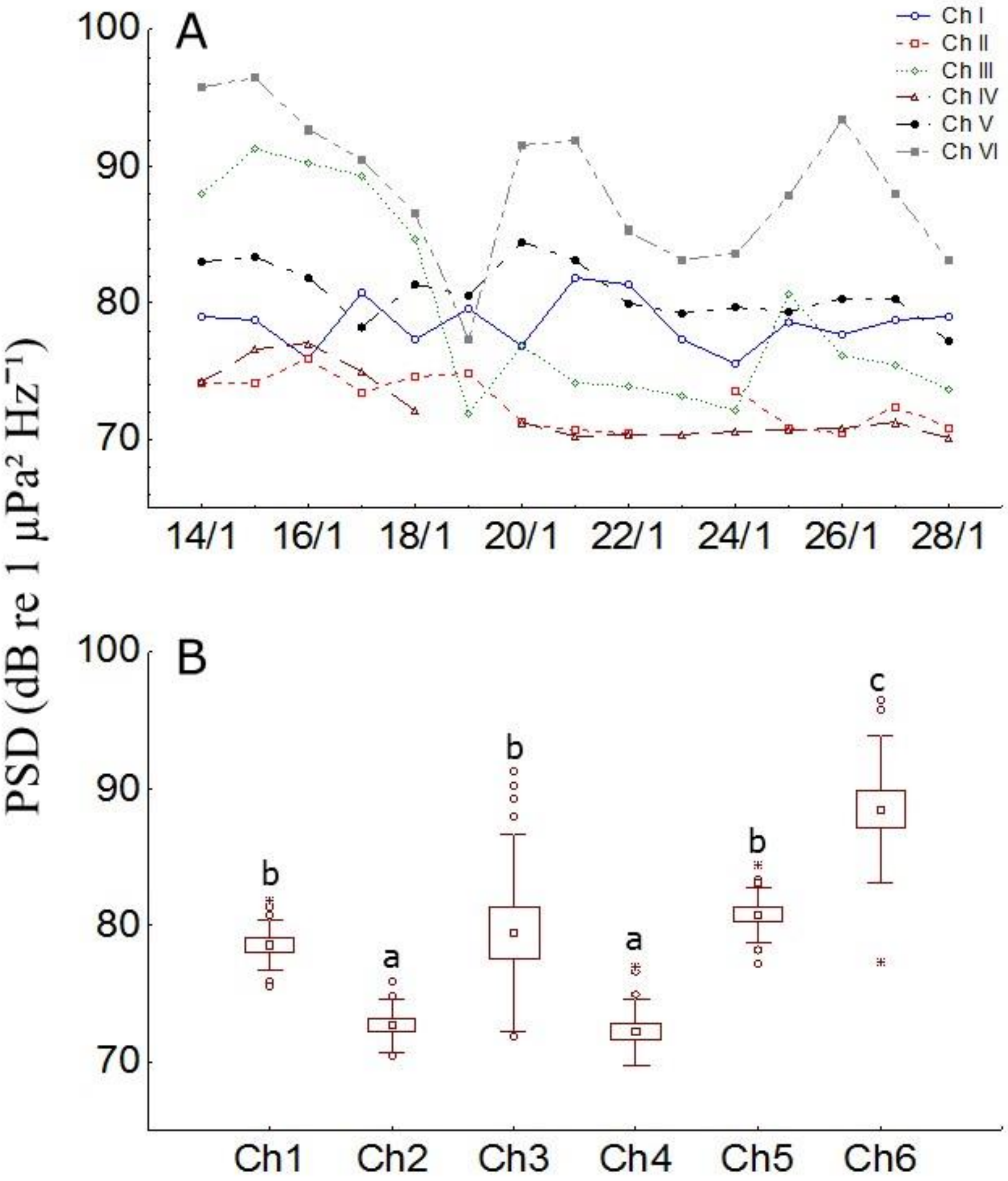

Figure 15. Daily energy pattern (dB re 1 μPa2 Hz-1) of fish choirs detected three days after the full moon in the Marine Life Preservation Zone of Tamandaré during the summer. Different letters indicate significance difference ($p<0.05$).

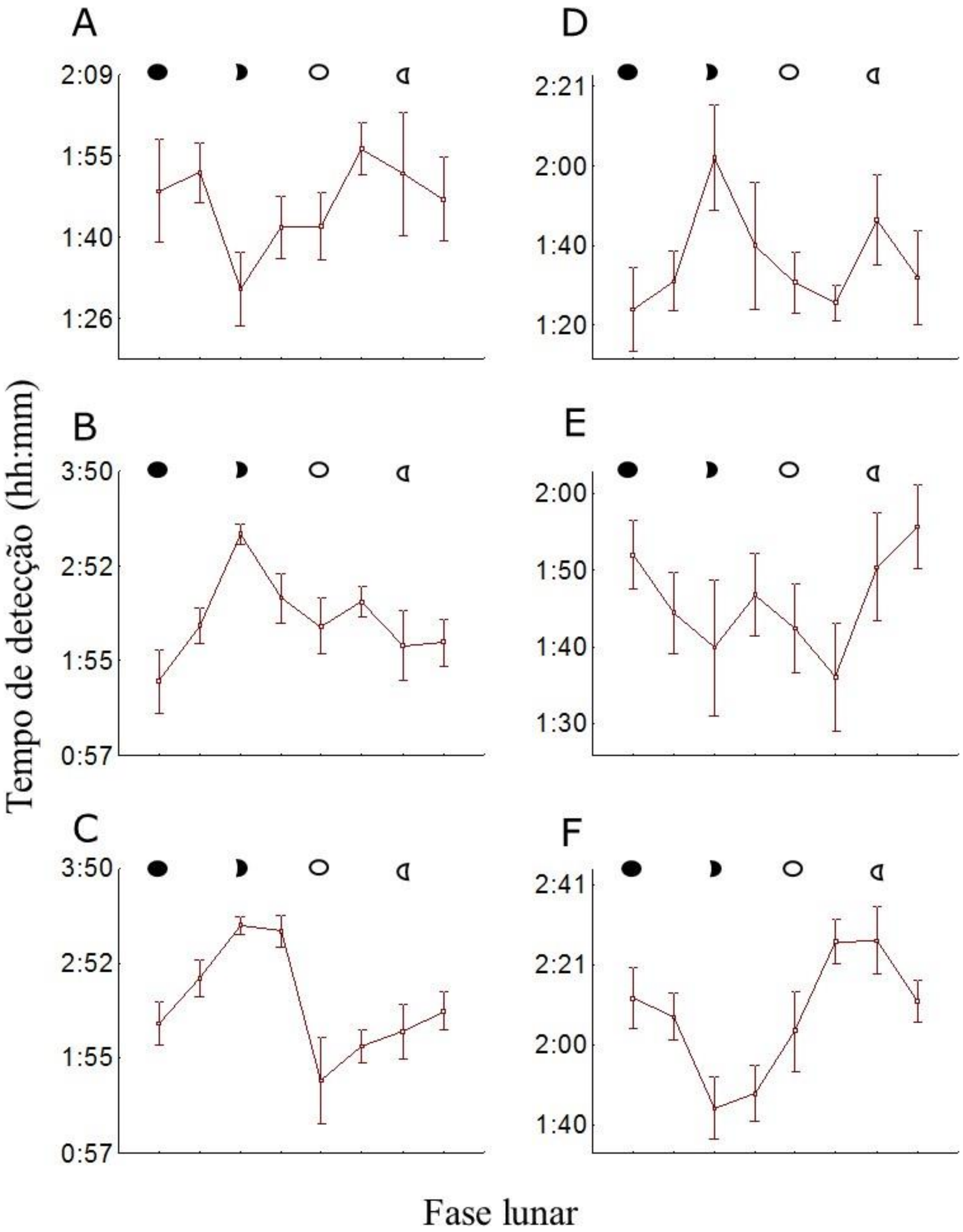

Figure 16. Patterns of total detection time of fish chorus in the different lunar phase. A. Chorus I; B. Chorus II; C. Chorus III; D. Chorus IV; E. Chorus V and F. Chorus VI.

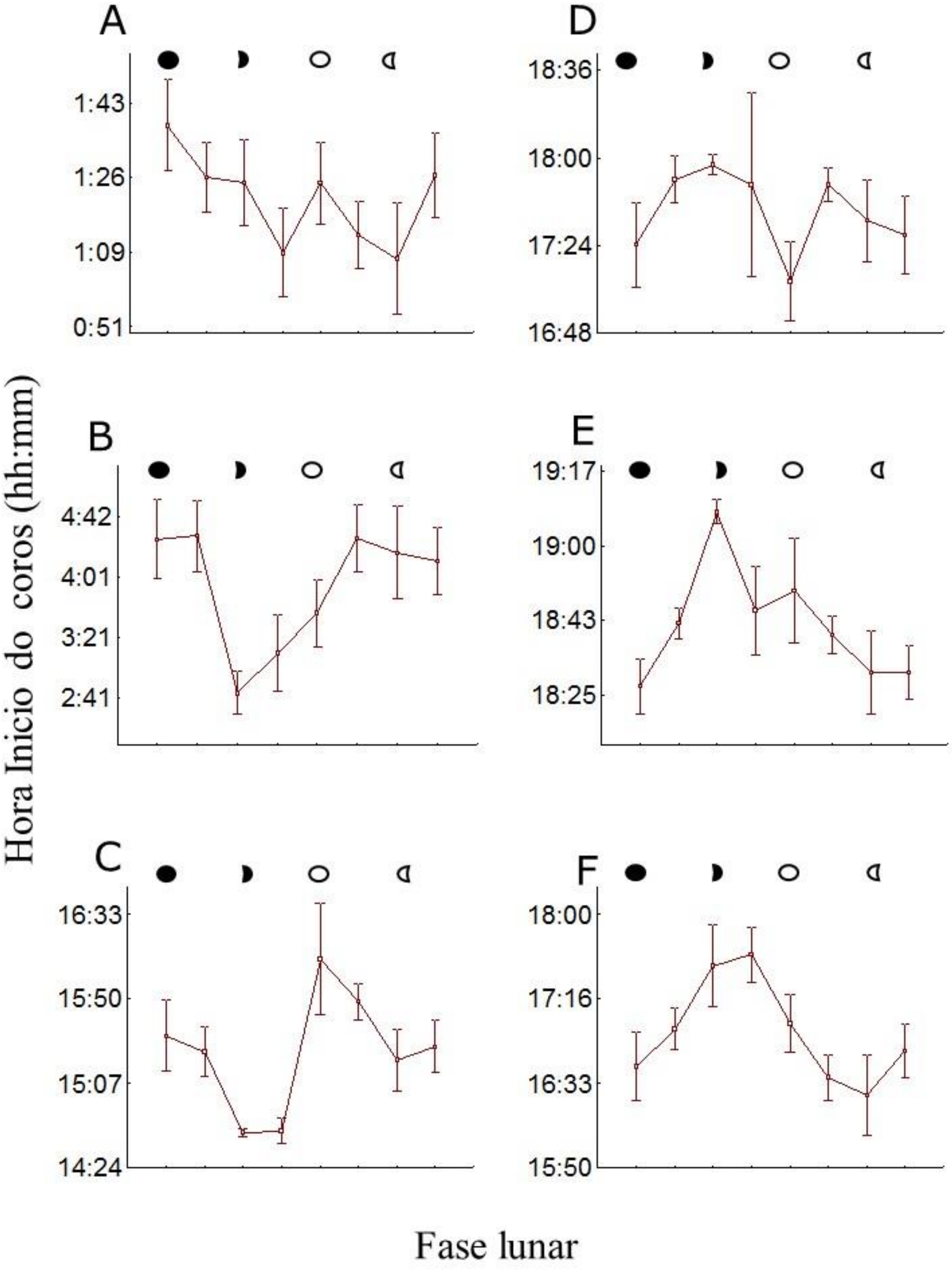

Figure 17. Patterns of Fish Chorus initial times in the different lunar cycle phase. A. Chorus I; B. Chorus II; C. Chorus III; D. Chorus IV; E. Chorus V and F. Chorus VI.

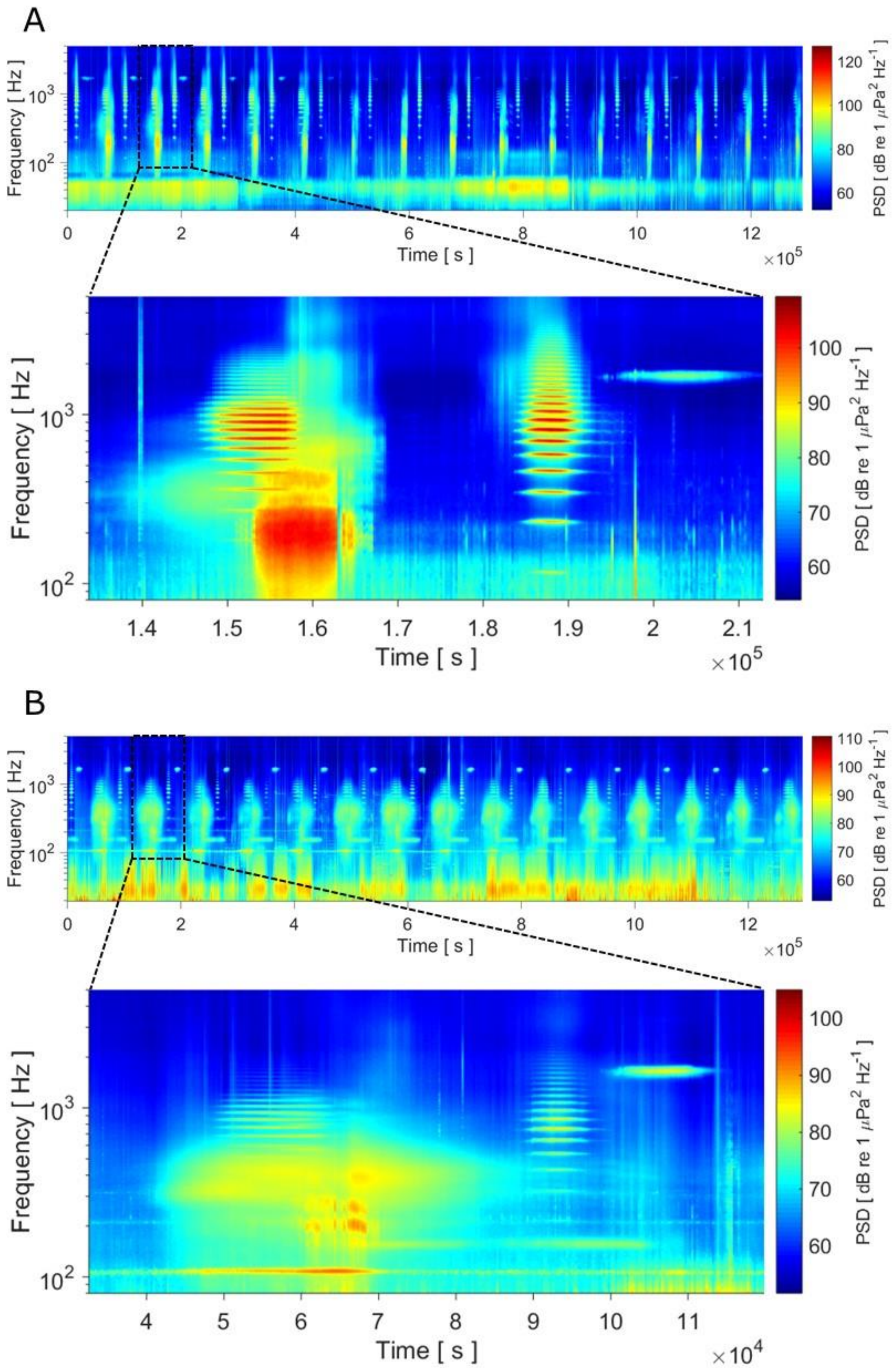

Figure 18. Spectrograms of 15 days after full moon in A. February, (summer) and B. June, 12-27 (winter). The spectrograms were produce using 1024 point Hanning window (50% overlap), plotted on a logarithmic frequency scale between 80 and 5000 Hz.

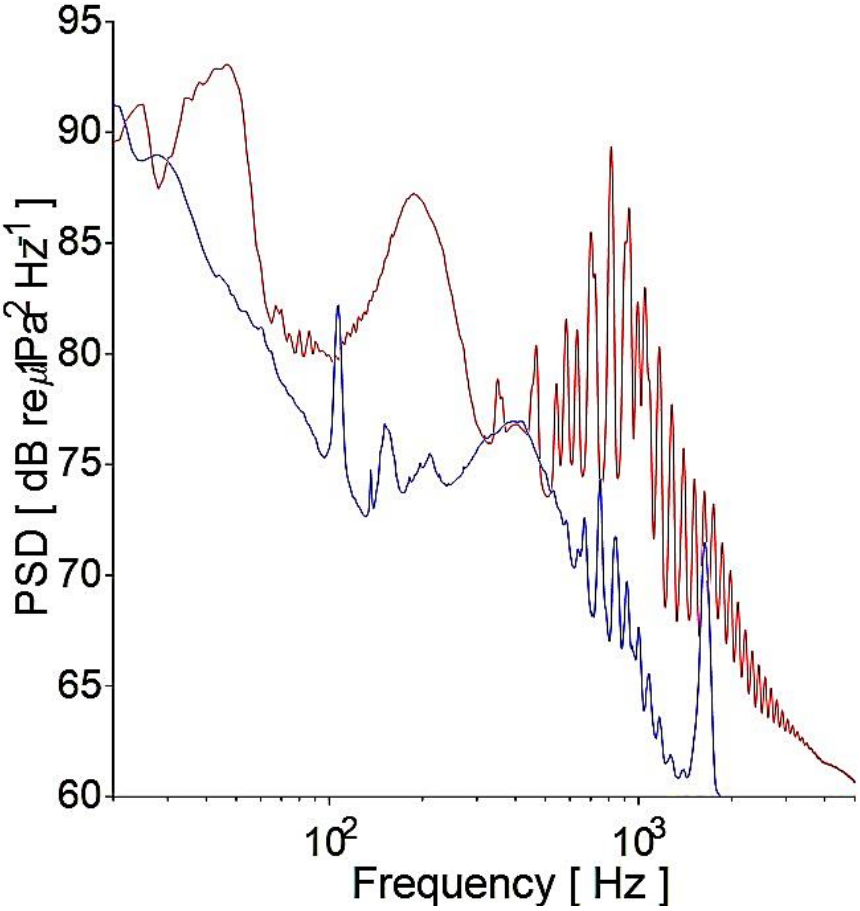

Figure 19. Spectrum of 15 days after full moon in February (red line) and B. June, 12-27 (blue line), plotted on a logarithmic frequency scale between 20 and 5000 Hz

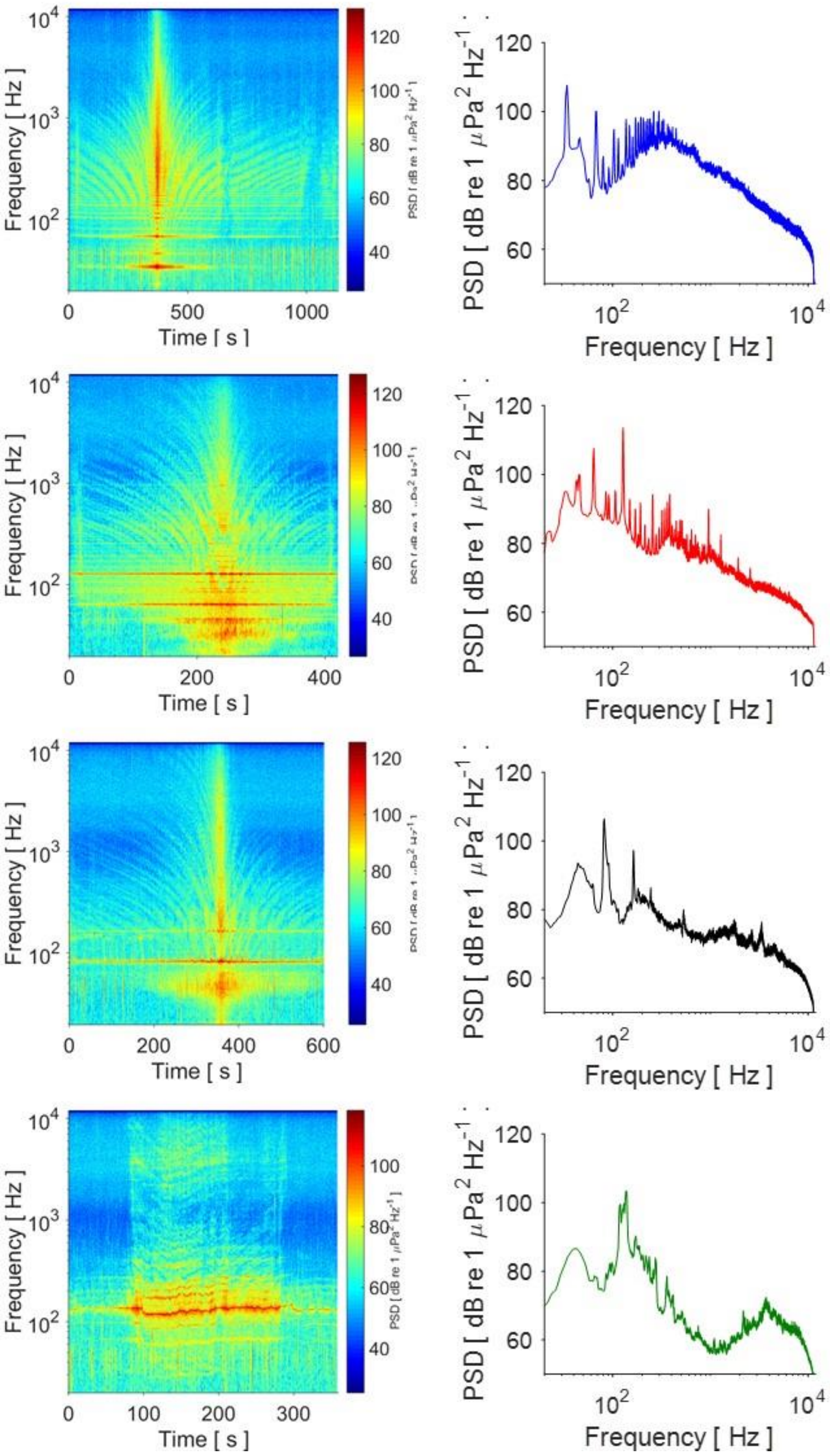

Figure 20. Spectrogram and power spectrum A. fishery vessel B. recreational boats. The spectrograms were produce using 1024 point Hanning window (99.9% overlap), plotted on a logarithmic frequency scale between 20 and 10000 Hz.

Table 4. Frequencies (Hz) acoustic parameters of the) detected in the different vessels. Mean ± Standard Deviation (Minimum - Maximum).

Vessel type	Limit of low frequency (Hz)	Limit of high frequency (Hz)	Dominant frequency (Hz)
Fishery	32 ± 12 (15 - 81)	8418 ±1803 (3720 -11330)	166 ± 288 (22 - 1060)
Recreational	50 ± 20 (18 - 84)	9428 ± 860 (7537 - 11329)	161 ± 73 (53 - 321)
Ship	55 ± 13 (36 - 666)	2058 ± 727 (1235 - 4196)	155 ± 248 (40 - 1000)

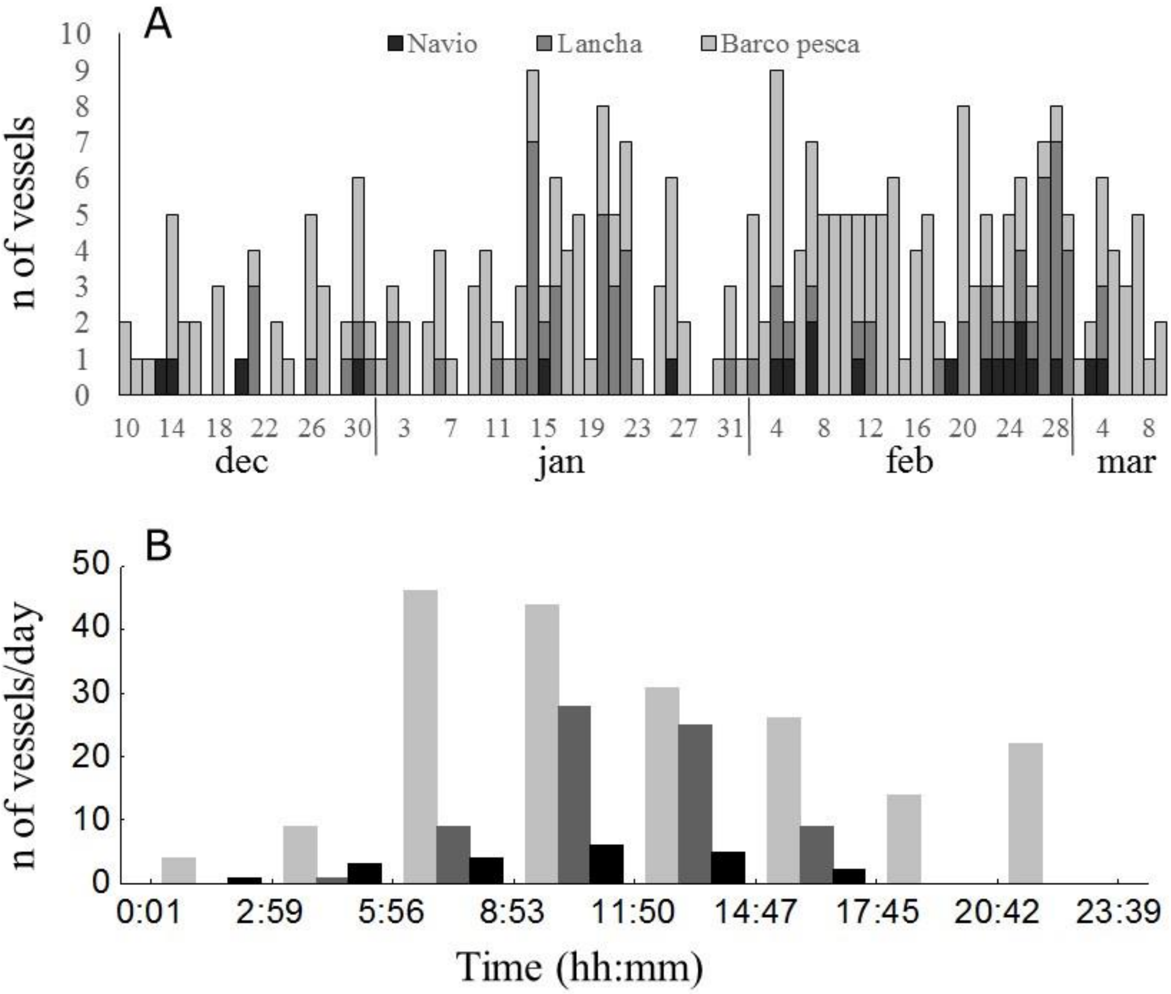

Figure 21. A. Number of vessels per day and B. standard of schedules of the total number of vessels detected.

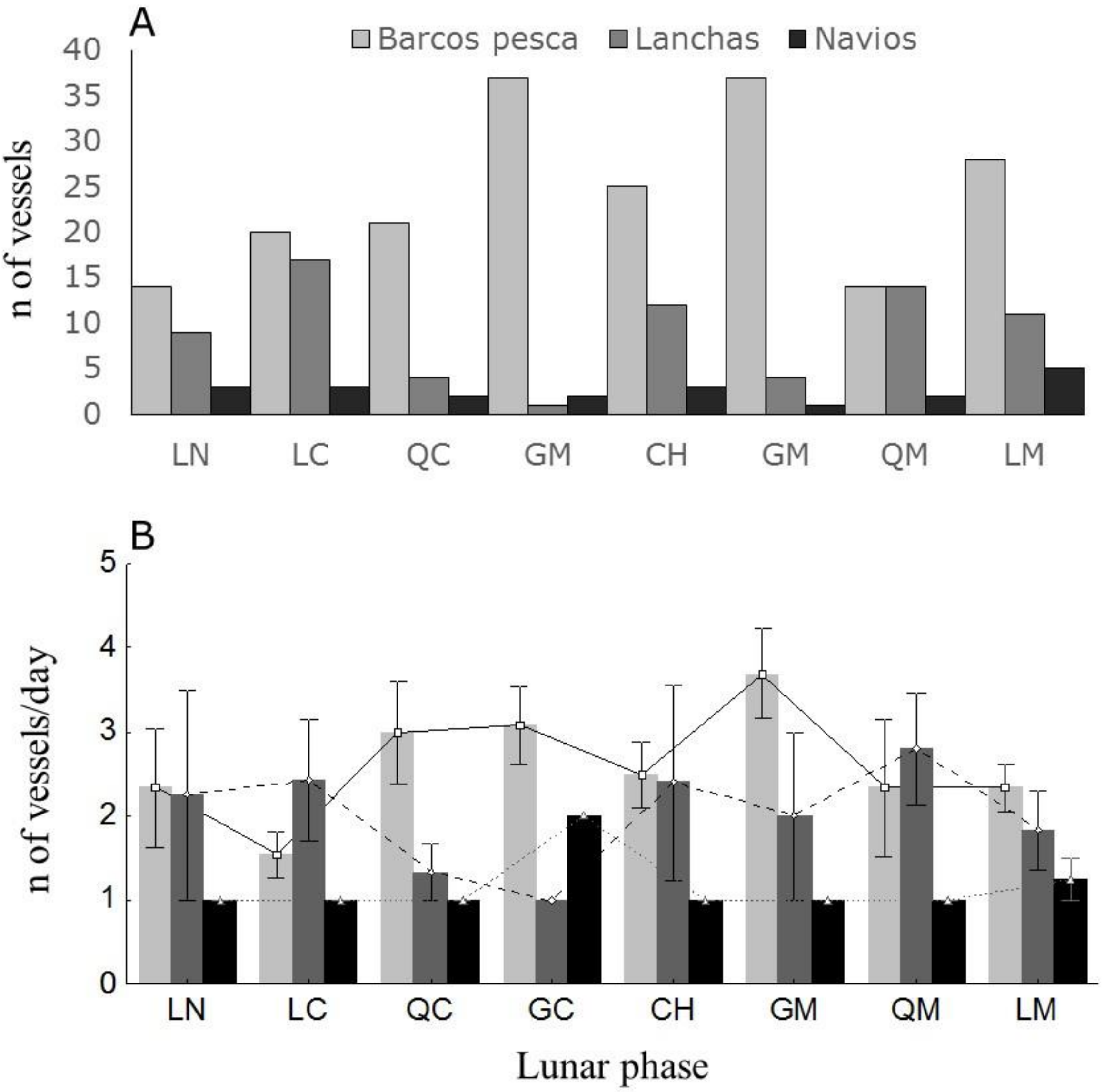

Figure 22. Number of fishing vessels A. total and B. per day (indicating mean ± standard error), detected in the different phases of the moon.

6. Artigo cientifico III

CALLS OF FIVE CHARACIFORM MIGRATORY SPECIES AND CHORUS IN SPAWNING SITES OF AMAZON RIVERS”

Abstract

Fishes produce sounds to defend territories, avoid predation, or during courtship/spawning. We used the passive acoustics evaluate species-specific sounds in five commercial characiform species (*Potamorhina latior*, *P. altamazonica*, *Psectrogaster amazonica*, *Semaprochilodus insignis* and *Prochilodus nigricans*). Hand-held disturbance calls in air and underwater recordings were made in river confluences in the Madeira basin during the high-water mating season (January and February). *P. latior* and *S. insignis* produced sounds with a similar pulse period and pulse rate in overlapping mating areas in the Guaporé River. *P. latior* preferred river confluences during calling and was the dominant sound-producing species during the study period. *S. insignis* shoals occurred mostly in the main river channel. Two other types of underwater sounds recorded in the mouths of Sotério and Machado Rivers were likely emitted by *P. nigricans* and *Anodus elongatus* respectively. Males of these species have an extrinsic sonic muscle associated with the first ribs and attached in an aponeurosis surrounding the lateral swimbladder. These results could help to identify and differentiate underwater sounds, evaluate breeding areas and courtship period, as a part of acoustics monitoring programs used in management strategies for these species.

Key-words: bioacoustics, communication, mating call, Curimatidae, Prochilodontidae, sonic muscle

Introduction

The Amazon river basin is the largest biodiversity center for freshwater fish of any other large river basins around the world (Junk et al., 2007), representing probably by more than 3500 specie which inhabit a dense network of rivers connected to extensive areas of floodplains (Junk et al., 1997). These wetlands oscillate between terrestrial and aquatic phases in an annual hydrological cycle that influence directly the fish migration strategies (Ruffino et al.,1995). Migratory fish are adapted to a life strategy strongly associated with the hydrological cycle of the Amazon Basin, during rising water levels, the spawning success of migrating species depends more on the hydrological conditions than on food supply (Worthmann 1982).

Some Amazon fish are famous for performing mass migrations associated with feeding and reproduction. The migration generally occur between resource-rich rivers (characterized by a turbid “white-water”) and floodplains. The spawning migrations start at the beginning of the flooding season; fishes move out from nutrient-poor tributaries (black- and clear-water rivers) downstream toward the confluence with white-water rivers. After breeding, these fish move toward the flooded forest to feed (Araujo-lima e Ruffino, 2003; McClain & Naiman 2008; Freitas et al., 2012).

Many of the most abundant migratory species are Characiform especies, which represent up to 70% of the fish biomass in South American freshwaters (Carolsfeld et al., 2003). Migrations of commercially important Characiforms are represented by species of Characidae, Prochilodontidae, Curimatidae and Hemiodontidae families, that form large migratory schools in the Rio Madeira basin (like others Amazon rivers) (Goulding 1979; Doria et al 2012). These families are consumed regionally, and are commercially important in the main cities of the Amazon basin, expressing more than 80% of total landings.

Characiforms can perform reproductive aggregations in confluence rivers areas (Junk 1987, Goulding 1980), and although there has been little formal work, migratory prochilodontid fishes of *Semaprochilodus* and *Prochilodus* genera may reveal their presence along the confluence areas by the sounds they make. *Prochilodus lineatus* perform large spawning schools that produce a characteristic “snoring” sound during reproduction when huge numbers of males can be heard (Pesoa and Schulz 2010).

Amazon fish produce sound using a variety of different mechanism, the most important comprise specially developed sonic muscles attached to the swimbladder, very know in piranhas (Eichelberg, 1977; Kastberger, 1981; Ladich and Bass, 2005; Oniki et al., 2006; Millot et al., 2011; Ladich, 2014 Mélotte et al., 2016). The sonic muscle also is use by males of the amazon croaker *Plagioscion squamosissimum*, developing during the mating season (Borie et al., 2014). Males of *S. insignis* also develop a muscle around the swimbladder, and by rhythmic contraction, the "drum muscle" causes the swimbladder vibration, resulting in loud sounds produced by males at the beginning of the spawning season, (Junk, et al. 1997). With the exception of studies on captive piranhas, bioacoustics work on characids, one of the most specious groups in South America fish fauna has been largely ignored. Shaller (1971, 1972) published two short notes on *S. insignis* showing evidences about sound and sonic muscle in this species.

The need for studies on the ecological aspects of the fish of the Madeira River is mainly due to the operation of two huge hydroelectric power dams located in the region that may affect the pattern of migration of fish species. In this sense, passive acoustics, a non-invasive tool, can be used to complement this study. Therefore, the present study focus in a characterization of disturbance sounds in five commercial amazon Curimatidae, like *Potamorhina latior* Spix & Agassiz, 1829; *P. altamazonica* Cope, 1878; *Psectrogaster amazonica* Eigenmann & Eigenmann, 1889; and the Prochilodontidae *Prochilodus nigricans* Spix & Agassiz, 1829; and *Semaprochilodus insignis* Jardine, 1841. In addition, this work verified the hypothesis of reproductive aggregations proposed by and Goulding 1980 Junk 1987 for characiforms, such as *P. latior*, *P. altamazonica* and *P. amazonica*, *S. insignis* and *P. nigricans* ocurrs in confluences rivers areas.

Material and Methods

Study area

The Madeira River Basin occupies an area twice the size of any other basin in the Amazon region and is considered to be the largest tributary of the Amazon River in terms of sediment discharge and the second largest in flow (Goulding et al., 2003). The fish richness of the Madeira River includes approximately 1000 species, being the largest ever recorded for any other Amazon basin and of the world (Queiroz et al. 2013; Ohara et al., 2013).

The Upper Madeira basin presents a wide range of climatic, geomorphological and biological features. Three of its four main tributaries, the Beni, Madre de Dios and Mamoré Rivers, originate in the Andes and strongly influence the hydrology, morphology, biogeochemistry and ecology of the Madeira River (Guyot 1993, McClain and Naiman 2008). Rivers originating in the Andes are considered a “white-water rivers” with a muddy colour, an alkaline to neutral pH, and a high sediment load. The Guaporé River (known as Itenez in Bolivia), is the only one that comes from the Brazilian shield, presenting “black-water” characteristics including an acidic pH, a high concentration of dissolved organic carbon, and lower suspended sediments (Araujo-lima e Ruffino, 2003).

Madeira Basin Rivers begin flooding in November and may continue to rise until June, rises and falls two months earlier than most of the rest of the Amazon. The flood pulse is regulated by the Andean thaw and regional rainfall concentrated between January and March. The ebb of the river begins in May and extends until November.

Sounds recordings

Hand-held disturbance calls were recorded in air using a digital recorder Olympus WS-400S (WMA format and sampling frequency of 44.1 kHz, and frequency respond of 80 Hz to 20 kHz) with an internal microphone. Natural advertisement calls in eight river confluences where recorder which an H_2a hydrophone (Aquarian Audio), with a sensitivity of -180dB re: 1V/µPa and useful range of 10 Hz to 100 KHz connected to a digital recorder. Recordings were made during the fish collecting campaign for the Ichthyological Collection of Rondônia Federal University (the largest and most complete collection of fish from the Madeira River basin), and also during the Fishery Monitoring Program of the Jirau Hydropower Dam.

Hand-held disturbance calls

Hand-held disturbance calls of Curimatidae and Prochilodontidae commercial species were recorded to support evidence of advertisement calls detected in river confluences. All recordings were made in the late afternoon and early evening (ca. 05:00 pm) in the spawning season during high-waters levels in January and February of 2013.

The characterization of disturbance sounds was performed by recording hand-held disturbance call calls on the boat. Curimatidae fish species were caught with a cast net in the confluence of Guaporé and Mamoré rivers, and the prochilodontidae *Semaprochilodus insignis* was recorded in the Guaporé upstream (Fig. 1) near to the river margin. After the catch, each fish was gently hand-held, until the emission of five calls or for 1 to 2 minutes, with the digital recorder placed 1-2 cm from its abdominal area.

Disturbance calls were recorded from the Curimatidae, commonly called “branquinhas,” including *Potamorhina latior* (9 fish with 15.0 ± 0.5 cm standard length, SL), *Potamorhina altamazonica* (11 fish with 13.3 ± 0.9 cm SL), *Psectrogaster amazonica* (7 fish with 11.6 ± 0.4 cm SL), and the Prochilodontidae including the “jaraqui” *S. insi*gnis (11 fish with 21.0 ± 1.6 cm SL). Also, adults of “curimba” *Prochilodus nigricans* were collected and recorded from the Santo Antonio hydropower reservoir in the Madeira River, and sound files were provided by the Fishery and Ichthyology Laboratory of Rondônia Federal University.

Soon after each recording, the fish were euthanized by cranial concussion for later investigation of the sonic mechanism. Work was done with the authorization for capture, collections and transportation of biological material of fish issued by the Brazilian Ministry of the Environment, through license number 83/2012.

Fish chorus distribution

The importance of river confluences to fish aggregation during the spawning season was evaluated through recordings in eight river confluences with different water characteristics in the Madeira Basin. The complex of Madeira/Mamore Rivers was considered as the main steam of Madeira Basin.

Recordings were made in the downstream to upstream direction, in the confluence of Madeira/Mamoré Rivers and "black-water" tributaries like Machado (and its last tributary, the Negro River), Pakaas, Sotério, Guaporé, and also in the Guaporé upstream tributaries like Cautário, and Andes "white-water" Machupe and Branco Rivers (Fig. 1).

Due to the strong current and lower or non-detection of sound in the Madeira/Mamore "white-water" rivers main channel, the recordings were mainly made in the mouth of its tributaries and sometime in the river plume, except for the Guapore River, where the recordings were made both at the mouths of the tributary rivers and on the main channel. The underwater recordings were made during two to three days for 1–2 hours day^{-1} in each river confluence.

Acoustic analysis

Recording files were downloaded to a portable computer for pre-evaluation. Segments containing acoustic recordings were transformer and saved in WAV format (16-bit amplitude resolution) and stored for later analyses. Audacity® (v.2.2) audio software was used to exclude mechanical and human noise from the selected parts of the sound files for individual signal analyses.

A one minute segment of each recording was selected for analysis of each call type. Segments were chosen according to signal quality and amplitude. Selected sections had minimal overlapping signals whenever possible. We measured the following acoustic parameters: 1. Number of pulses per call (n); 2. Call duration (ms); 3. Pulse period (ms); 4. Pulse rate (pulse s^{-1}), 5. Pulse cycle period of the greatest amplitude cycle within a pulse (ms); 6. Dominant frequency (Hz) of call; pulse cycle period, and calculated from the cycle period. The spectrogram and power spectra employed 256 points Hann windows with 98.8% overlapping. The acoustic parameters were measured using oscillograms and spectrograms, with a resolution of Discrete Fourier transform (DFT) of 512 and overlap of 98.8% analysed in Raven Pro 1.4 software (Cornell Laboratory of Ornithology).

To evaluate the frequency band distribution over time and the PSD "Power Spectral Density" (dB re 1 μPa^2 / Hz) of sound in each river confluence, spectrograms and power spectra were elaborated using the PAMGuide tool (Merchant et al., 2015) in MATLAB 2016. These spectra were visually evaluated for general trends in underwater and disturbance recordings. The spectrograms were plotted with a frequency band between 100 to 4000 Hz. A band between 100 and 2000 Hz was used for the power spectra analysis of underwater and disturbance recordings. The Mean Square Root (RMS), and the percentiles (1, 5, 50, 95 and 99%) levels of the PSD values were calculated.

The acoustic parameters of each sound signal were compared using non-parametric Kruskal-Wallis multiple comparison tests between the different groups for each variable using STATISTICA 7 (Dell Inc.). The principal components analysis and canonical modules were used for identifying important dimensions in a set of variables with similar characteristics after testing the assumption of independence between the acoustic variables.

Results

Hand-held and underwater voluntary calls

Species captured on the mouth of Guapore River (confluence with the Madeira/Mamore Rivers) were from the family Curimatidae (*P. latior*, *P. altamazonica*, *P. amazonica*), and the catches were only of *S. insignis* in Guapore upstream (Figure 1). The occurrence of the species were responsible for the chorus in these areas and it was possible to hear the sounds of the aggregations on board, passing over the water interface. Captured fish were individually recorded, producing sounds in the air and audibly distinguishable between the species.

Underwater individual voluntary calls (Figure 26) were found in the Guaporé and Sotério Rivers (defined as Chorus I and Chorus II, respectively). The individual calls of Chorus I were recorded near to the *P. latior* occurrence, with similar acoustic characteristics. Calls of Chorus II presented similar acoustic parameters to *P. nigricans* (Table 6).

Each species emitted different types of hand-held disturbance grunts, also were produced a sound similar to a bladder being emptied (Supplementary Figure S1). Different grunts sounds were composed by trains of pulses. The pulses were composed of different oscillations and number of cycles (Figure 24), in the case of *P. amazonica* a single cycle (Figure 24C). Pulse cycle period of the greatest amplitude within a pulse was 2.4 ms in *P. nigricans*, and 1.2 ms in *S. insignis*. This is related to the low levels of dominant frenaqucy in this species, presenting means of 0.5 and 0.9 kHz respectively. Among the curimatids, *P. latior* had a higher dominant frequency, with an average of 1.3 kHz, being 0.6 kHz in *P. altamazonica* and 0.8 kHz in *P. amazonica* (Table X).

The number of pulses was higher in *S. insignis*, averaging 28 pulses per call, being lower in the species *P. altamazonica* and *P. amazonica*, both with an average of 5 pulses (Table X). The number of pulses was related to the duration of call, and despite the larger number in *S. insignis*, the call duration was higher in *P. nigricans*, with mean duration of 830.2 ms. The number of pulse and call duration was directly associated to the pulse period (Figure XX), and it was longer in *P. nigricans*, with mean of 48.6 ms, similar to Chorus II. The pulse period was very similar in *P. latior, S. insignis* and Chorus I, presenting around 13 ms between pulses, this species also showed a similarity of pulse rate values (Table X).

The greater values of pulse rate were found in *P. amazonica*, with a mean of 122 pulses per second. On the other hand, was lower in *P. nigricans* (20 pulse/s). As the pulse rate increases, the dominant frequency also tends to increase, showing smaller values in P. *nigricans* and Chorus II. On the other hand, the greater pulse period in these species implies in lower the dominant frequency.

The power spectra showed a series of peaks amplitude of *P. latior*, *P. altamazonica*, *P. amazonica* and *S. insignis* (Figure 3), increasing as they reach to the peak frequency. For *P. latior* peaks occurred at intervals from ~100 Hz to the peak frequency at ~1200 Hz (Figure 2.A). In other species of Curimatidae the interval between frequency peaks varies from~50 Hz in *P. altamazonica*, to 200 Hz in *P. amazonica* (Figure 3. B and C). The pattern of intervals between peaks in *P. latior* appears to be similar for *S. insignis*, reached the peak frequency at ~500 Hz and presented a second peak at ~1200 Hz (Figure 3. D). Peak intervals were not observed clearly in *P. nigricans*, presented a range of 500 Hz (Figure 3. E).

These calls overlapped in the pulse rate, pulse period, and dominant frequancy (Figure 6A) with *P. latior* and S. *insignis*, and Chorus I. However, Chorus II was more similar to the sound emitted by *P. nigricans* (Figure 6. A and B).

The Kruskal-Wallis test applied for each acoustic parameter indicated a significant difference between the Chorus I and II ($p<0.05$). There was no significant difference ($p> 0.05$) of sounds produced by *P. latior* and Chorus I for pulse rate, pulse period, dominant frequency and cycle frequency (Table 1). The disturbance sound of *P. nigricans* and voluntary Chorus II were not significant ($p > 0.05$) for all acoustics parameters (Table 1).

The original set of 8 acoustic parameters was transformed by PCA into two Factors that accounted for 69.7% of the total variance (Factor 1 = 50.1%, Factor 2 = 19.6%). In particular, the Factor 1 was highly correlated (> 0.70) and most correlated with the variables Pulse rate, Cycle frequency (Hz), Frequency calculated (Hz) (high negative correlations), and Pulse period (ms), Pulse cycle (ms) (high positive correlations), whereas Factor 2 with Pulses per call and Call duration (ms) (Table 2).

Canonical discriminant analyses was not allow to discriminate between the acoustic signals of *P. latior*, *S. insignis* and Chorus I. However, there is a greater proximity between *P. latior* and Chorus I, indicated by the mean values of the canonical variables. This was also observed between Chorus II and the sound of *P. nigricans*. On the other hand, the characteristics and acoustic patterns of *P. altamazonica* and *P. amazonica* were isolated, with *P. amazonica* plotted further to the right (Figure 7).

Chorus in the river confluences

Different fish sounds were recorded in all river confluence during this study, and sometimes being audible on board. The frequency band was similar in Negro, Machado, Pakaas, Cautário and Machupe rivers, with a frequency band of 250 to 2000 Hz. On the other hand, Sotério and Branco Rivers presented a low frequency band reaching 1000 Hz. All rivers confluence presented relative PDS above 120 dB re 1 $\mu Pa^2 Hz^{-1}$.

The Negro and Machado Rivers presented similar patterns, possibly due to their proximity. It was possible to differentiate between the sounds made by different species. It was generally possible to distinguish choruses from more than one species in all confluences, except in Guaporé and Cautário Rivers. The choruses overlapping made it impossible to isolate individual callers in the confluences, with the exception of the nears areas of mouth of the Guaporé and Sotério Rivers. There was also possible to detect individual calls, which were analysed with the same acoustics parameters used in disturbance sounds. The individual calls detected in the Sotério and Guapore Rivers were analysed and defined as Chorus I and II respectively (Table 2).

The power spectra confirmed the idea that more than one fish chorus could be found in the Madeira confluences rivers. It seems that in the Guaporé and Cautário Rivers a single chorus probably produced by *P. latior* occurs due to the similarities of characteristic in the series of peaks energy. In this sense, the chorus found in the Sotério River was comparable to *P. nigricans* and Chorus II, presenting different spectral characteristics compared to other rivers. The peak frequency of ~ 250 Hz is similar to the spectrum found in *P. nigricans* (Figure 5. D).

The occurrence of *P. latior* becomes more evident in the mouth of the Guaporé River and its tributary, the Cautário River, (similar peak characteristics between 100 Hz and the peak of 1000 Hz (Figure 5. E F).It was possible to detect the occurrence of *P. latior* and *S. insignis* clos e to each other in the same area of the Machupe and Branco Rivers (Figure 5.G and H), although the peak frequency of *S. insignis* was below (~ 400 Hz).

In general, the power spectra shows that the biological acoustic signals are above 100 dB re 1 $\mu Pa^2\ Hz^{-1}$ within the 1%, and above 140 dB re 1 $\mu Pa^2\ Hz^{-1}$ when they reach the peak of the frequency in the 99% of observations. The range of both percentiles was maintained until the 2 kHz, decaying after this.

Sonic apparatus

Curimatid and prochilodontid species use a pair of similar extrinsic intercostal sonic muscles present only in males. The sonic muscles are attached to the first rib in Curimatidae or firts ribs in Prochilodontidae. The muscle uscle was located in the anterior (most part) and posterior part of the innervated first rib in *P. latior*. In contrast, this muscle occurs only from the first rib, projecting forward to the front of swimbladder in *P. altamazonica* and *P. amazonica*. In Prochilodontidae, the sonic muscle was located between the first four ribs in *S. insignis*, and in the first five in *P. nigricans* (few observations in laboratory). Similarly, in *Anodus elongatus* (Hemiodontidae), the most captured species at the confluence of the Negro and Machado Rivers, a pair of sonic muscles was also present only in males and associated with the first ribs were observed.

Two-chambers compose the swimbladder and the muscles surrounding the lateral surface would are likely cause the bladder to be compressed. The big cycle of the sound likely reflects sonic muscle contractions and later cycles likely are caused by repeated cycling of the ribs and/or the Heba tendon (see Parmentier and Fine, 2016).

Discussion

The sounds produced by the fish during recordings out of water, evidenced an audible distinction between the different species. Thus, even species of the same family and even of the same genus may have very specie-specific sounds. This has already been observed in other species of Amazonian fish such as piranhas, which although they present a similar sound-producing apparatus between the different species, the sounds are clearly distinguishable (Méllote et al., 2016). Despite this, and as seen in this study, species of different families such as *Potamorhina latio*r and *Semaprochilodus insgnis* may present quite similar acoustic characteristics and parameters, being difficult to differentiate.

Within Characiforms, several extrinsic drumming mechanisms evolved, which move the swimbladder indirectly. In the jaraqui *Semaprochilodus insignis* rapid contractions of well-developed intercostal muscles vibrate the swimbladder (Schaller, 1971), allowing these fish in the Amazonian river system to produce loud evening choruses. Schaller (1972) affirm that even closely related species, have developed very different drumming muscle, present only in males, during the breeding season, with the males produce a loud noise in chorus. The intercostal muscles between the first four ribs are easily recognized by their deep red coloration in fresh specimens. In the closely related branquinha *Potamorhina (= Anodus) laticeps* a paired sonic muscle surrounds the anterior part of swimbladder, running between the left and right first ribs (Dorn and Schaller, 1972), similar to the sonic muscle found in *P. amazonica*.

Other Amazon sonic characids, piranhas, produce aggressive sounds in the red piranha *Pygocentrus nattereri* (Millot et al., 2011). This species and *Serrasalmus rhombeus* have extrinsic sonic muscles (Ladich and Bass 2005). For the red piranha *Pygocentrus nattereri* and *Serrasalmus rhombeus*, the sonic muscles originate on vertebral processes and insert on a broad tendon that surrounds the bladder ventrally. Contrary to the majority of sonic fishes, the sonic muscles in piranhas are innervated by the 3rd and 4th true spinal nerves (Markl 1971, Ladich and Bass 2005).

This work shows strong evidence of mating aggregations of different species at the confluences of the main tributaries of the Madeira/Mamore and the lower Guapore Rivers. In most river confluences, it is possible to audibly detect the occurrence of more than one species. *Potamorhina latior* is the most represented species in these confluences. The occurrence of *P. latior* coincides most with the meeting of waters. Precisely this was the most abundant species in this region (Cella-Ribeiro et al 2016). Also, the curimatidae family was the most abundant (56%) in 1 km stretch of the black-water tributary (Belmont River) upstream from its mouth and connection with the Madeira River (Araújo et al., 2009).

The mating aggregation in the Machado and Guaporé River occurs inside the mouth and in the plume of those rivers (lentic area), not in the main channel of Madeira and Mamoré rivers. This makes sense since the flux velocity of these Andean rivers is high and probably affects the egg fertilization when compared with the slower Guaporé. In black-water rivers the current velocity averages 0.4 m s^{-1} in the flood season, and the Andean river averages 1.3 m s^{-1} (Barbosa et al., 2016). In Madeira River the channel has a water velocity of 0.9–1.4 m s^{-1}. Therefore, the fish capacity to maintain swimming speed higher than the water velocity necessitates substantial energy cost (Torrente-Vilara et al., 2011). This cost can be reduced by schooling (but see a review in Blake 2004). Even so, Characiforms are considered efficient swimmers (Santos et al. 2007; Makrakis et al. 2010).

P. latior appears to prefer river confluences for courtship and was the dominant species during the study period. *S. insignis* shoals occurred in the main river channel of black-water Guapore River. *P. latior* and *S. insignis*, produced similar acoustic patterns and these species could be found mating sympatrically in the Guaporé River.

Both audible and spectra similarity, indicated that that choruses detected in the mouths of the Machado, Negro (with dominant frequency of ~ 800 Hz in both) and Pakaas rivers (with frequency peak of 1000 Hz) included *P. latior* (Figure 5. A, B and C) and *Anodus elongatus* (Hemiodontidae), which was the most frequently captured species during the collections for the ichthyofaunal inventory of the Madeira River basin conducted by UNIR. No live individuals were obtained for recording disturbance calls, due to their capture by gillnet.

The chorus found in Curimatidae and Prochilodontidae species come from huge schools, containing hundreds of thousands of individuals (Araujo-Lima e Ruffino 2003) during the mating season. The larval drift indicates an annual reproductive period for the curimatidae and prochilodontidae families, between December and April (Barthen et al., 2014).

Prochilodontid species like *P. nigricans* and other congeners also are sound-producers (GOULDING, 1979). Godoy (1975) observed males of the "curimbata" (*Prochilodus scrota*) in a stretch of 1.5 kilometers in the Mogi-Iguassu River emitting a characteristic croaking sound during spawning activity. In captive conditions, mature *P. argenteus* males emit sounds and release seminal fluid when a slight pressure was applied to their ventral region (SATO & GODINHO, 2003; SATO et al., 2003). *Prochilodus insignis* hand-held conditions during breeding season produce drumming sounds of 300-600 Hz and 300 ms (KASTBERGER, 1978). Similar to the results for *P. nigricans* found in this work, the sounds of three species of Prochilodontidae were similar in nature, with series of low frequency pulses that lasted from 1 to 11 s, often with an initial rapid rise followed by a slow tapering of pulse amplitude. There was also a significant linear relationship between the dominant frequency of pulses and trains (Smith et al., 2017).

This work verified the hypothesis of reproductive aggregations proposed by Junk 1987 and Goulding 1980 for characiforms, such as *P. latior*, *P. altamazonica* and *P. amazonica*, *S. insignis* and *P. nigricans* ocurrs in confluences rivers areas. Using passive acoustic we showed the specificity of the fish, useful as a method of both species and area of reproduction identification. Although the authors propose that these areas are in the main river channel, in this case the Madeira River, these results indicate that reproductive aggregations occur in sheltered areas of the mouths of tributary rivers and could help to develop long-term acoustics monitoring programs as basis for management strategies policies for those species.

In addition to river confluences, there are other well-known Amazonian aquatic environments such as floodplains, lakes, channels and streams, which are considered essential habitats for fish. Certainly also in these environments acoustic signals representative of the species of fish that can be found in these habitats.

References

ARAÚJO, T. R. D. et al. Composition and trophic structure of the ichthyofauna from a stream downriver from Santo Antonio Falls in the Madeira River, Porto Velho, RO. **Biota Neotropica**, v. 9, n. 3, p. 21-29, set. 2009. ISSN 10.1590/S1676-06032009000300001.

ARAUJO-LIMA, C.; RUFFINO, M.. Brazilian Amazon migratory fish. in **Migratory Fishes of South America:** Biology, Fisheries and Conservation Status. [S.l.]: International Bank for Reconstruction and Development / The World Bank, 2003. 388 p. ISBN 978-0-9683958-2-0.

BARBOSA, P. M. et al. Diffusive methane fluxes from Negro, Solimões and Madeira rivers and fringing lakes in the Amazon basin. **Limnology and Oceanography**, v. 61, p. S221-S237, 2016. ISSN 10.1002/lno.10358.

BARTHEM, R. et al. Diversity and abundance of fish larvae drifting in the Madeira River, Amazon Basin: sampling methods comparison. In: **Biodiversity-The Dynamic Balance of the Planet**. InTech, 2014.

BORIE, A. et al. Spatiotemporal variability and sound characterization in silver croaker plagioscion squamosissimus (sciaenidae) in the central amazon. **PLoS ONE**, v. 9, n. 8, 2014. ISSN 10.1371/journal.pone.0099326.

DORN, E.; SCHALLER, F. Uber den Lautapparat von Amazonasfischen, II. **Die Naturwissenschaften**, v. 59, n. 4, p. 169-170, abr. 1972. ISSN 10.1007/BF00637366.

EICHELBERG, H. Fine structure of the drum muscles of the piranha (serrasalminae, characidae). **Cell and Tissue Research**, v. 185, n. 4, p. 547-555, dez. 1977. ISSN 10.1007/BF00220658.

F. SCHALLIGR. Uber den Lautapparat von Amazonas-Fischen, v. 11, 1971.

GODOY, M. P. **Peixes do Brasil - Subordem Characoidei:** Bacia do rio Mogi Guassu. [S.l.]: Piracicaba (Brazil) Edit. Franciscana, 1975. 846 p.

GOULDING, Michael. **Ecologia da pesca do rio Madeira**. Conselho Nacional de Desenvolvimento Científico e Tecnológico, Instituto Nacional de Pesquisas da Amazônia, 1979.

GOULDING, M.; BARTHEM, R.; FERREIRA, E. J. G. **The Smithsonian atlas of the Amazon**. [S.l.]: Washington, DC (USA) Smithsonian Books, 2003. 253 p. ISBN 1588341356.

GUYOT, J. L.; JOUANNEAU, J. M.; WASSON, J. G. Characterisation of river bed and suspended sediments in the Rio Madeira drainage basin (Bolivian Amazonia). **Journal of South American Earth Sciences**, v. 12, n. 4, p. 401-410, 1 jul. 1999. ISSN 10.1016/S0895-9811(99)00030-9.

INTERNATIONAL CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT.; ICLARM--THE WORLD FISH CENTER.; WORLDFISH CENTER. **Naga:** the ICLARM quarterly. [S.l.]: International Center for Living Aquatic Resources Management, 1986. ISBN 0116-290X.

JUNK, W. J. General Aspects of Floodplain Ecology with Special Reference to Amazonian Floodplains. [S.l.]: Springer, Berlin, Heidelberg, 1997. p. 3-20. ISBN 10.1007/978-3-662-03416-3_1.

JUNK, W. J.; SOARES, M. G. M.; BAYLEY, P. B. Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. **Aquatic Ecosystem Health & Management**, v. 10, n. 2, p. 153-173, 8 jun. 2007. ISSN 10.1080/14634980701351023.

KASTBERGER, G. Lautproduktion bei Fischen: die Trommellaute der Jaraquí-Männchen (Prochilodus insignis-Pisces, Characiformes, Tetragonopteriadae, Prochilodontinae, 1978.

LADICH, F. Fish bioacoustics. **Current Opinion in Neurobiology**, v. 28, p. 121-127, 2014. ISSN arXiv:1011.1669v3.

LADICH, F.; BASS, A. H. Sonic motor pathways in piranhas with a reassessment of phylogenetic patterns of sonic mechanisms among teleosts. **Brain, behavior and evolution**, v. 66, n. 3, p. 167-76, 2005. ISSN 10.1159/000087157.

MAKRAKIS, S. et al. Ascent of neotropical migratory fish in the Itaipu Reservoir fish pass. **River Research and Applications**, v. 27, n. 4, p. 511-519, 1 maio 2011. ISSN 10.1002/rra.1378.

MARKL, H. Schallerzeugung bei Piranhas (Serrasalminae, Characidae). **Zeitschrift f�r Vergleichende Physiologie**, v. 74, n. 1, p. 39-56, 1971. ISSN 10.1007/BF00297789.

MCCLAIN, M. E.; NAIMAN, R. J. Andean Influences on the Biogeochemistry and Ecology of the Amazon River. **BioScience**, v. 58, n. 4, p. 325-338, 1 abr. 2008. ISSN 10.1641/B580408.

MÉLOTTE, G. et al. Interspecific variation of warning calls in piranhas: a comparative analysis. **Scientific Reports**, v. 6, n. 1, p. 36127, 26 dez. 2016. ISSN 10.1038/srep36127.

MERCHANT, N. D. et al. Measuring acoustic habitats. **Methods in Ecology and Evolution**, v. 6, n. 3, p. 257-265, 2015. ISSN 10.1111/2041-210X.12330.

MILLOT, S.; VANDEWALLE, P.; PARMENTIER, E. Sound production in red-bellied piranhas (Pygocentrus nattereri, Kner): an acoustical, behavioural and morphofunctional study. **Journal of Experimental Biology**, v. 214, n. 21, p. 3613-3618, 2011. ISSN 10.1242/jeb.061218.

PEIXES do Brasil, subordem Characoidei: bacia do rio Mogí Guassú - Google Livros. [S.l.]: [s.n.].

PESOA, N. A.; SCHULZ, U. H. Diel and seasonal movements of grumatã Prochilodus lineatus (Valenciennes 1836) (Characiformes: Prochilodontidae) in the Sinos River, Southern Brazil. **Brazilian journal of biology**, v. 70, n. 4 Suppl, p. 1169-77, 2010. ISSN 10.1590/S1519-69842010000600006.

QUEIROZ, L. J. et al. Peixes do rio madeira VOLUME I, v. I, p. 402, 2013. ISSN CDD-597.09811.

RIBEIRO, A. C. et al. Primeiras hidrelétricas em rio de águas brancas na Amazônia: resistência da ictiofauna bentônica, TESE 10 jun. 2016.

RUFFINO, MAURO LUIS; ISAAC, VICTORIA JUDITH. Life cycle and biological parameters of several Brazilian Amazon fish species. **Naga, the ICLARM Quarterly**, v. 18, n. 4, p. 41-45, 1995.

SATO, Y.; GODINHO, H. P. Migratory fishes of the São Francisco river. **CAROLSFELD, J.; HARVEY, B.; ROSS, C**, p. 195-232, 2003.

SANTOS, A. C. D. A.; CARAMASCHI, É. P. Composition and seasonal variation of the ichthyofauna from upper Rio Paraguaçu (Chapada Diamantina, Bahia, Brazil). **Brazilian Archives of Biology and Technology**, v. 50, n. 4, p. 663-672, jul. 2007. ISSN 10.1590/S1516-89132007000400012.

SCHALLER, F. Uber den Lautapparat von Amazonas-Fischen. **Die Naturwissenschaften**, v. 58, n. 11, p. 573-574, nov. 1971. ISSN 10.1007/BF00598735.

SCHALLER, F.; KRATOCHVIL, H. Lautbildung bei Fischen. **Biologie in unserer Zeit**, v. 11, n. 2, p. 43-47, 1 abr. 1981. ISSN 10.1002/biuz.19810110205.

TORRENTE-VILARA, G. et al. Effects of natural rapids and waterfalls on fish assemblage structure in the Madeira River (Amazon Basin). **Ecology of Freshwater Fish**, v. 20, n. 4, p. 588-597, 1 dez. 2011. ISSN 10.1111/j.1600-0633.2011.00508.x.

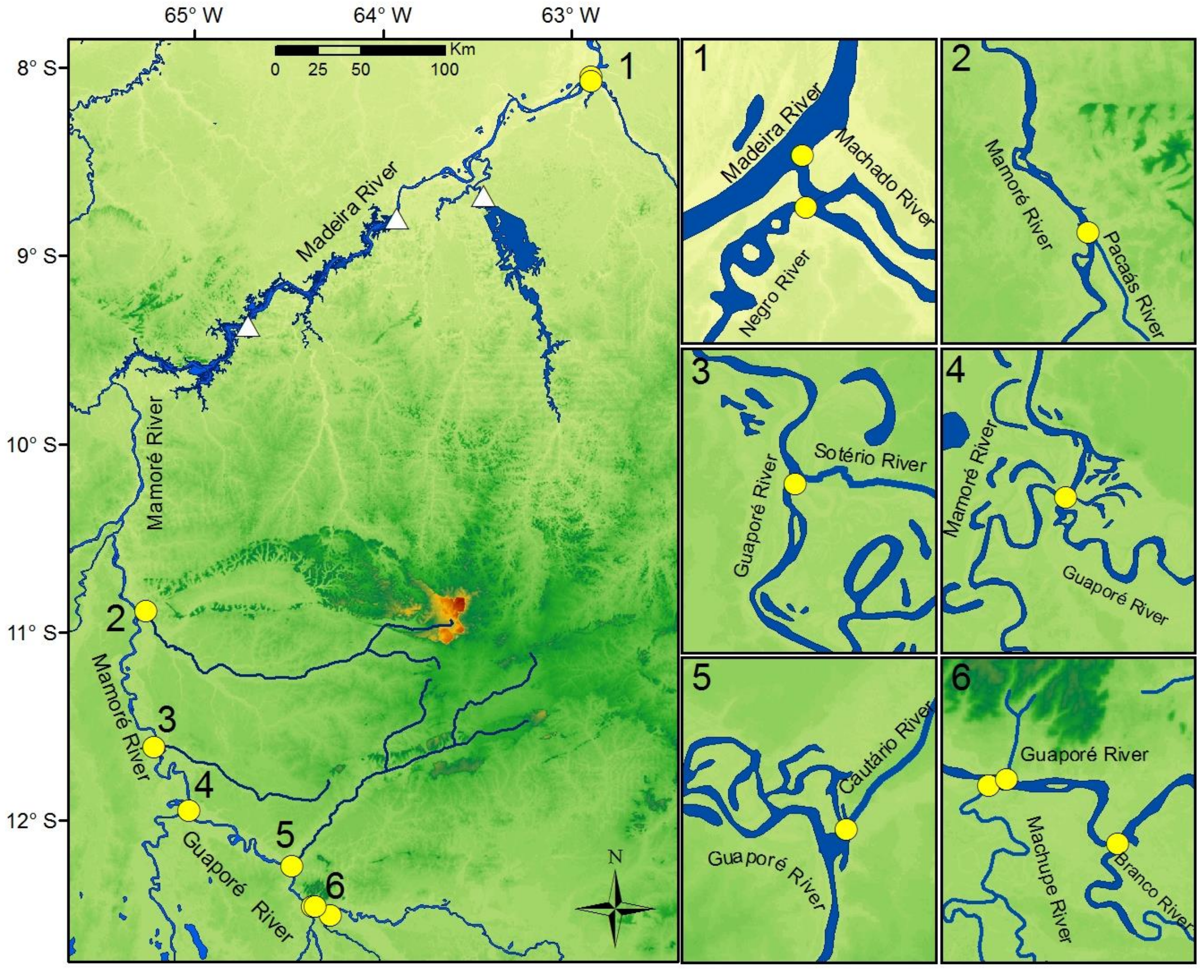

Figure 23. Study area of recordings in confluence Rivers of Madeira/Mamoré River and "black-waters" tributaries. 1. Machado (and their last tributary, the Negro River); 2. Pacaás; 3. Sotério; 4. Guaporé; 5. Cautário, and Andes "white-water" 6. Machupe and Branco Rivers. Disturbance calls were recorded in area 4 (Curiamatidae) and 6 (S. insignis).

Figure 24. Amazon Characiformes sound-producing fish

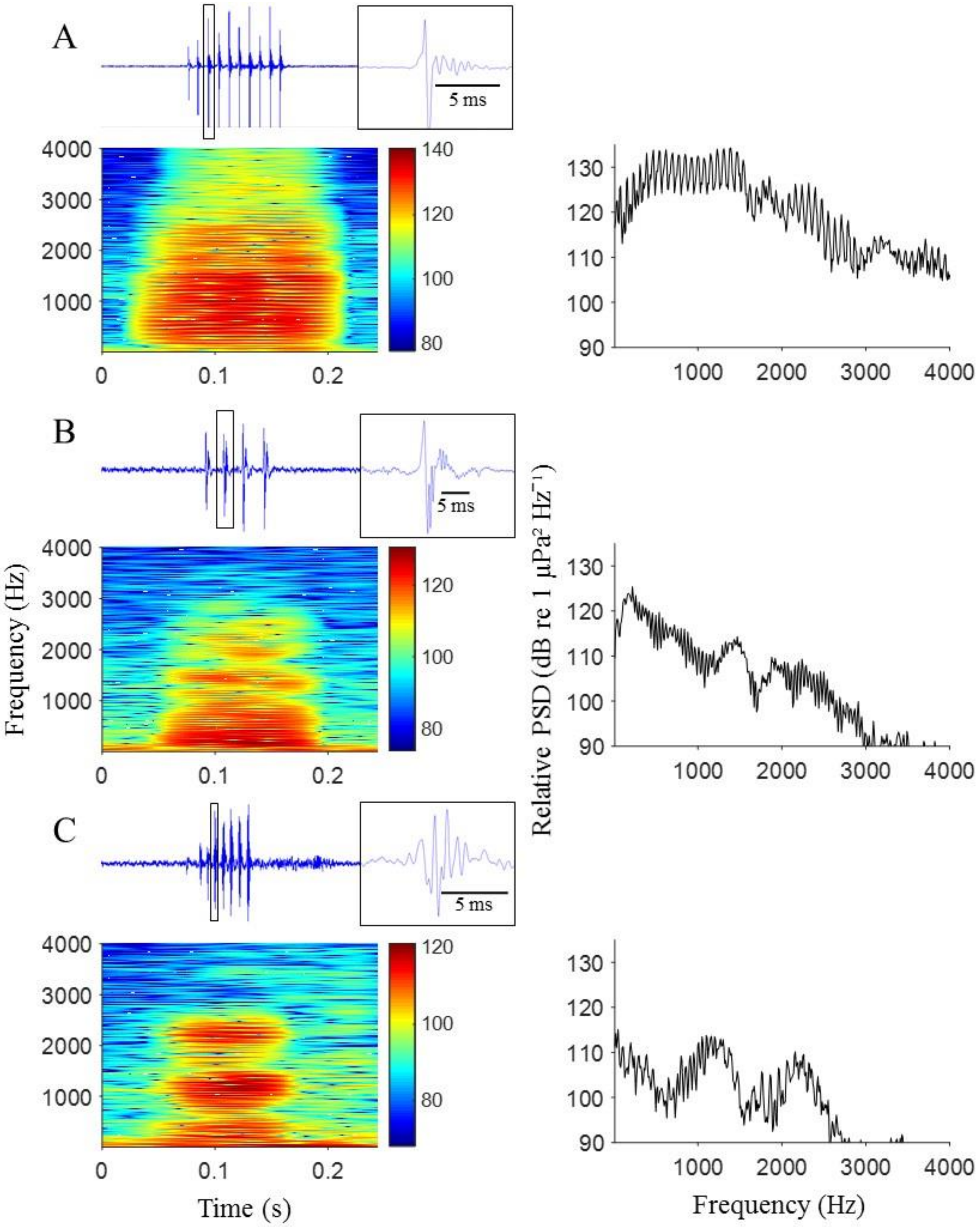

Figure 25. Curimatidae disturbance calls spectrograms, waveforms (individual pulse indicated by squares) and spectrum of A) P. latior; B) P. altamazonica; C) P. amazonica. Hann windows type, DFT size 512 and overlap of 98.8%. Low and high frequency limits of 100- 4000 Hz. Hann windows type, window length 0.1s.

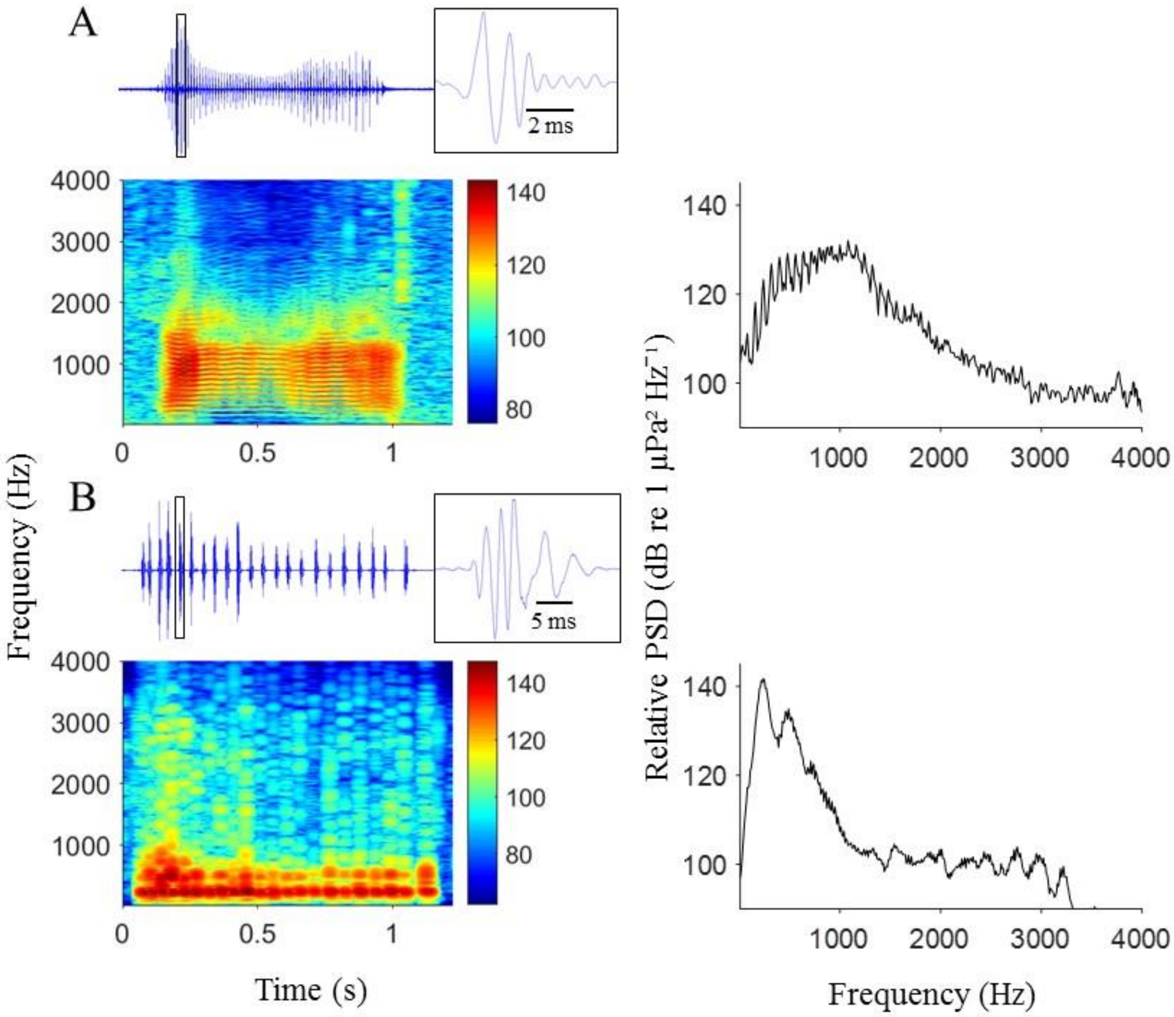

Figure 26. Prochilodontidae disturbance calls spectrograms, waveforms (individual pulse indicated by squares). A) *S. insignis* and B) *P. nigricans*. Hann windows type, DFT size 512 and overlap of 98.8%. Low and high frequency limits of 100- 4000 Hz. Hann windows type, window length 0.1s.

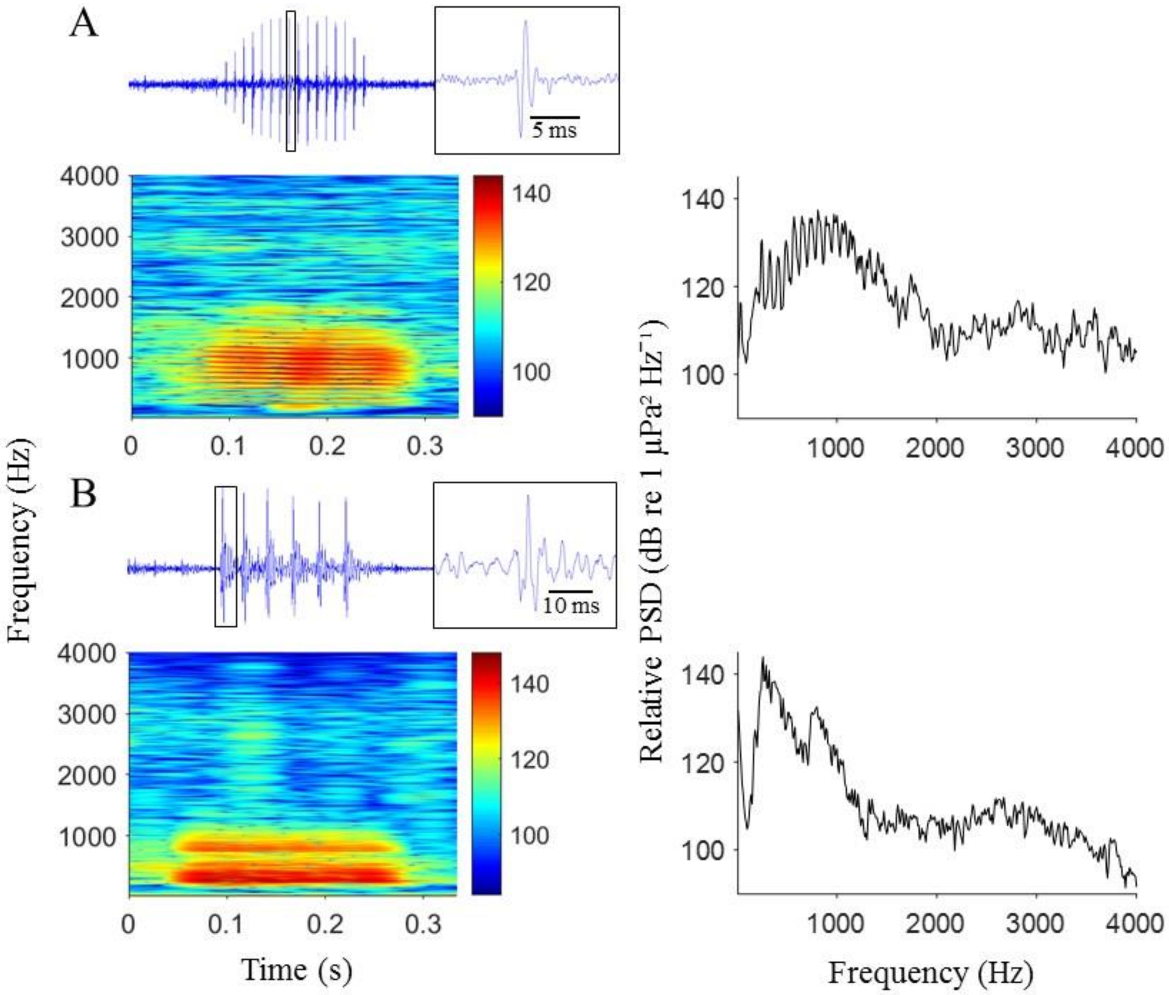

Figure 27. Underwtaer voluntary calls spectrograms, waveforms (individual pulse indicated by squares). A) Chorus 1 and B) Chorus II. Hann windows type, DFT size 512 and overlap of 98.8%. Low and high frequency limits of 100- 4000 Hz. Hann windows type, window length 0.1s.

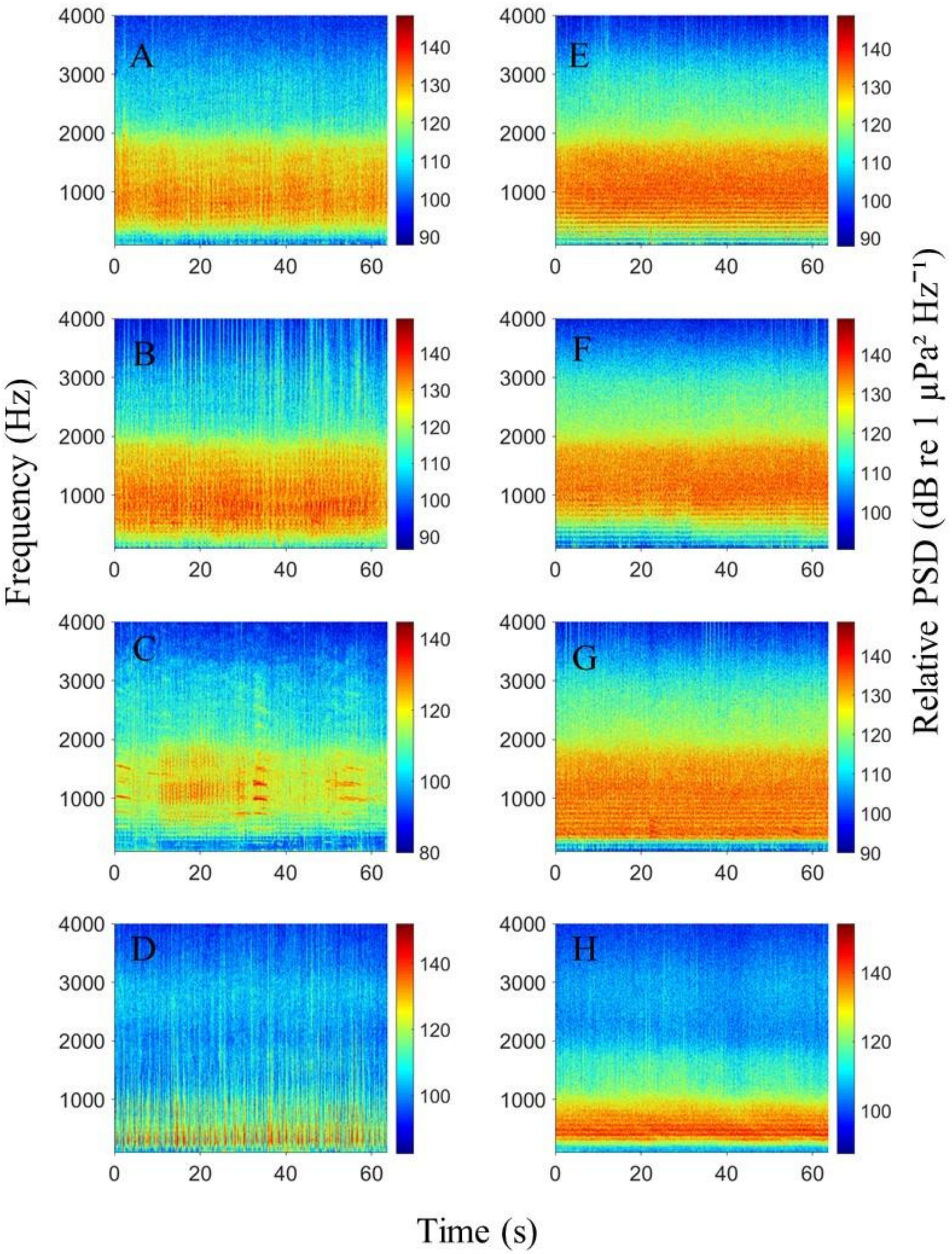

Figure 28. Sonograms of underwater recordings from various rivers: A) Negro; B) Machado; C) Pakaas; D) Soterio, E. Guapore, F) Cautario; G) Machupe and H) Branco. Low and high frequency limits of 100- 4000 Hz, Hann window, window length 0.1s.

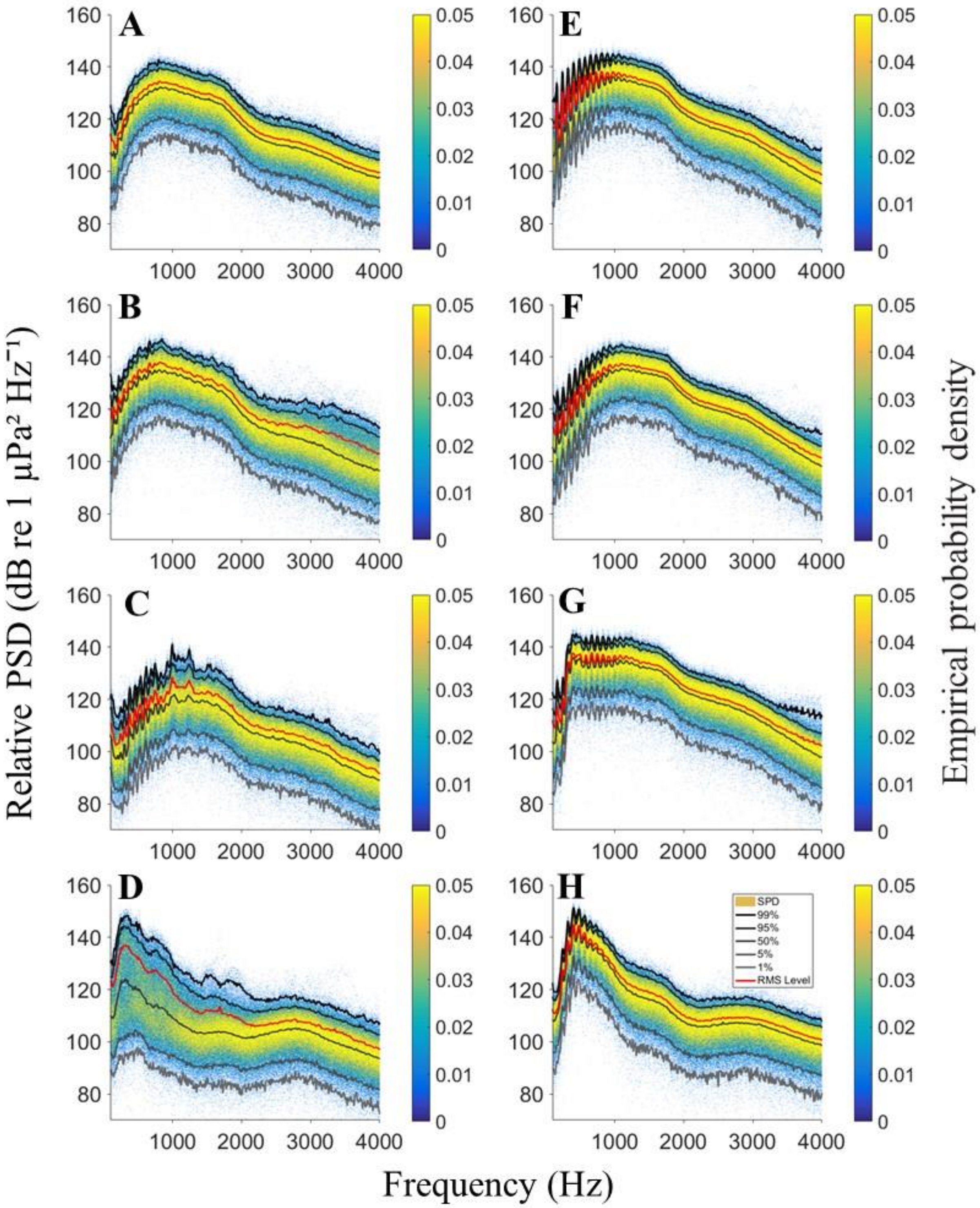

Figure 29. Power spectra of continuous sounds from rivers confluences. A) Negro, B. Machado, C. Pakaas, D. Soterio, E. Guapore, F. Cautario, G. Machupe and H. Branco. Low and high frequency limits of 100- 4000. Hann windows type, window length 0.1s and overlap of 50%.

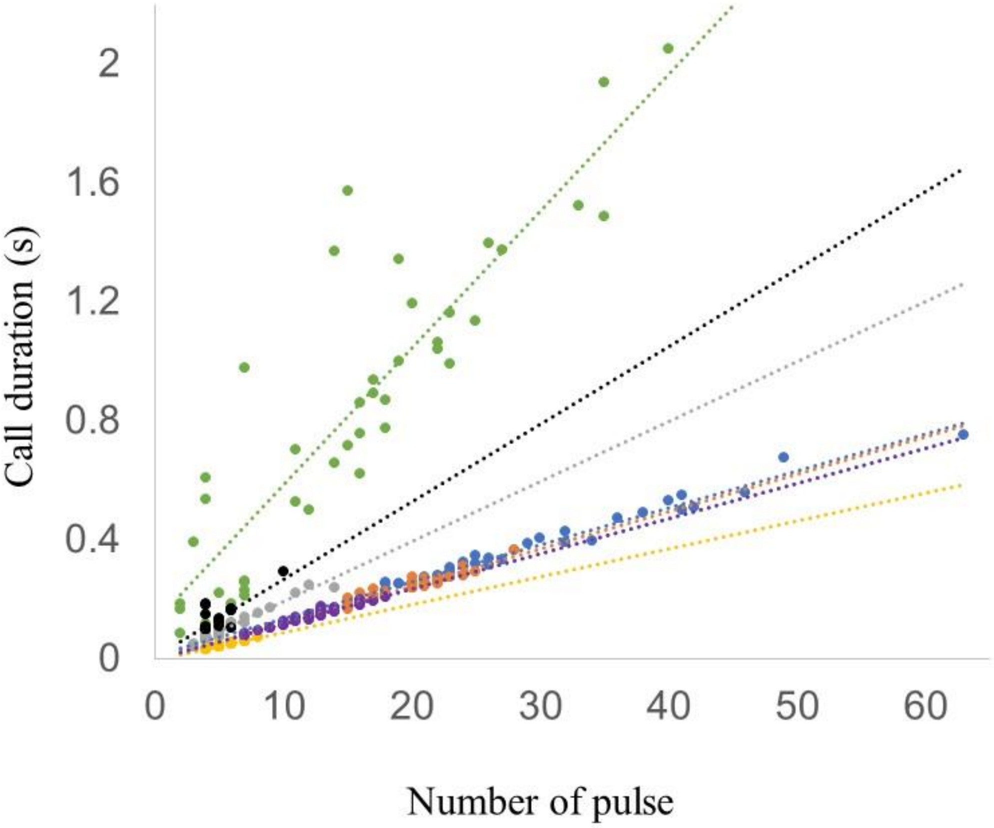

Figure 30. Positive relationship of call duration and number of pulse in disturbance and voluntary calls detected in the Madeira basin

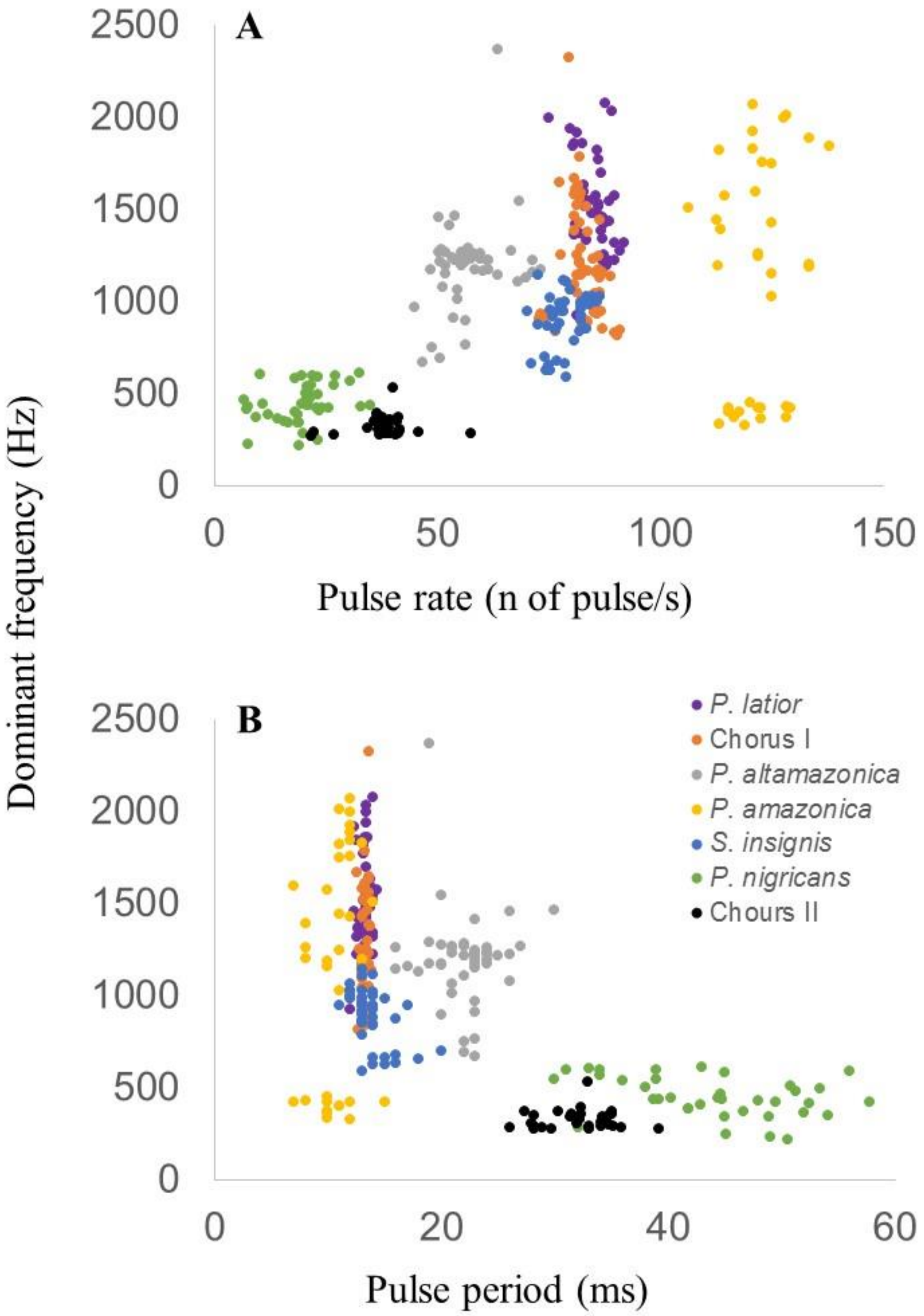

Figure 31. Relationship between acoustic parameters in the disturbance calls of five characids and two underwater choruses. A. Call duration (ms) and number of pulses (Put pulses not pulse on the x-axis), B. Dominant frequency (Hz) and pulse rate, and C. Dominant Frequency (Hz) and pulse period (pulse/s).

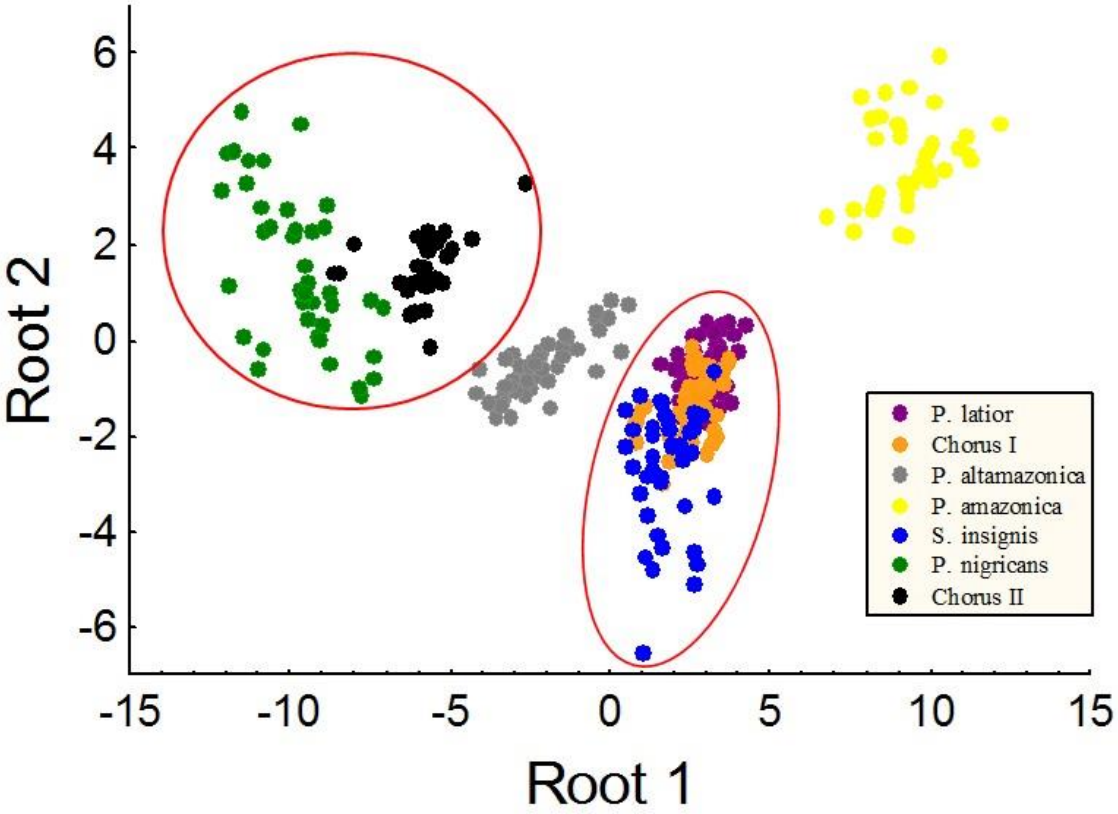

Figure 32. Canonical discriminant scores generated by the first two discriminant functions to illustrate the separation of different Amazon fish using acoustic parameters. Based on a total of 8 discriminant functions, with the factor 1 and 2 was 69.7%.

Table 5. PCA factors used in separating the calls of five characid species

	Factor 1	Factor 2
Pulses per burst	-0.006	**0.913**
Burst duration (ms)	0.567	**0.745**
Pulse rate (pulse/s)	**-0.826**	-0.118
Pulse period (ms)	**0.824**	0.041
Peak Frequency (Hz)	-0.563	0.248
Pulse cycle (ms)	**0.856**	-0.234
Cycle Peak Frequency (Hz)	**-0.727**	0.196
Frequency calculated (Hz)	**-0.864**	0.076
% Total variance	50.1	19.6
Cumulative %	50.1	69.7

Table 6. Acoustic parameters (mean, range and SE) of the calls of five characids. Different letters indicate significant differences based on ANOVA and Tukey posthoc test

	P. latior	**Chorus I**	***P. altamazonica***	***P. amazonica***	***S. insignis***	***P. nigricans***	**Chorus II**
Pulses per burst	12 (7-18) ± 3 ^{a}	17 (10-28) ± 4 ^{c,d}	5 (3-14) ± 2 ^{b}	5 (4-8) ± 1 ^{b}	28 (14-63) ± 11 ^{c}	15 (2-40) ± 10 ^{a,d}	5 (4-10) ± 1 ^{b}
Burst duration (ms)	141.0 (80-210) ± 31 ^{a}	210.9 (120-370) ± 50 ^{c}	99.4 (40-250) ± 47 ^{a,b}	44.9 (30-70) ± 11 ^{b}	354.7 (160-750) ± 132 ^{c,d}	830.2 (90-2000) ± 500 ^{d}	132.2 (100-290) ± 42 ^{a}
Pulse period (ms)	13.3 (12-14) ± 0.5 ^{a}	13.3 (13-14) ± 0.3 ^{a}	22.1 (16-30) ± 2.7 ^{b}	10.8 (7-15) ± 1.8 ^{c}	13.8 (11-20) ± 1.8 ^{a}	48.6 (30-111) ± 18.0 ^{d}	32.2 (26-40) ± 3.0 ^{b,d}
Burst Peak Frequency (kHz)	1.3 (0.3-2.4) ± 0.6 ^{a}	1.1 (0.7-1.6) ± 0.2 ^{a}	0.6 (0.3-1.9) ± 0.4 ^{b}	0.8 (0.2-2.4) ± 0.6 ^{b,c}	0.9 (0.5-1.6) ± 0.3 ^{a,c}	0.5 (0.3-0.9) ± 0.2 ^{b}	0.3 (0.2-0.5) ± 0.06 ^{b}
Pulse cycle (ms)	0.7 (0.4-1.1) ± 0.1 ^{a}	0.9 (0.4-1.2) ± 0.2 ^{b}	0.9 (0.4-1.5) ± 0.2 ^{b}	1.4 (0.4-3.1) ± 1.0 ^{b,c}	1.2 (0.8-1.7) ± 0.2 ^{c}	2.4 (1.6-4.6) ± 0.7 ^{d}	3.1 (1.8-3.7) ± 0.4 ^{d}
Cycle Peak Frequency (kHz)	1.1 (0.2-2.1) ± 0.5 ^{a}	1.2 (0.7-1.9) ± 0.3 ^{a}	0.9 (0.2-2.1) ± 0.5 ^{a}	1.0 (0.2-2.4) ± 0.6 ^{a}	0.7 (0.5-1.4) ± 0.2 ^{a}	0.4 (0.2-0.5) ± 0.1 ^{b}	0.3 (0.3-0.5) ± 0.05 ^{b}
Frequency calculated (kHz)	1.5 (0.9-2.1) ± 0.3 ^{d}	1.2 (0.8-2.3) ± 0.3 ^{a}	1.2 (0.7-2.4) ± 0.3 ^{a}	1.1 (0.3-2.1) ± 0.6 ^{a,b}	0.9 (0.6-1.1) ± 0.2 ^{b}	0.4 (0.2-0.6) ± 0.1 ^{c}	0.3 (0.3-0.5) ± 0.05 ^{c}

Considerações finais

A paisagem acústica nos ambientes marinhos e continentais estudados foi composta pela produção de sons de crustáceos e principalmente por coros de peixes. No total, foram detectados seis tipos de coros de peixes marinhos e cinco de espécies de água doce.

No ambiente marinho, verificou-se a ocorrência de seis tipos de coros de peixes depois da última linha recifal, produzindo sons com maior energia principalmente na área de exclusão de Tamandaré. Energia inferior foi detectada em Porto de Galinhas, com diferença de até 20 dB re 1 $\mu Pa^2\ Hz^{-1}$, onde apenas foi mais evidente a ocorrência o Chorus I e VI. Mostrando a importância da área marinha protegida de Tamandaré, e a necessidade de aplicar o Monitoramento Acústicos Passivo (PAM) como ferramenta complementar para a avaliação da paisagem acústica que contribuam para a implementação de novas áreas.

As embarcações detectadas no ambiente marinho foram na sua maioria pesqueiras, e ocorreram principalmente no período diurno, assim como lanchas e navios. Embarcações de pesca foram recorrente durante as fases de gibosa crescente e minguante, antes e após a lua cheia respectivamente. Com frequências dominante <100 Hz, e energia alcançando 7000 Hz, sendo mais que suficiente para mascarar os sons emitidos pelos peixes.

Tanto em ambientes marinho como de água doce, a frequência dominante emitida pelos peixes foi entre os 200 e 2000 Hz, banda de frequência comumente encontrada para esse grupo. Peixes maiores emitem uma baixa frequência com relação a peixes menores, isto de alguma forma pode ajudar a elucidar os sons encontrados no ambiente marinho. Machos das espécies (incluindo os Prochilodontidae analisados) exibiram uma musculatura sonoro associada as primeiras costelas, responsáveis pelara vibração e produção de sons através da bexiga natatória. Esta hipótese também pode ser aplicada as espécies marinhas de "roncadores", que utilizariam um mecanismo sonoro similar, associado com a bexiga natatória e muito comum na família Sciaenidae.

Em áreas de confluências dos afluentes com o rio Madeira foram detectados e identificados choros de espécies de importância comercial, como o Jaraqui (*S. insignis*) e a branquinha (*P. latior*), ambas com características acústicas similares e utilizando o mesmo nicho no rio Guapore. *P. latior* foi a mais recorrente durante o período de estudo. Nesse trabalho corroborarmos que áreas de confluências de rios são habitat essências para a reprodução destas espécies de peixes, sendo necessário maiores estudos para melhor entendimento do uso desse habita ao longo do ano.

Portanto, conhecendo o momento e o lugar de ocorrência das diferentes espécies, tanto em ambientes marinhos e de água doce, permite o uso da ferramenta acústica passiva como metodologia não invasiva e não destrutiva. Apesar da acústica passiva ser limitada as espécies que emitem sons naturalmente, esta ferramenta permite para o monitoramento e conservação de diversos organismo aquáticos, já que muito são capazes de emitir sons. Sendo assim, maiores esforços ainda precisam ser feitos para a identificação das especeis, juntamente com avaliar biomassa das populações, como também padrões de movimentação, uso de habitat e interação com outras espécies.

Anexo I

Caracterização dos sons produzidos por peixes e crustáceos em áreas estuarinas do Rio Maracaípe, nordeste brasileiro

Foram realizados três “drifts” em áreas intermedia e inferiores do Rio Maracaípe entre novembro e janeiro. As gravações foram realizadas durante a mare cheia. Durante os drift foram detectados diferentes sinais biológicos, produzidos por peixes e crustáceos em diferentes áreas do mangue. Na zona estuarina intermediaria ou media

Figura 1. Área estuarina do rio Maracaípe onde foram detectados sinais acústicos biológicos.

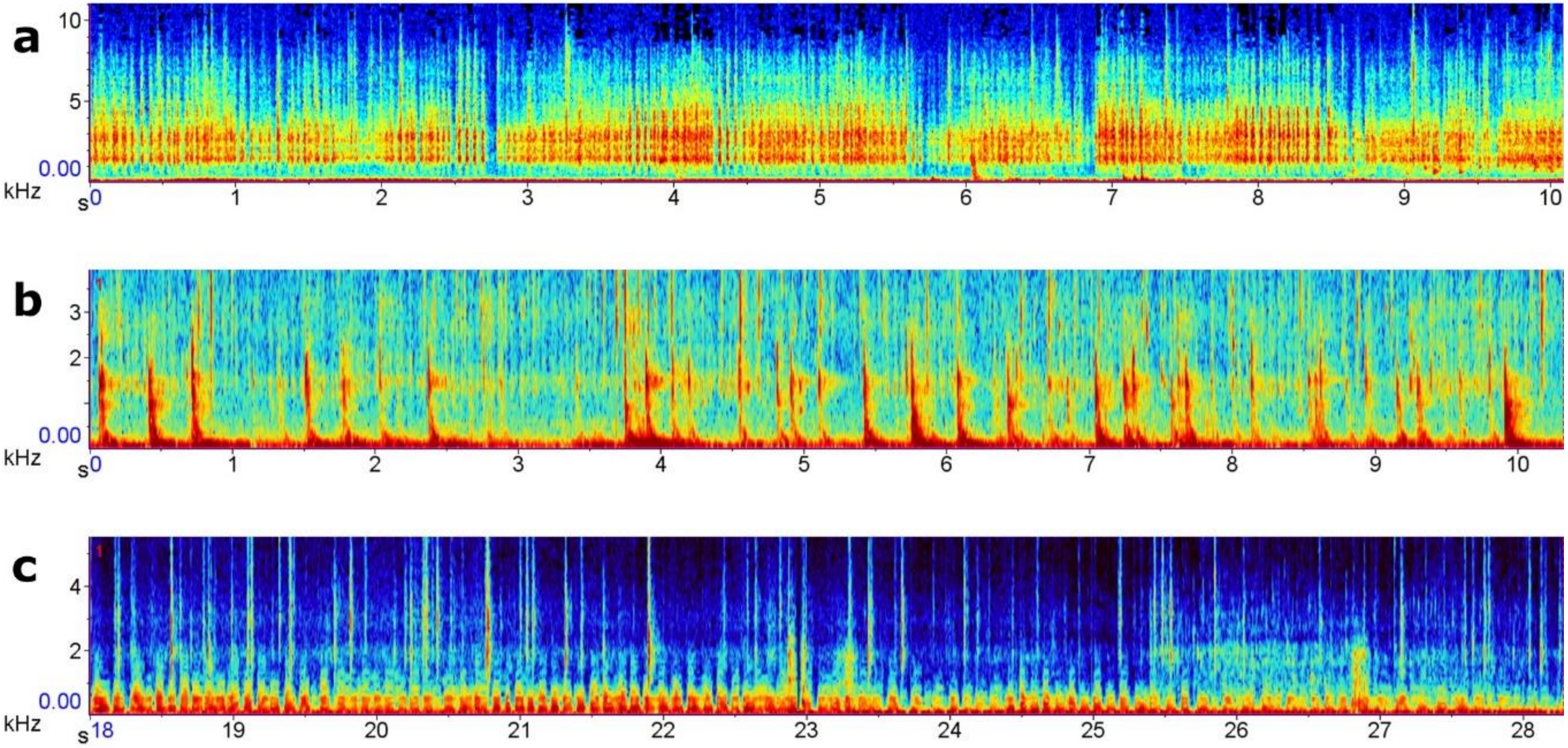

Figura 1. Espectrogramas dos sons detectados no estuário de Maracaípe. **a.** agregação de caranguejos e **b.** e **c.** peixes.

Anexo II

Produção de sons voluntários e estresse na lagosta verde (*Panulirus argus*) em cativeiro.

As gravações foram realizadas em ambiente controlado, nas instalações do Despertamento de Pesca e Aquicultura da UFRPE. Utilizando um gravador subaquático autônomo (SoundTrap 300) que permitiu gravações continuas para avaliar sons voluntários da lagosta verde, como também sons de estresse provocados durante a manipulação.

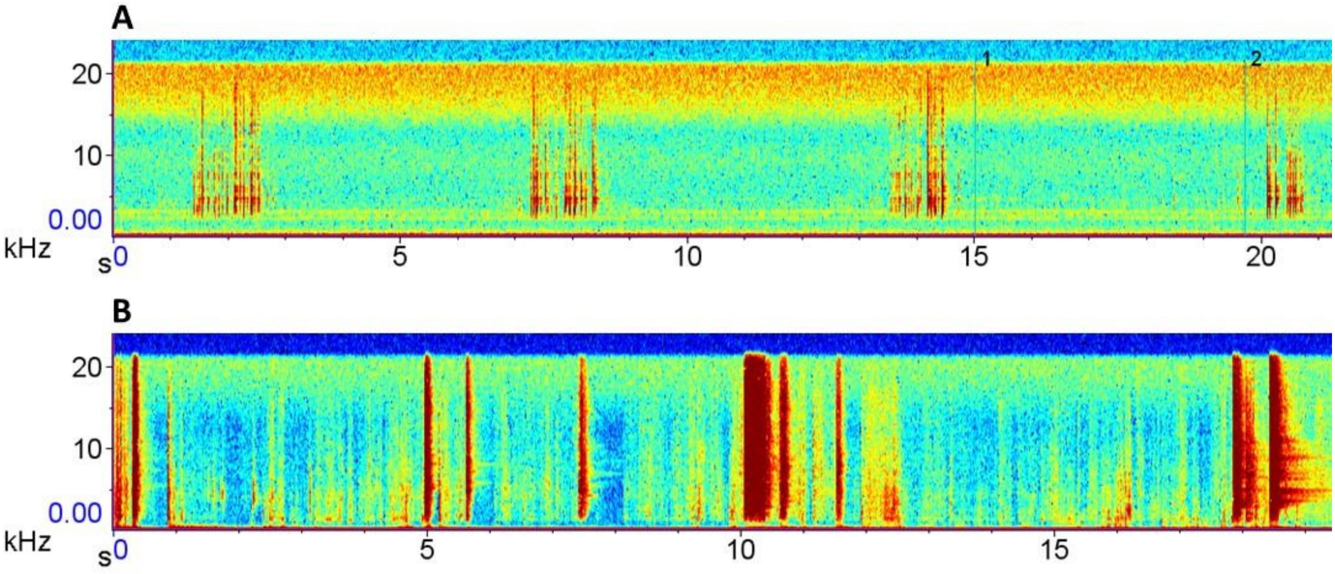

Figura 1. Espectrogramas dos sons A. voluntários e B. estresse produzidos por *P. argus*

Anexo III

Sons gravados nos peixes amazônicos *mandi, jau, piranha-caju* e *branquinha* em condições de estresse.

Os sons forma gravados na região do Distrito do Iata no Rio Mamore durante pescarais artesanais realizadas em pequenas embarcações, utilizando malhadeiras no caso de *Pimelodus blochii*, *Pygocentrus nattereri* e *Potamorhina latior,* e espeinhel de surpeficie para a captura do *Zungaro Zungaro*. Os sons foram gravados utilizando um hidrofone H2a (Aquarian Audio, Anacortes, USA), conectado a um gravador digital Olympus WS-400S (com microfone interno).

Tabela 1. Parâmetros acústicos dos peixes. Mean±DP

	NP	***LF***	***HF***	***DF***	***CD***	***PP***	***PR***
P. blochii							
Type I	10.5±3.2	82.5±31.7	585.5±80.8	379.0±92.6	69.3±17.8	6.0±0.6	151.4±23.3
Type II	11.8±6.3	981.9±214.5	3068.0±583.6	1877.7±156.2	45.4±13.9	5.0±3.8	259.7±100.2
Z. zungaro							
Type I	4.5±0.5	55.3±24.9	455.0±7.1	125.6±96.7	63.6±5.9	11.8±0.2	71.0±8.0
Type II	9.8±1.5	487.3±53.7	3011.7±271.5	1378.1±689.1	60.1±10.9	5.2±0.2	164.4±27.7
P. nattereri	11.1±1.4	53.0±9.6	616.8±88.9	232.6±81.7	108.8±12.0	9.8±0.4	101.8±4.0
P. latior	--	514.9±53.8	3240.9±434.3	1541.8±246.6	164.3±27.9	--	--

NP=numero de pulsos; LF=low frequency (Hz); HF=high frequency (Hz); DF= dominant frequency (Hz); CD=call duration (ms); PP=pulse period (ms); PR=pulse rate (pulse/s)

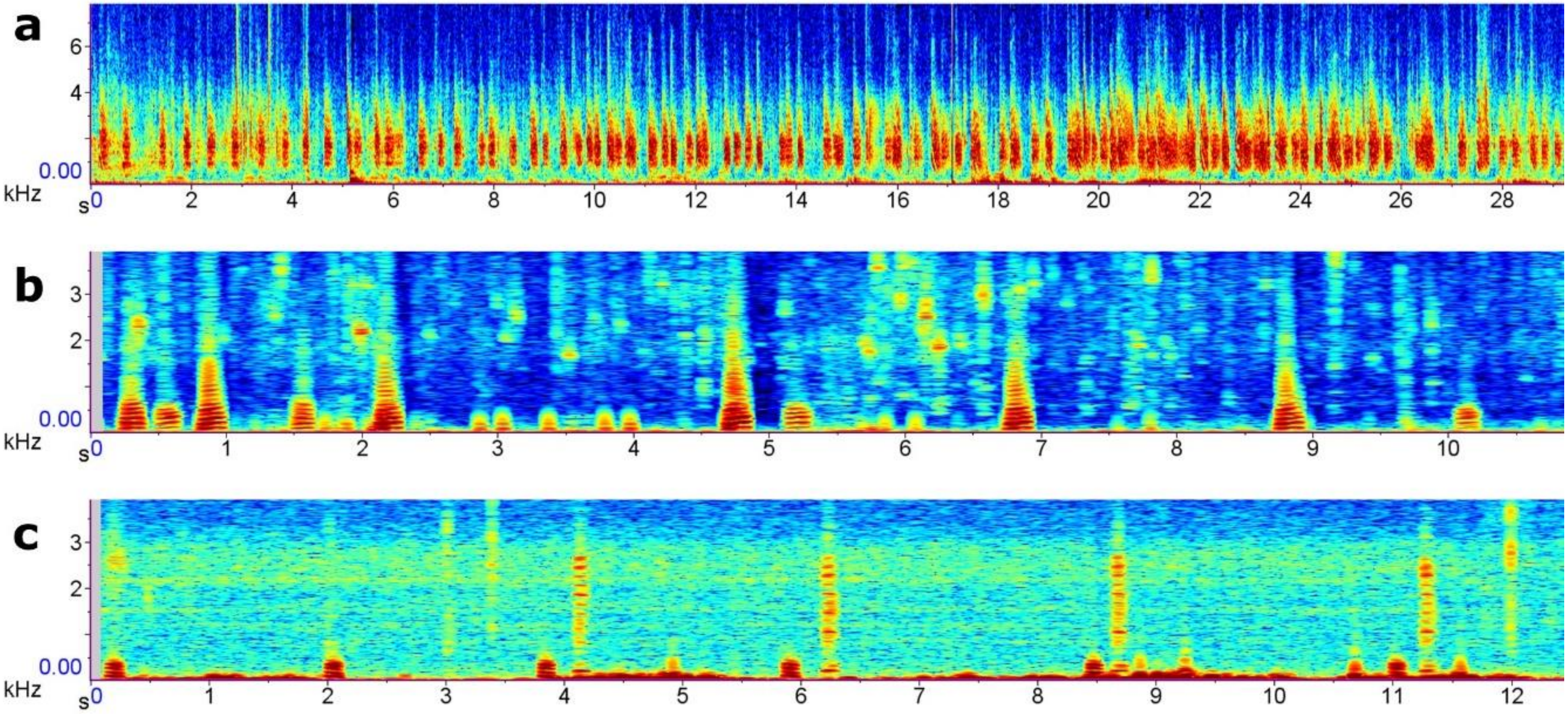

Figura 1. Espectrogramas dos sons de estresse gravados de **a.** *Potamorhina latior* e **b.** *Pimelodus blochii*, dentro da agua quando capturados utilizando rede de emalhe; c. *Zungaro Zungaro*, fora da agua e capturado com espinhel de superfície.