



MARINHA DO BRASIL

INSTITUTO DE ESTUDOS DO MAR ALMIRANTE PAULO MOREIRA

UNIVERSIDADE FEDERAL FLUMINENSE

PROGRAMA ASSOCIADO DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA
MARINHA

ALÉXIA ANTONIA LESSA DA COSTA

**Impactos do ruído antropogênico no comportamento do peixe-donzela,
Stegastes fuscus (Cuvier, 1830)**

ARRAIAL DO CABO, 2022

ALÉXIA ANTONIA LESSA DA COSTA

**Impactos do ruído antropogênico no comportamento do peixe-donzela,
Stegastes fuscus (Cuvier, 1830)**

Dissertação de mestrado apresentada ao Instituto de Estudos do Mar Almirante Paulo Moreira e à Universidade Federal Fluminense, como requisito parcial para a obtenção do grau de Mestre em Biotecnologia Marinha.

Orientador: Prof. Dr. Carlos Eduardo Leite Ferreira

Coorientador: Prof. Dr. Fábio Contrera Xavier

ARRAIAL DO CABO, 2022

ALÉXIA ANTONIA LESSA DA COSTA

**Impactos do ruído antropogênico no comportamento do peixe-donzela,
Stegastes fuscus (Cuvier, 1830)**

Dissertação apresentada ao Instituto de Estudos do Mar Almirante Paulo Moreira e à Universidade Federal Fluminense, como requisito parcial para a obtenção do título de Mestre em Biotecnologia Marinha.

COMISSÃO JULGADORA:

Dr. Carlos Eduardo Leite Ferreira
Universidade Federal Fluminense
Professor Orientador – Presidente da Banca Examinadora

Dr. Eduardo Barros Fagundes Netto
Instituto de Estudos do Mar Almirante Paulo Moreira

Dra. Lis Bittencourt Vilas Boas
Universidade do Estado do Rio de Janeiro

Dr. Bernardo Antonio Perez da Gama
(Suplente)
Universidade Federal Fluminense

Arraial do Cabo, 11 de Julho de 2022

AGRADECIMENTOS

Em primeiro lugar à minha família e aos meus amigos pelo apoio. Obrigada por terem tornado tudo mais fácil! Um obrigada especial para a minha mãe que sempre acreditou nos meus sonhos e me incentivou a continuar lutando por eles!

Ao Guilherme, por me apoiar, incentivar e me ajudar nos momentos mais difíceis.

Às minhas liros (Ana, Mayra, Nayara, Paula e Marina), que sempre me apoiaram e estiveram comigo desde o começo.

Aos meus orientadores, Fábio Contrera e Carlos Ferreira, por todos os conselhos e orientação. Obrigada pela confiança para desenvolver esse projeto.

Ao Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico pelo financiamento da bolsa de mestrado.

À Marinha do Brasil e ao Departamento de Oceanografia do IEAPM pelo suporte logístico dos campos, em especial ao primeiro tenente Fernando Ribeiro.

Ao LECARdume por toda ajuda e parceria em todo o processo.

A todos que me ajudaram nos campos (Pedro, Celine, Rafael, Cesar, Samia, Vinicius). Sem vocês esse trabalho não teria acontecido.

RESUMO

Ruídos antropogênicos representam uma constante ameaça aos ecossistemas marinhos. Visto que organismos marinhos utilizam o som em diversas atividades do seu ciclo de vida, o aumento do tráfego marítimo pode ter efeitos significativos na dinâmica e resiliência de sistemas naturais. O peixe-donzela *Stegastes fuscus* é uma espécie chave na dinâmica de sistemas recifais rasos, visto papel como herbívoros e comportamento territorialista. Pertencendo a uma família que tem biofonia conhecida e associada a comportamentos diversos, representa uma boa espécie modelo para a avaliação de impactos causados por atividades antropogênicas. O objetivo deste trabalho foi de avaliar o impacto do ruído gerado por embarcações de turismo náutico no comportamento de *Stegastes fuscus* em costões rochosos de Arraial do Cabo ($22^{\circ} 58' S$ $42^{\circ} 01' W$). Para isso foram realizados experimentos *in situ*. Territórios de *S. fuscus* foram selecionados em cada site de modo que uma fonte acústica ficasse posicionada centralmente a três territórios (=indivíduos). Para cada indivíduo foi aplicado um dos tratamentos: Tratamento 1: 10 min sem a transmissão de som (controle) + 10 minutos de *playback* de barcos (200 Hz-1 kHz); Tratamento 2: 10 minutos sem a transmissão de som (controle) + 10 minutos de um som artificial (400Hz). As faixas de som foram feitas usando 30s de som intercalados com 30s de silêncio em *looping* por 10 minutos. Respostas comportamentais foram analisadas através de filmagens remotas, considerando as frequências de comportamentos agonísticos (núm. de chases/minuto), taxas de forrageamento (núm. de mordidas/minuto) e o tempo de refúgio (tempo gasto escondido). As observações foram divididas em três períodos: 10 minutos antes do som ("pré"), os 10 minutos de exposição a um dos tratamentos ("durante"), e os 10 minutos seguintes ("após"). Para avaliar se existiram diferenças nos comportamentos entre os períodos analisados foi usado um teste de Friedman ($\alpha < 0.05$). Os peixes apresentaram mudanças comportamentais significativas associadas aos períodos de exposição ao som. Os indivíduos aumentaram significativamente seu tempo de refúgio durante a exposição do som, em comparação ao momento pré-exposição (Tratamento 1: $p = 0.009$; Tratamento 2, $p = 0.008$). Além disso, quando submetidos aos diferentes sons os indivíduos diminuíram significativamente suas taxas de forrageamento (Tratamento 1: $p = 0.003$; Tratamento 2, $p = 0.01$). Tais mudanças nas taxas de forrageamento e defesa do território podem ter efeitos potenciais sobre o *fitness* populacional. Já que a espécie possui um papel funcional importante nos sistemas recifais rasos, como herbívoros territoriais mais abundante em toda a costa brasileira. Os efeitos detectados influenciaram significativamente comportamentos chaves no ciclo de vida da espécie, o que pode gerar efeitos críticos na dinâmica dos recifes como um todo. A paisagem acústica submarina está mudando rapidamente e com isso há uma necessidade urgente de avaliar os impactos dessa pressão antropogênica sobre espécies e processos, de modo a fornecer medidas de mitigação desse impacto ainda negligenciado.

Palavras-chave: etologia; bioacústica; antropofonia

ABSTRACT

Anthropogenic noise is a major threat to biodiversity. Since marine organisms use sound in many activities in their life cycle, increased nautical traffic can have significant effects on critical behaviors, such as foraging and territoriality. The damselfish *Stegastes fuscus* (Pomacentridae) is a key species influencing the dynamics of shallow reef systems along the Brazilian coast. This species represents an important model to assess the impacts caused by anthropogenic activities. The present work aims to evaluate the impact of noise generated by nautical tourism vessels on the behavior of *S. fuscus* in subtropical coastal rocky reefs of Arraial do Cabo (22°58'S 42°01'W). For this, in situ experiments were carried out. Territories of *S. fuscus* were randomly selected from different sampling sites and an underwater loudspeaker was placed in a central position, at least 1 m from the individual's territories, so that it could cover more than one territory (= individual) at once. For each replicate it was applied: Treatment 1: 10 min without sound transmission (control) + 10 minutes of boat playback (200Hz-1kHz) or Treatment 2: 10 minutes without sound transmission (control) + 10 minutes of an artificial sound (400Hz). Tracks were made using 30s of noise and 30s of silence, in a loop for 10 minutes. Behavioral responses were analyzed by remote filming, considering the frequencies of agonistic behaviors (chases/minute), foraging rates (bites/minute) and refuge time (time spent hiding). Observations were divided in three periods of time: 10 min prior to the sound treatment ('pre'), as our control, the 10 min trials of the soundtrack ('during') and the 10 minutes following the sound treatment ('post'). To evaluate differences between behavior within periods of time a Friedman test was used ($\alpha < 0.05$). Fish had significant behavior modifications associated with different sound exposures. Individuals increased their refuge time when exposed to sound compared to the moment of pre-exposure (Treatment 1: $p = 0.009$; Treatment 2, $p = 0.008$) in both treatments. And also had lower foraging rates during noise exposure period compared to pre-exposure (Treatment 1: $p = 0.003$; Treatment 2, $p = 0.01$). Such changes in foraging and territory defense rates may have potential effects on population fitness. Since the species has a functional role as the most abundant territorial herbivore in the system, the detected effects can significantly alter the reef dynamics. The underwater acoustic soundscape is changing rapidly and with it there is an urgent need to assess the impacts of this anthropogenic pressure and provide measures to mitigate this still-neglected impact.

Keywords: ethology; bioacoustics; anthrophony

LISTA DE FIGURAS

Figura 1. Método de animal focal, adaptado Altman, 1974. A) Estrutura de filmagem remota utilizada para monitoramento dos comportamentos ao longo dos experimentos em campo. B) Recorte do vídeo analisado.....	13
Capítulo Único: <i>Exposure to motorboat noise playback impairs anti-predator responses in a damselfish</i>	
Figura 1. Mapa de Arraial do Cabo indicando os sítios amostrais (1 - Maramutá, 2 - Pedra Vermelha).....	27
Figura 2. Visão geral dos experimentos mostrando a linha do tempo	28
Figura 3. Espectrogramas da paisagem acústica submarina durante os experimentos em campo, para uma janela de 30s das gravações de áudio de cada tratamento. Espectrogramas da esquerda representam o período antes, os do meio durante a exposição sonora e os da direita representam o após. FFT = 1024, Hamming evaluation window, 50% window overlap, 0–1500 Hz.....	30
Figura 4. Respostas comportamentais comparativas para ambas as abordagens experimentais durante três períodos (-antes, -durante, -após a exposição ao ruído). Os boxplots representam os intervalos interquartis e as linhas dentro das caixas representam valores médios em 19 indivíduos para o <i>playback</i> (<i>T1</i>) e 16 para o tom puro. Cada ponto representa um indivíduo. (a) taxas de mordida no <i>playback</i> ; (b) perseguição no <i>playback</i> ; (c) refugiar-se no <i>playback</i> ; (d) taxas de mordida no tom puro; (e) perseguição no tom puro; (f) refúgio no tom puro. * indica o nível de significância: ** < 0,01; ns: resultado não significativo do teste de Friedman.....	33

LISTA DE TABELAS

Tabela 1. Comparação dos comportamentos antes, durante e após a exposição sonora. Resumo do teste de Friedman testando o efeito no comportamento entre períodos para <i>Stegastes fuscus</i> . Negrito denota diferença significativa em 0.05.....	32
---	----

SUMÁRIO

1. INTRODUÇÃO GERAL	10
1.1 Mudanças na paisagem acústica submarina	10
1.2 Bioacústica em peixes recifais: peixes-donzela como modelo	11
2. OBJETIVOS	15
2.1 OBJETIVO GERAL	15
2.2 OBJETIVO ESPECÍFICO	15
3. HIPÓTESES	15
4. REFERÊNCIAS	16
Capítulo Único	22
1. INTRODUÇÃO	24
2. MATERIAIS E MÉTODOS	26
2.1 Área de estudo	26
2.2 Visão geral do experimento	27
2.3 Dados acústicos	28
2.4 Análise comportamental	29
2.5 Análises estatísticas	30
3. RESULTADOS	30
4. DISCUSSÃO	33
5. CONCLUSÕES	36
6. REFERÊNCIAS	37
7. CONCLUSÕES GERAIS	44

1. INTRODUÇÃO GERAL

1.1 Mudanças na paisagem acústica submarina

A paisagem acústica submarina (PAS) é formada por sons bióticos e abióticos de um ecossistema, juntamente com o ruído antropogênico (gerado pelo homem) (Pijanowski et al., 2011). Esses sinais podem ser classificados em dois principais tipos: os de alta frequência, como o ruído produzido por canhões de ar, sonares militares e pesquisas sísmicas, ou de baixa frequência (abaixo de 1 kHz), como o ruído de embarcações motorizadas (Slabbekoorn et al., 2010). As mudanças na PAS provenientes de atividades antropogênicas têm sido uma preocupação da comunidade científica (Duarte et al., 2021), visto que esta representa um importante *proxy* para entender diversos processos ecológicos. Dessa forma, o número de estudos que buscam avaliar os impactos do ruído antropogênico em diferentes escalas ecológicas e temporais vem aumentando cada vez mais (Ferrier-Pagès et al., 2021).

O turismo, principalmente aquele associado às atividades náuticas, se caracteriza como indústria crescente em todos os continentes e oceanos (Boljat et al., 2021; Duarte et al., 2021). Devido à facilidade de acesso e alta densidade demográfica, sistemas costeiros são os mais impactados (Weatherdon et al., 2016). Os impactos associados às atividades náuticas incluem distúrbios como danos físicos em organismos bentônicos (Giglio et al., 2017), despejo de poluentes pelas embarcações (Halfwerk e Slabbekorn, 2015), e a poluição sonora (Boljat et al., 2021). Apesar do grande avanço em estudos reportando a influência de impactos antropogênicos nas taxas de perda de biodiversidade em sistemas marinhos diversos, como superexploração e eutrofização (Bellwood et al., 2012; Bender et al., 2014), pouco se conhece sobre os efeitos da poluição sonora em espécies e processos ecológicos (Popper & Hawkins, 2019). Embora a maioria dos trabalhos existentes no tema dizem respeito ao comportamento de aves e mamíferos marinhos (Shannon et al., 2016), peixes recifais constituem um dos grupos potencialmente vulneráveis aos efeitos das atividades náuticas. O aumento nos níveis de ruído podem interferir de diversas maneiras, incluindo a detecção e resposta ao som, que pode acarretar efeitos ecológicos na dinâmica e resiliência

dos sistemas naturais (Bellwood et al., 2012; Kunc et al., 2016). Dessa forma, torna-se evidente a necessidade de avaliar os impactos dessa pressão antropogênica em espécies e processos no ambiente marinho, de modo a desenvolver medidas de mitigação para este impacto ainda negligenciado.

Embarcações motorizadas, incluindo grandes navios e pequenas embarcações de turismo, representam uma das principais fontes de ruído antropogênico em ambientes costeiros (Haviland-Howell et al., 2007; Whitfield e Becker, 2014; Bittencourt et al., 2014; Sertlek et al., 2019; Bittencourt et al., 2020). Nesses ambientes, o tráfego náutico contribui principalmente com o aumento nos níveis de ruídos de baixa frequência (Codarin et al., 2009) e muitas espécies detectam sons nessa faixa de frequência (Popper, 2003). Diversos estudos mostram os efeitos negativos desse tipo de ruído no comportamento (Bracciali et al., 2012; Nedelec et al., 2017; Sebastianutto et al., 2011), na fisiologia (Simpson et al., 2016; Mills et al., 2020), e na sobrevivência de muitas espécies (McCormick et al., 2018). Alguns estudos avaliaram a poluição sonora submarina por pequenas embarcações na costa brasileira (Bittencourt et al., 2014; Campbell et al., 2019), mas poucos investigaram seus efeitos na vida marinha, especialmente em espécies-chave, como os peixes-donzela (Leduc et al., 2021).

1.2 Bioacústica em peixes recifais: peixes-donzela como modelo

Os sinais acústicos possuem um papel crucial para diversos organismos marinhos em diversas atividades do seu ciclo de vida (Hawkins and Popper, 2019). Muitas espécies de peixes desenvolveram mecanismos capazes de detectar, produzir e interpretar diferentes tipos de sons com finalidades distintas. Por exemplo, o som resultante de comunidades recifais funcionam como pistas acústicas que podem influenciar nos padrões de recrutamento e auxiliar na orientação de larvas de peixes (Lillis et al., 2013; Popper and Hawkins, 2019). Outros sinais acústicos podem garantir a atração de parceiros e sucesso reprodutivo, bem como evitar eventos de predação (Cole et al., 2010; de Jong et al., 2020).

Os peixes-donzela (Teleostei: Pomacentridae) compõe uma família diversa e amplamente distribuída em recifes tropicais e subtropicais (Fréderich & Parmentier, 2016). Atualmente é composta por mais de 400 espécies distribuídas em cerca de 29 gêneros (Fricke et al., 2020), sendo considerada uma das mais representativas em sistemas recifais em todo o mundo (Bellwood and Wainwright, 2002; Ferreira et al., 2004; Fréderich & Parmentier, 2016). Constitui uma das famílias de peixes recifais mais estudadas, visto sua diversidade e abundância de espécies nas comunidades onde ocorrem (Ferreira et al., 1998; Ceccarelli et al. 2005; Barneche et al. 2009; Vazzana et al., 2017; Chase et al., 2020; da Silva-Pinto et al., 2020). Essa família é formada por espécies com diferentes estratégias tróficas, sendo em sua maioria onívoras e planctívoras (Choat, 1991; Ceccarelli et al., 2001; Hata e Ceccarelli, 2016). Algumas espécies (e.g. *Stegastes* spp.) são reconhecidamente de hábitos herbívoros, consumindo diferentes tipos de algas no substrato bentônico. Dentro do grupo de herbívoros estão incluídos os herbívoros territoriais também chamados de “fazendeiros” (= *farmers*), com cultivos de algas específicas em seu território (Ferreira et al., 1998; Ceccarelli et al., 2005). Tais espécies são extremamente importantes na manutenção e dinâmica dos sistemas recifais, visto seus aspectos únicos de comportamento alimentar e territorial (Ferreira et al., 1998).

A produção de sons em peixes-donzela é reconhecida em cerca de 8 gêneros (Fréderich e Parmentier, 2016), e está associada a diversas interações ecológicas, como as interações agonísticas de defesa e reprodução (Cole, 2010; Parmentier et al., 2010). Essa produção de sons se dá por um mecanismo estridulatório pela colisão entre os dentes, induzida pelo ligamento cerato-mandibular (c-md) que une o músculo hióide e permite um rápido fechamento da boca e consequente produção de som (Olivier et al., 2014). Além disso, o grupo possui uma estratégia exclusiva, onde a bexiga natatória é utilizada como amplificador de som (Fréderich e Parmentier, 2016).

Stegastes fuscus (Cuvier, 1830) é a espécie herbívora territorial mais abundante em ambientes recifais brasileiros (Ferreira et al., 2004). É endêmica da província brasileira, com sua distribuição indo do Rio Grande do Norte até Santa Catarina. Possui um papel ecológico importante na estruturação da comunidade

bentônica dos sistemas recifais brasileiros, pelo processo de herbivoria e hábito territorialista (Ferreira et al., 1998; Ceccarelli et al., 2001; 2005; Barneche et al., 2009). Espécies do gênero *Stegastes* produzem sons em faixas de frequência de 250-1500 Hz (Myrberg, 1972; Myrberg and Spires, 1972) e detectam sons em faixas de 500 Hz (Myrberg and Spires, 1980; Egner and Mann, 2005), sobrepondo-se com o som de baixa frequência produzido por embarcações motorizadas. Assim, essa espécie representa um importante modelo ecológico na compreensão do impacto do ruído antropogênico. Estudos anteriores com peixes-donzelas indicam estes como importantes bioindicadores de impactos sonoros devido a sensibilidade e diversidade de vocalizações existentes (Picciulin et al., 2010; Bracciali et al., 2012; Vazzana et al., 2017; Holmes et al., 2017), especialmente as espécies territorialistas, que devido à sua área de vida limitada são mais vulneráveis a impactos (Daros et al., 2016, Benevides et al., 2019).

Neste trabalho foram realizados experimentos em campo para investigar os efeitos do ruído antropogênico no comportamento da espécie de peixe recifal *Stegastes fuscus* (Cuvier, 1830) em uma reserva extrativista marinha (Resex-Mar de Arraial do Cabo – 23° 44'S - 42° W). As respostas comportamentais de cada indivíduo foram analisadas através de filmagens remotas (Figura 1), considerando as frequências dos comportamentos agonísticos (número de interações agonísticas/minuto), taxas de forrageamento (número de mordidas/minuto) e tempo de refúgio (tempo gasto escondido/minuto). É previsto que a exposição ao ruído afetará o comportamento da espécie, ocasionando a diminuição das taxas de forrageamento e aumento do tempo de refúgio. Além disso, os indivíduos podem ter efeitos residuais em resposta ao estresse agudo causado pela exposição ao som. Esse dados são essenciais para a gestão do tráfego local da Reserva Extrativista Marinha de Arraial do Cabo, de modo a auxiliar na criação e implementação de normativas marinhas para este impacto ainda negligenciado.

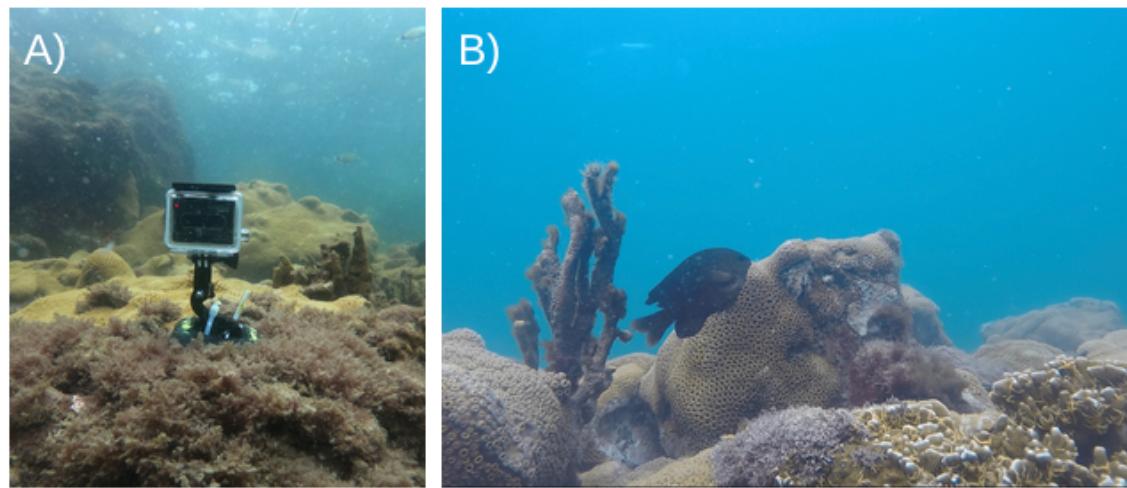


Figura 1: Método de animal focal, adaptado Altman, 1974. A) Estrutura de filmagem remota utilizada para monitoramento dos comportamentos ao longo dos experimentos em campo. B) Recorte do vídeo analisado mostrando a espécie em estudo *Stegastes fuscus* (Cuvier, 1830).

2. OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o impacto do ruído antropogênico gerado por embarcações de pequeno porte no comportamento da espécie de peixe recifal *Stegastes fuscus* (Pomacentridae).

2.2 OBJETIVO ESPECÍFICO

- Avaliar diferenças comportamentais (taxas de forrageamento, frequência de chases, tempo de refúgio) de *Stegastes fuscus* em resposta ao *playback* de embarcações de pequeno porte (200 Hz - 1 kHz);
- Avaliar diferenças comportamentais (taxas de forrageamento, frequência de chases, tempo de refúgio) de *Stegastes fuscus* em resposta ao tom puro (400 Hz) ;
- Avaliar possíveis efeitos residuais à exposição ao som;

3. HIPÓTESES

- O comportamento dos indivíduos do peixe-donzela *Stegastes fuscus* é influenciado pelo ruído antropogênico.
- O ruído apresenta efeitos residuais no comportamento do peixe-donzela.

4. REFERÊNCIAS

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49, 227–267.

Barneche, Diego R., et al. "Feeding macroecology of territorial damselfishes (Perciformes: Pomacentridae)." *Marine Biology* 156.3 (2009): 289-299.

Bellwood, David R., and Peter C. Wainwright. "The history and biogeography of fishes on coral reefs." *Coral reef fishes: dynamics and diversity in a complex ecosystem* 5 (2002): 32.

Bellwood, David R., Andrew S. Hoey, and Terence P. Hughes. "Human activity selectively impacts the ecosystem roles of parrotfishes on coral reefs." *Proceedings of the Royal Society B: Biological Sciences* 279.1733 (2012): 1621-1629.

Bender, Mariana G., et al. "Local ecological knowledge and scientific data reveal overexploitation by multigear artisanal fisheries in the Southwestern Atlantic." *PLoS One* 9.10 (2014): e110332

Benevides, Larissa J., et al. "Fear-induced behavioural modifications in damselfishes can be diver-triggered." *Journal of Experimental Marine Biology and Ecology* 514 (2019): 34-40.

Bittencourt, L., et al. "Anthropogenic noise influences on marine soundscape variability across coastal areas." *Marine Pollution Bulletin* 160 (2020): 111648.

Bittencourt, L., et al. "Underwater noise pollution in a coastal tropical environment." *Marine pollution bulletin* 83.1 (2014): 331-33.

Boljat, Helena, Neven Grubišić, and Merica Slišković. "The Impact of Nautical Activities on the Environment—A Systematic Review of Research." *Sustainability* 13.19 (2021): 10552.

Bracciali, Claudia, et al. "Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*)." *PloS one* 7.7 (2012): e40582.

Campbell, Daniel, et al. "Underwater soundscape pattern during high season of nautical tourism in Cabo Frio Island, Brazil." *Proceedings of Meetings on Acoustics 5ENAL*. Vol. 37. No. 1. Acoustical Society of America, 2019.

Ceccarelli, Daniela M., Geoffrey P. Jones, and Laurence J. McCook. "Effects of territorial damselfish on an algal-dominated coastal coral reef." *Coral Reefs* 24.4 (2005): 606-620.

Ceccarelli, Daniela M., Geoffrey P. Jones, and Laurence J. McCook. "Territorial damselfish as determinants of structure of benthic communities on coral reefs." *Oceanogr Mar Biol* 39 (2001):355–389

Chase, T. J., M. S. Pratchett, and M. O. Hoogenboom. "Behavioral trade-offs and habitat associations of coral-dwelling damselfishes (family Pomacentridae)." *Marine Ecology Progress Series* 633 (2020): 141-156.

Choat JH (1991) The biology of herbivorous fishes on coral reefs"". In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press, San Diego, pp 120–155

Codarin, Antonio, et al. "Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy)." *Marine pollution bulletin* 58.12 (2009): 1880-1887.

Cole, Kathleen Sabina, ed. *Reproduction and sexuality in marine fishes: patterns and processes*. Univ of California Press, 2010.

Daros, Felippe Alexandre, et al. "Otolith fingerprints of the coral reef fish Stegastes fuscus in southeast Brazil: a useful tool for population and connectivity studies." *Regional Studies in Marine Science* 3 (2016): 262-272.

da Silva-Pinto, Thalles, et al. "Damselfish face climate change: Impact of temperature and habitat structure on agonistic behavior." *PloS one* 15.6 (2020): e0235389.

de Jong, Karen, et al. "Predicting the effects of anthropogenic noise on fish reproduction." *Reviews in Fish Biology and Fisheries* 30.2 (2020): 245-268.

Duarte, Carlos M., et al. "The soundscape of the Anthropocene ocean." *Science* 371.6529 (2021): eaba4658.

Egner, Sarah A., and David A. Mann. "Auditory sensitivity of sergeant major damselfish *Abudefduf saxatilis* from post-settlement juvenile to adult." *Marine Ecology Progress Series* 285 (2005): 213-222.

Ferreira, C. E. L., et al. "Trophic structure patterns of Brazilian reef fishes: a latitudinal comparison." *Journal of Biogeography* 31.7 (2004): 1093-1106.

Ferreira, Carlos Eduardo L., et al. "Herbivory by the dusky damselfish *Stegastes fuscus* (Cuvier, 1830) in a tropical rocky shore: effects on the benthic community." *Journal of Experimental Marine Biology and Ecology* 229.2 (1998): 241-264.

Ferrier-Pagès, Christine, et al. "Noise pollution on coral reefs?—A yet underestimated threat to coral reef communities." *Marine Pollution Bulletin* 165 (2021): 112129.

Frederich, Bruno & Parmentier, E.. (2016). *Biology of Damselfishes*.

Fricke R, Eschmeyer WN, Fong JD (2020) Eschmeyer's catalog of fishes: species by family/subfamily, <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>

Giglio, Vinicius J., et al. "Anchoring damages to benthic organisms in a subtropical scuba dive hotspot." *Journal of Coastal Conservation* 21.2 (2017): 311-316.

Halfwerk, Wouter, and Hans Slabbeekhoorn. "Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance." *Biology letters* 11.4 (2015): 20141051.

Hata, Hiroki, and Daniela M. Ceccarelli. "Farming Behaviour of Territorial Damselfishes." *Biology of damselfishes*. CRC Press, 2016. 140-170.

Haviland-Howell, Genevieve, et al. "Recreational boating traffic: a chronic source of anthropogenic noise in the Wilmington, North Carolina Intracoastal Waterway." *The Journal of the Acoustical Society of America* 122.1 (2007): 151-160.

Hawkins, Anthony D., and Arthur N. Popper. "Effects of man-made sound on fishes." *Effects of anthropogenic noise on animals*. Springer, New York, NY, 2018. 145-177.

Holmes, Lucy J., et al. "Juvenile damselfish are affected but desensitize to small motor boat noise." *Journal of Experimental Marine Biology and Ecology* 494 (2017): 63-68.

Kunc, Hansjoerg P., and Rouven Schmidt. "The effects of anthropogenic noise on animals: a meta-analysis." *Biology Letters* 15.11 (2019): 20190649.

Leduc, Antoine OHC, et al. "Land-based noise pollution impairs reef fish behavior: a case study with a Brazilian carnival." *Biological Conservation* 253 (2021): 108910.

Lillis, Ashlee, David B. Eggleston, and DelWayne R. Bohnenstiehl. "Oyster larvae settle in response to habitat-associated underwater sounds." *PLoS one* 8.10 (2013): e79337.

McCormick, Mark I., et al. "Boat noise impacts risk assessment in a coral reef fish but effects depend on engine type." *Scientific reports* 8.1 (2018): 1-11.

Mills, Suzanne C., et al. "Hormonal and behavioural effects of motorboat noise on wild coral reef fish." *Environmental Pollution* 262 (2020): 114250.

Myrberg, A. A., and Spires, J. Y. (1980). "Hearing in damselfishes—An analysis of signal-detection among closely related species," *J. Comp. Physiol.* 140, 135–144.

Myrberg, Arthur A., and Juanita Y. Spires. "Sound discrimination by the bicolor damselfish, *Eupomacentrus partitus*." *Journal of experimental biology* 57.3 (1972): 727-735.

Myrberg Jr, Arthur A. "Ethology of the bicolor damselfish, *Eupomacentrus partitus* (Pisces: Pomacentridae): a comparative analysis of laboratory and field behaviour." *Animal Behaviour Monographs* 5 (1972): 197-IN2.

Nedelec, Sophie L., et al. "Repeated exposure to noise increases tolerance in a coral reef fish." *Environmental pollution* 216 (2016): 428-436.

Olivier, D et al. The cerato-mandibular ligament: a key functional trait for grazing in damselfishes (Pomacentridae). *Frontiers in Zoology* 11.1 (2014): 1-14.

Parmentier, Eric, et al. "Call properties and morphology of the sound-producing organ in *Ophidion rochei* (Ophidiidae)." *Journal of Experimental Biology* 213.18 (2010): 3230-3236

Picciulin, Marta, et al. "In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a Marine Protected Area." *Journal of Experimental Marine Biology and Ecology* 386.1-2 (2010): 125-132.

Pijanowski, Bryan C., et al. "Soundscape ecology: the science of sound in the landscape." *BioScience* 61.3 (2011): 203-216.

Popper, Arthur N., and Anthony D. Hawkins. "An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes." *Journal of fish biology* 94.5 (2019): 692-713.

Popper, Arthur N. "Effects of anthropogenic sounds on fishes." *Fisheries* 28.10 (2003): 24-31

Sebastianutto, Linda, et al. "How boat noise affects an ecologically crucial behaviour: the case of territoriality in *Gobius cruentatus* (Gobiidae)." *Environmental biology of fishes* 92.2 (2011): 207-215.

Sertlek, H. Ozkan, Hans Slabbekoorn, and Michael A. Ainslie. "The contribution of shipping sound to the dutch underwater soundscape: Past, present, future." *Proceedings of Meetings on Acoustics 5ENAF*. Vol. 37. No. 1. Acoustical Society of America, 2019.

Shannon, Graeme, et al. "A synthesis of two decades of research documenting the effects of noise on wildlife." *Biological Reviews* 91.4 (2016): 982-1005.

Simpson, Stephen D., et al. "Anthropogenic noise increases fish mortality by predation." *Nature communications* 7.1 (2016): 1-7.

Slabbekoorn, Hans, et al. "A noisy spring: the impact of globally rising underwater sound levels on fish." *Trends in ecology & evolution* 25.7 (2010): 419-427.

Vazzana, Mirella, et al. "Noise elicits hematological stress parameters in Mediterranean damselfish (*Chromis chromis*, perciformes): a mesocosm study." *Fish & shellfish immunology* 62 (2017): 147-152.

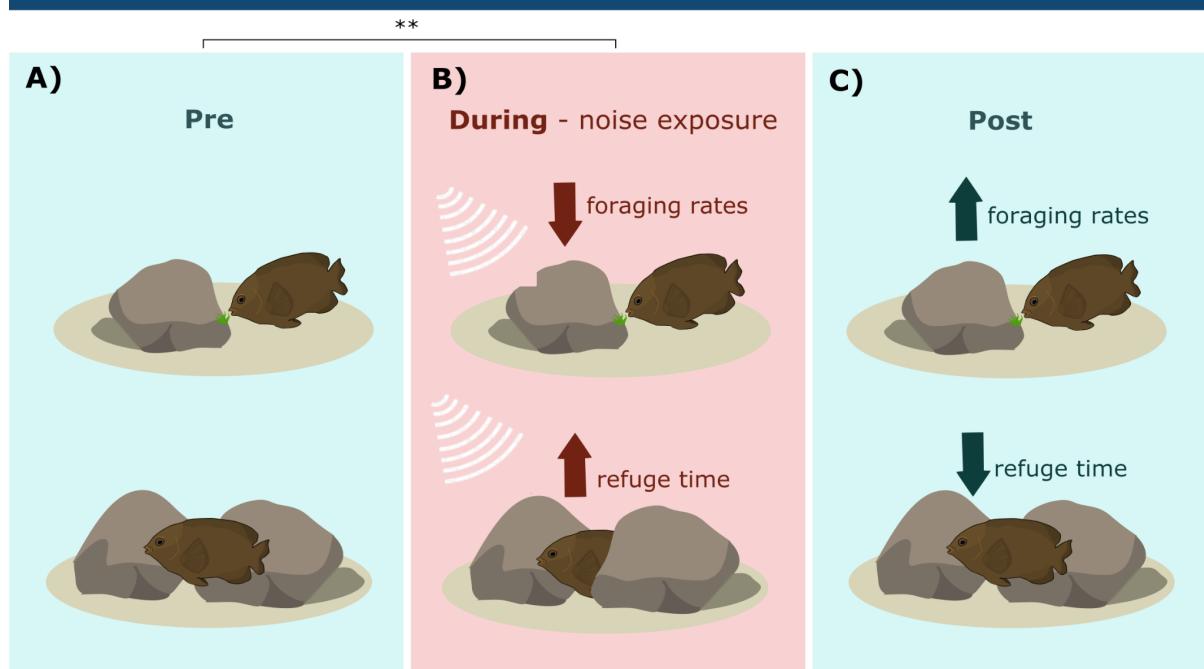
Weatherdon, Lauren V., et al. "Observed and projected impacts of climate change on marine fisheries, aquaculture, coastal tourism, and human health: an update." *Frontiers in Marine Science* (2016): 48.

Whitfield, A. K., and A. Becker. "Impacts of recreational motorboats on fishes: a review." *Marine pollution bulletin* 83.1 (2014): 24-31.

Capítulo Único

*Exposure to anthropogenic noise impairs anti-predator responses in wild
damselfishes*

Formatado para a revista "Environmental Pollution"



*Graphical Abstract: Behavioral responses of the dusky damselfish *Stegastes fuscus* pre, during and post exposure to anthropogenic noise. Significant changes are marked with ** ($p < 0.01$). During sound exposure individuals increased their refuge time and decreased foraging rates. After the noise stimulus ceased, individuals returned behaviors to pre-exposure levels.*

1. INTRODUCTION

Reef ecosystems, despite supporting great biodiversity, and providing many ecosystem services to coastal communities (Worm et al., 2006) are constantly threatened by several human activities globally, including coastal urbanization, overfishing, and climate change (Bellwood et al., 2004; Pandolfi et al., 2005, Weatherdon et al., 2016). Noise pollution, as an unintended by-product or not, is a major threat to biodiversity and its increase has been documented worldwide, in both coastal and oceanic environments (Andrew et al., 2002; Tyack, 2008; Hildebrand et al., 2009; Bittencourt et al., 2014). The anthropogenic (human-generated) noise is derived from many sources and can be divided into two main groups. Firstly, the transient and high peak pressure sounds which are produced by pile driving, air guns, military sonar, and seismic surveys; and secondly, the continuous and low-frequency ones (<1 kHz) that are produced by shipping (e.g., commercial and recreational boats), seabed drilling, and more (Slabbekoorn et al., 2010; Hawkins & Popper, 2018; Popper & Hawkins, 2019). The underwater soundscapes are rapidly changing (Duarte et al., 2021), and there is an urgent need to assess the impacts of this anthropogenic driver on marine life and fill the remaining gaps to deliver mitigation measures to this yet neglected impact.

Sound plays a vital role for marine organisms in many important daily life activities, including foraging, territoriality and mating. Acoustic cues also guide orientation, settlement, habitat use, as many other critical life functions (Cole et al., 2010; Radford et al., 2011; Jolivet et al., 2016; Gordon et al., 2018), while had been detected to indicate habitat quality (Freeman et al., 2016). In coastal areas, nautical traffic contributes to increasing low-frequency noise levels (Codarin et al., 2009) and many fish species can detect sounds in this frequency range (Popper, 2003). Increased noise levels can interfere in a number of ways, including individuals' sound detection and response, which may have ecological effects on the dynamics and resilience of natural systems (Kunc et al., 2016; Popper and Hawkins, 2019). Therefore, research investigating the effects of human-produced noise on important key-species, such as the dusky damselfish (Bracciali et al., 2012; Leduc et al., 2021), is critical to understand sound impact on the reef scape.

Motorized vessels, including large ships and recreational boats, represent a prevalent and increasing source of anthropogenic noise (Haviland-Howell et al., 2007; Whitfield and Becker, 2014; Bittencourt et al., 2014; 2020; Sertlek et al., 2019). There is mounting evidence showing that noise produced by such boats have impacts on behavior (e.g. foraging, agonistic interactions, anti-predator responses, parental care) (Picciulin et al., 2010; Sebastianutto et al., 2011; Bracciali et al., 2012), on physiology, (e.g. increases in metabolic, ventilation and heart rates) (Simpson et al., 2016; Mills et al., 2020), and on survival of many reef fish species (McCormick et al., 2018). Recent studies have assessed the underwater noise pollution by small vessels in the Brazilian coast (Bittencourt et al., 2014; Campbell et al., 2019), but few have investigated its effects on marine life, especially on key-species, such as damselfishes (Leduc et al., 2021).

Damselfishes represent a keystone group for the diversity of benthic communities (Hata & Ceccarelli., 2016). Many of them are territorial farmers that control algal diversity and coral zonation (Ceccarelli et al., 2001). Sound production in damselfishes is well known and is highly associated with different ecological interactions, including agonistic ones during territory and nest defense (Parmentier et al., 2010), plus for recognition during reproduction (Cole, 2010). The dusky damselfish *Stegastes fuscus* (Cuvier, 1830) is the most common territorial herbivorous in shallow Brazilian reefs (Ferreira et al., 2004). It plays a critical ecological role in structuring benthic communities through grazing and territoriality (Ceccarelli et al., 2005; Barneche et al., 2009). By maintaining a territory and aggressively excluding other herbivores, diversity of algae and associated cryptozoa are higher inside territories of *Stegastes fuscus*. In addition, primary productivity is higher inside their territories, indicating that this species plays an important trophodynamic role in shallow habitats (Ferreira et al. 1998). *Stegastes* spp. produce sound at frequencies from 250 to 1500 Hz (Myrberg, 1972; Myrberg and Spires, 1972), and detect sounds at 500 Hz bands (Myrberg and Spires, 1980; Egner and Mann, 2005), overlapping with the broadband sound produced by motorboats. Thus, these fishes represent an optimal ecological model for understanding the consequences of anthropogenic noise. Previous studies on related species indicated damselfishes as important bioindicators for noise impacts (Picciulin et al., 2010; Bracciali et al., 2012; Vazzana et al., 2017; Nedelec et al.,

2016; Holmes et al., 2017), especially the territorialist species, that due to their limited home range are more vulnerable to such impacts (Daros et al., 2016, Benevides et al., 2019).

Here we carried out field experiments to investigate the effects of noise on *Stegastes fuscus* (Cuvier, 1830) behavior in a multi use marine reserve. Our specific goals were: (i) to assess the effect of noise on different behaviors (foraging, refuging and agonistic interactions) under natural conditions, and (ii) to investigate residual effects of noise exposure. We predict that exposure to both noise treatments would cause changes in behavior (e.g. decreased foraging rates, increased aggression and hiding). Moreover, individuals may have carry-over effects in response to acute stress caused by previous noise stimulus. These data are essential to implement local normatives for such neglected impact.

2. MATERIALS AND METHODS

2.1 Study Area

Field experiments were carried out in shallow rocky reefs inside the Arraial do Cabo Marine Reserve, Rio de Janeiro state, Brazil ($23^{\circ} 44'S$ - $42^{\circ} W$), during a 10-day period between January and May 2022. This MPA is a multi use reserve where only local traditional fishermen are allowed to exploit natural resources. Despite some fishing restrictions, there are no effective no-take areas or control of fishing activities, and different fishing gears are used (Bender et al., 2014). The Arraial do Cabo region consists of an isthmus and two islands dominated by rocky shores. Upwelling events in the region are triggered by a combination of prevailing winds and coastal morphology, which leads to distinct habitat conditions: the western side of the isthmus that is affected directly by cold, upwelling waters ($<18^{\circ} C$), and the eastern side with comparative higher mean water temperatures (averaging $22^{\circ} C$) (Cordeiro et al, 2020). The study sites, namely Pedra Vermelha and Maramutá (Figure 1), are sheltered from both wave exposure and local upwelling, and present tropical characteristics on its composition, clear waters, and a mean average water temperature of $22^{\circ}C$ (Valentin, 1984a; 1984b). The benthos coverage is

predominantly composed by epilithic algal matrix, zoanthids, massive corals and millepores (Cordeiro et al, 2014). The community of the dusky damselfish *Stegastes fuscus* includes mainly adult individuals (13-17 cm TL), which territories occupies a great part of the substrate in the rocky reef zone ranging from 2 to 5 m depth (Ferreira et al., 1998). The sites were selected based on similarity of environment conditions and its restricted nautical traffic, allowing less external noise during the field experiments, while providing great abundance of the target species.

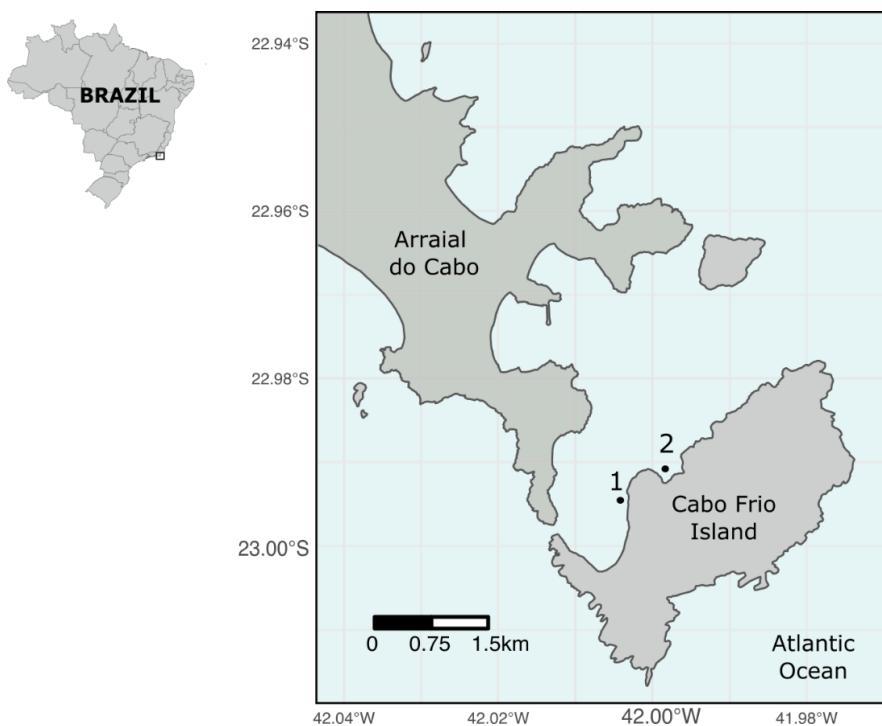


Figure 1. Map of Arraial do Cabo showing study sites (1 - Maramutá, 2 - Pedra Vermelha).

2.2 Experimental overview

We randomly selected territories of *S. fuscus* in the study sites, totaling 35 territories (19 for playback, 16 for pure tone) separated by each other by at least 2 m. All experiments were developed between 9 and 12 am due to the peak of activity of the target species, and during week to avoid high-nautical-traffic-days, and thus to minimize possible interferences during the experiments. An underwater loudspeaker (Lubell LL-1424HP - (200 Hz - 9 kHz), 197dB re 1uPa @ 1m at 600Hz, source level 150 dB, approximately) was placed in a central position, at least 2 m from the individual's territories, so that can cover more than one territory at once (Figure 2).

The speaker was kept off during the first 15 minutes, as a 5 min acclimation period (Nanninga et al., 2017) and a 10 min to collect baseline behavior. In each territory, we applied one of the following experimental approaches: Treatment 1 (T1) - 10 min of playback of sound of small vessels, collected in days of intense traffic (200 Hz-1kHz) and Treatment 2 (T2) - 10 min of a pure tone in the frequency range of the studied interactions (400 Hz) (Olivier et al., 2014). All individuals were equally subjected to control and treatment. When submitted to noise exposure all fish received 30s of noise every minute for a total time of 10 minutes. Playbacks of small boats noise were used since it is the most common source of anthropogenic noise in the area. Individuals' responses to the experimental approaches were assessed with remote underwater videos (RUVs). Video cameras (GoPro Hero 4) were settled on the rocky substrate at 1 m from the individuals' territories, comprehending an area of 2m², carefully delimited by scuba diver. One video camera was used in each territory to avoid further interference. After settled, the camera was left recording for at least 45 minutes without interruption and the diver kept a 10 m distance to avoid interferences on the experiments (Longo & Floeter, 2012). A RUVs sample consisted of a 30-minute video shot.

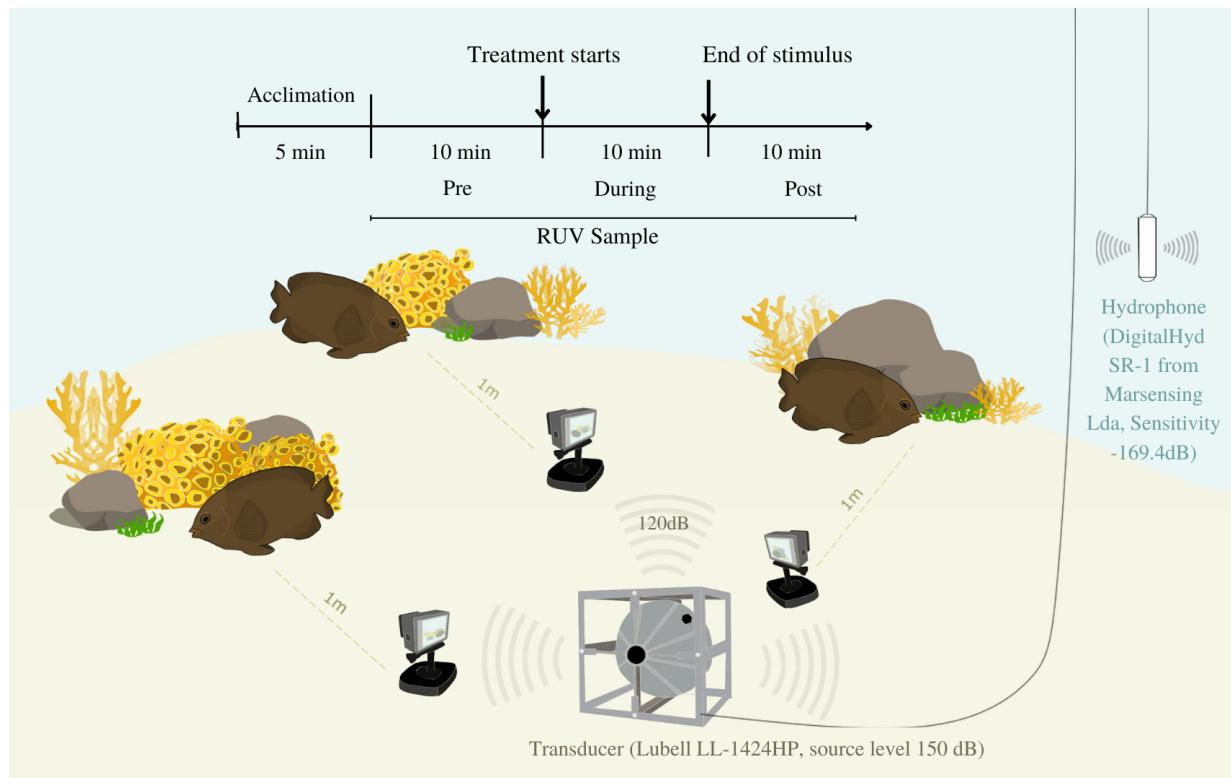


Figure 2. Experimental overview showing timeline of trials.

2.3 Acoustic data

Playback treatments were created using 30s of noise and 30s of silence. For the first noise stimulus (T1), playback tracks were constructed using recordings of noise of small tourism vessels passing by the area, and played in a loop for 10 minutes. The recordings occurred during daytime of intense nautical traffic in the Arraial do Cabo Marine Reserve ($23^{\circ}44'S$ - $42^{\circ}W$). The projected noise was recorded by a hydrophone (DigitalHyd SR-1 from Marsensing Lda, sensitivity of -169.4dB at 1000Hz), to compare playbacks with real sound sources and to validate trials. For the second noise stimulus (T2), we used a tone of 400 Hz in a loop for 10 minutes, also using 30s of noise and 30s of silence. The volume of the playback was adjusted and the signal was previously calibrated. The median distance between the hydrophone and the loudspeaker was 20m. Sound travel between experimental sites have been limited to distance and the presence of rocky reefs, as sound treatments at one site could not be heard from the other (verified through recordings). To calibrate and characterize the underwater soundscape during trials, the acoustic activities detected were analyzed via spectrograms with Raven Pro 1.6 bioacoustics software (The Cornell Lab of Ornithology, Ithaca, NY). A 30-second-window of the audio recordings were used to illustrate the soundscape changes within periods.

2.4 Behavioral analysis

In the laboratory, only the central minutes of all video recordings were analyzed to minimize the diver effect over fish behavior (Longo et al., 2014). Each fish had its length visually estimated, which varied between 13 cm and 17 cm, and its behavior aspects quantified. The three behaviors analyzed were: (1) Agonistic interactions - the number of chasing/aggressive strikes towards other fishes per minute. We considered as a chase all events in which a fish swam rapidly towards another fish with subsequent escape of the latter, with or without contact between them; (2) Foraging - the number of bites per minute on the substrate or at a drift particle in the water column. We considered a bite every time a fish stroked the benthos with its jaws opened, closing its mouth subsequently, regardless ingestion (Longo et al., 2014); (3) Refuging - Time hiding in a shelter structure, with at least 50% of the body within the shelter, per minute. All observations lasted 30 minutes, divided in three periods of time: 10 min prior to the sound treatment ('pre'), as our control, the 10 min

trials of the soundtrack ('during') and the 10 minutes following the sound treatment ('post'). The observer watched the videos without audio to remain naïve to treatments. Clips of each behavior are presented in Supplementary material.

2.5 Statistical analysis

Differences between behaviors (e.g foraging rates, frequency of chases, time refuging) within periods (=pre, during noise, post) were analyzed through a Friedman test. Pairwise Wilcoxon signed-rank tests were conducted comparing behaviors within periods for each treatment separately. The waveform and spectrogram graphics of the analyses were performed in Python (Python Software Foundation, 2022). All statistical analyses and other plots were performed in R software (R Core Team, 2022). A significance level of $\alpha < 0.05$ was considered in all analyses.

3. RESULTS

There were significant differences between control and treatments in terms of sound pressure levels. While sound treatments were active, power spectral density levels were substantially higher than the ambient soundscape during pre and post periods (Figure 3).

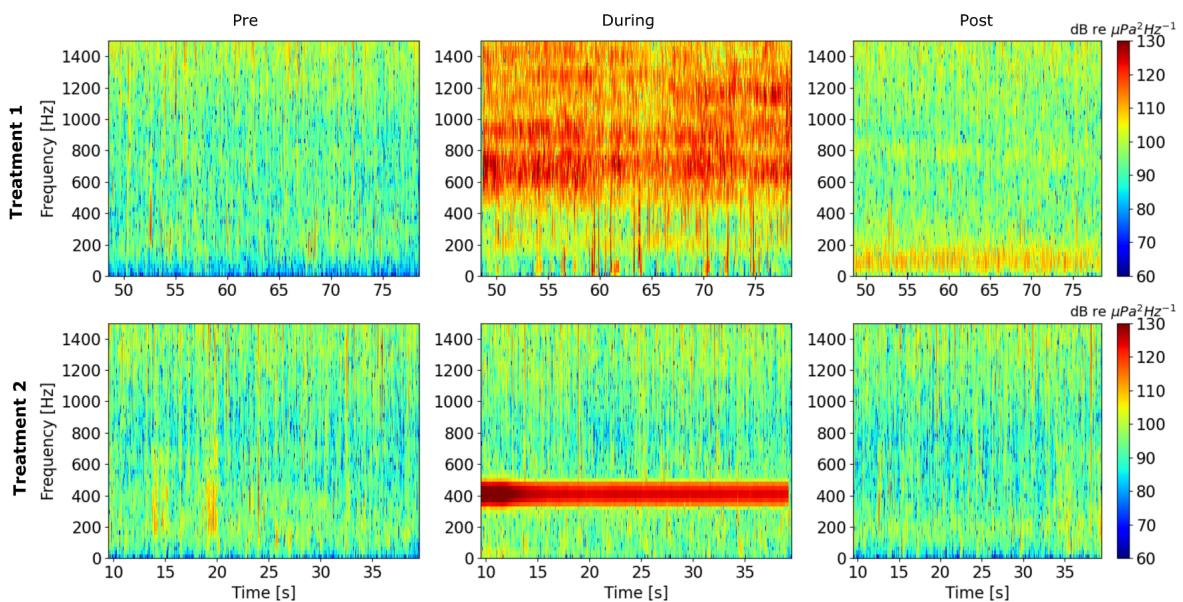


Figure 3. Spectrograms of the underwater soundscape during field experiments, for a 30-second-window of audio recordings of each treatment (treatment 1 = [playback; treatment 2 = pure tone). The left spectrograms represent the before period, the middle ones are during noise exposure and the ones on the right are after. FFT length = 1024, Hamming evaluation window, 50% window overlap, 0–1500 Hz.

Fish behavior of refuging and foraging varied significantly within periods in both treatments (bite = playback, $X^2(2) = 6.91$, $p = 0.032$, $n=19$; pure tone, $X^2(2) = 23.5$ $p < 0.0001$, $n=16$; refuge = playback, $X^2(2) = 10.03$, $p = 0.006$, $n=19$; pure tone, $X^2(2) = 14.4$, $p < 0.001$, $n=16$) (Figure 4). Fish spent more time hiding during the 10 min period of noise than in the 10 min pre-noise (playback: $p = 0.009$; pure tone, $p = 0.002$). Individuals had also lower foraging rates during noise exposure compared to pre-exposure (playback: $p = 0.003$; pure tone, $p = 0.0004$). In the 10-min-period after noise exposure, fish behavior returned to similar values of both foraging rates and hiding behavior to the moment pre-exposure, for both treatments (Table 1). Chasing activity of the dusky damselfish was reduced for 45,8% of individuals during playback and 65% during pure tone, but no significant differences within periods were observed ($p > 0.05$, Figures 4b-e).

*Table 1. Comparisons of behaviors under either pre, during and post noise exposure. Summary of Friedman test results of behaviors testing the effect within periods for Stegastes fuscus. Bold type denotes significant differences at $\alpha = 0.01$. * indicate significance level: * < 0.05; ** < 0.01*

Source of variation	df	X^2	p-value
<i>Between subjects</i>			
<i>Treatment 1 = (playback)</i>			
Bites x time	2	6.91	0.003**
Refuge x time	2	10.03	0.006**
Chases x time	2	6.86	0.032*
<i>Treatment 2 = (pure tone)</i>			
Bites x time	2	23.5	0.000007**

Refuge x time	2	14.4	0.00075**
Chases x time	2	4.69	0.0958
<hr/>			
<i>Within subjects</i>			
<hr/>			
<i>Treatment 1 (playback)</i>			
<hr/>			
Bites pre x noise	2	153	0.003**
Bites pre x post	2	131	0.153
Refuge pre x noise	2	20.5	0.009**
Refuge pre x post	2	82	0.813
Chases pre x noise	2	84	0.421
Chases pre x post	2	120	0.04*
<hr/>			
<i>Treatment 2 (pure tone)</i>			
<hr/>			
Bites pre x noise	2	0	0.00048**
Bites pre x post	2	49	0.339
Refuge pre x noise	2	102	0.002**
Refuge pre x post	2	17	0.554
Chases pre x noise	2	36	0.933
Chases pre x post	2	63.5	0.657
<hr/>			

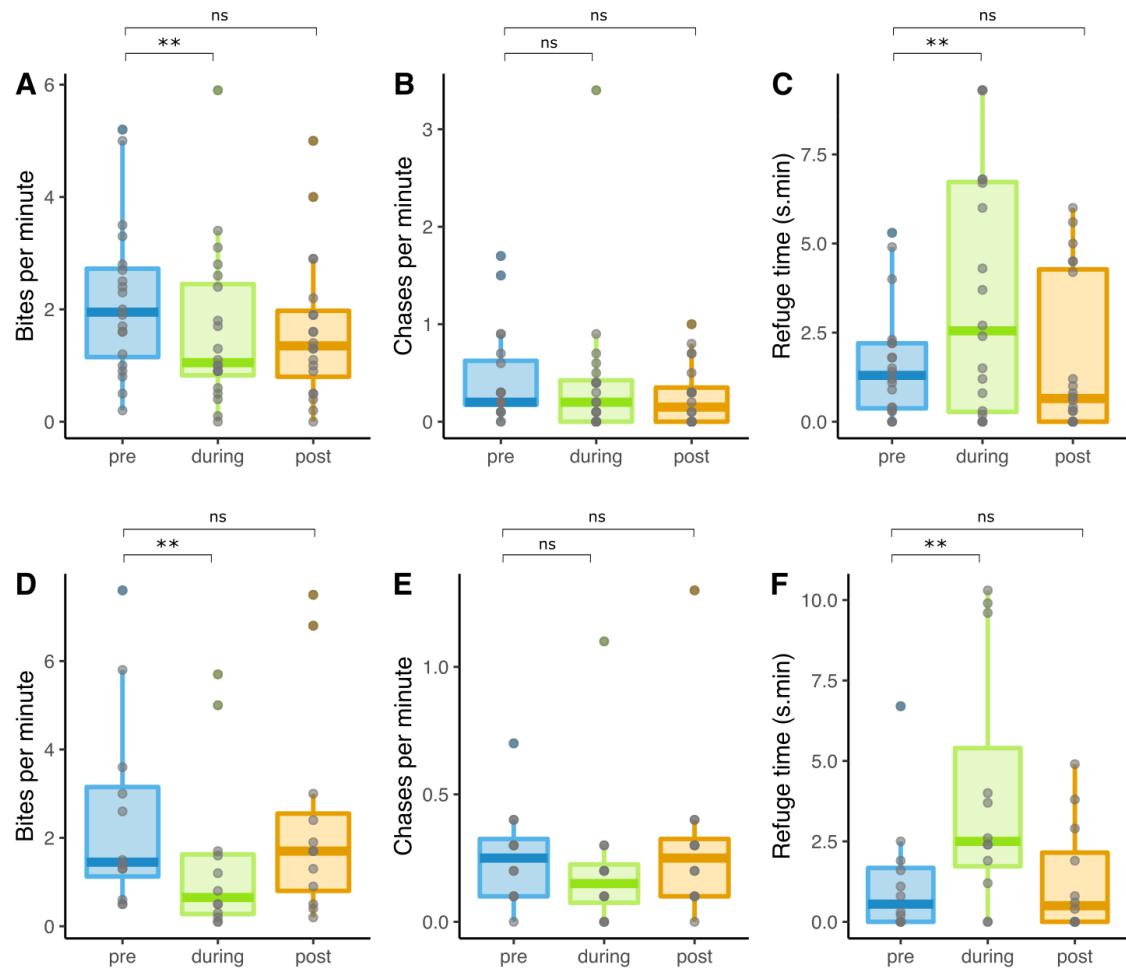


Figure 4. Comparative behavior responses for both experimental approaches during three periods (-pre, -during, -post noise exposure). Boxes represent interquartile ranges and lines within boxes represent median values across 19 individuals for the playback and 16 for the pure tone. Each dot represents an individual. (a) bite rates in playback; (b) chasing in playback; (c)refuging in playback; (d) bite rates in pure tone; (e) chasing in pure tone; (f) refuging in pure tone. * indicate significance level: ** < 0.01; ns: non-significant result from Friedman test.

4. DISCUSSION

Our study demonstrated noise-induced behavioral changes in a conspicuous damselfish of the Southern Atlantic reefs. Two key behaviors, foraging and refuging, were significantly disrupted when different noises, a pure tone (400 Hz) and playback (200 Hz - 1 kHz), were experimentally applied *in situ* for this species. Individuals were more likely to seek refuge and spent less time foraging during noise

exposure than in pre and post exposure periods. Previous studies with damselfishes found similar patterns. For instance, Nedelec et al (2016) observed that *Dascyllus trimaculatus* increased refuging during motorboat playback exposure, and Leduc et al. (2021) observed that land-based noise pollution, from carnival commemoration, had significant effect on *S. fuscus* foraging rates. Our results provide evidence of underwater anthropogenic noise impact on this key species. As changes in behavior are the initial response to different environmental pressures, further investigations on whether and how these changes affect the population's dynamics and functional role are required.

Refuging is a common behavioral outcome associated with animals' space use when facing any threat (Cooper and Blumstein, 2015). In another recent experiment in the same region, Benevides et al., (2019) reported that the dusky damselfish increased refuging time in the presence of a threat, in this case, a diver presence. Fish in our experiments had similar anti-predator responses to noise stimulus, which indicates noise being seen as a threat. Similar refuging patterns were found for the red-mouth goby *Gobius cruentatus* (Picciulin et al., 2010), and for the domino damselfish *Dascyllus trimaculatus* (Nedelec et al., 2016). When territorial farmers, such as the dusky damselfish, increase their refuge time, important feeding opportunities are missed and territory defense is compromised. Given the importance of the functional role of damselfishes (Hata & Ceccarelli et al., 2016), this could negatively affect the dynamics and structure of the community, as the time available for crucial daily activities is reduced.

Foraging is an important daily activity in fishes and apparently the first to be disrupted facing any threat. Bracciali et al (2012) found that *Chromis chromis*, a planktivorous fish, also significantly reduced foraging rates when subjected to intense boat noise and nautical traffic. Voellmy et al (2014) investigated how increased exposure to noise changed foraging responses of two sympatric freshwater fish species. They found that both species, *Gasterosteus aculeatus* and *Phoxinus phoxinus*, had a similar reduction in food intake. The long-term implications of reduced foraging should be taken into consideration, as decreased foraging rates could directly affect individuals' *fitness*. If they spend less time foraging, their body condition and growth may be reduced, as their ability to breed and survive (King et

al., 2015). Moreover, the energetic costs of a reduced likelihood of feeding events could also have other population-level changes, as fish may alter their behavior to compensate for food intake during less propitious conditions (Bracciali et al., 2012). In this case, the trade-off between predator avoidance and foraging activity may vary between individuals and should be taken into consideration in future studies.

It is expected that organisms exposed to anthropogenic stressors, such as noise, may have increased levels of agonistic interactions due to acute stress. For instance, the anemonefish *Amphiprion chrysopterus* increased its levels of aggression when exposed to motorboat noise playback, in short- and long-term experiments (Mills et al., 2020). Contrary to our hypothesis, the Dusky damselfish did not show any significant changes in chasing behavior within periods, although a reduced pattern was found during noise exposure. The main reason that could be attributed to these contrasting results may rely on risk assessment. Despite its benefits, territory maintenance has high costs in terms of energetic expenditure (Eurich et al., 2018). In this case, *S. fuscus* elevating chasing to keep defending its territory during noise exposure may not seem to outweigh the costs, as organisms spent more time hiding. The reflections of this pattern over time should be evaluated, as this could affect individuals' reproductive opportunities and also productivity inside territories. Clearly, more research is required to better understand the direct impacts of noise on territory maintenance.

Our findings show that anthropogenic noise, as applied here, for 10 minutes, had no carry-over effects on fish behavior, as individual's return to pre-exposure levels after noise has ceased. Mills et al., (2020) found short-term (5 minute) carry-over effects of motorboat playback on behavior and physiology of the orange-fin anemonefish *Amphiprion chrysopterus*. Nedelec et al. (2017) also found behavioral carry-over effects for cleaning interactions after 5 minutes, where the number of jolting clients remained elevated even after noise ceased. However, damselfishes in our experiment returned to pre-exposure levels on both foraging and refuging behavior after sound had ceased in both treatments, which indicates a fast response of this species' behavioral mechanisms to environmental conditions. The present study was a short-term evaluation (30-minute-long-trials), and for that reason we cannot say that these responses would persist over time, or if fish would

habituate to longer noise exposures. Still, experimental designs with longer observations and using different metrics (e.g. physiological markers) should be considered, as other studies indicate carry-over effects on other critical life functions beyond behavior, such as physiology (Mills et al., 2020).

In general, pure tones seem to have a weaker effect on fish' behavior (Cox et al., 2020). However, our results showed that fish behavioral responses to the playback were similar, but more pronounced to the pure tone. The frequency range in our study was based on Olivier et al. (2014) that registered 400 Hz as the main frequency of sound production during foraging and agonistic interactions of *Stegastes rectifraenum*. These results may indicate an overlap of the emitted frequency with the signals emitted by the species, resulting in a more pronounced response.

5. CONCLUSIONS

The increase of anthropogenic noise in coastal underwater soundscapes is well documented (Duarte et al., 2021). Noise produced by small vessels dominates shallow underwater soundscapes (Campbell et al., 2019) and the effects of these changes are affecting different species and processes (Kunc et al., 2016; 2019; Shannon et al., 2016). In our *in situ* experimental study two different noises were used as stressors, and both had significant effects on *Stegastes fuscus* behavior. Fish were short-term-impacted by noise through increased anti-predator responses. As the density of boats is relatively high and frequent around the coast, understanding the consequences of prevalent exposure to noise events is critical.

The effects of underwater noise on behavior, as highlighted here, are extremely relevant for management and species conservation, as increased anti-predation behavior could lead to alterations in population dynamics and thereby compromise key ecological processes (e.g. herbivory) . The more we know about the sublethal impacts of noise on such key species, the more we can do for conservation and management efforts across different coastal environments. Moreover, subsequent studies combining different approaches should examine all the effects of noise on wildlife, in both behavioral and physiological aspects. Then, we will be able to identify

noise susceptibilities in reef dynamics and have a more complete understanding of the effects of this neglected impact.

6. REFERENCES

Andrew, Rex K., et al. "Ocean ambient sound: comparing the 1960s with the 1990s for a receiver off the California coast." *Acoustics research letters online* 3.2 (2002): 65-70.

Barneche, Diego R., et al. "Feeding macroecology of territorial damselfishes (Perciformes: Pomacentridae)." *Marine Biology* 156.3 (2009): 289-299.

Bellwood, David R., Andrew S. Hoey, and Terence P. Hughes. "Human activity selectively impacts the ecosystem roles of parrotfishes on coral reefs." *Proceedings of the Royal Society B: Biological Sciences* 279.1733 (2012): 1621-1629.

Bellwood, David R., et al. "Confronting the coral reef crisis." *Nature* 429.6994 (2004): 827-833.

Bender, Mariana G., et al. "Local ecological knowledge and scientific data reveal overexploitation by multigear artisanal fisheries in the Southwestern Atlantic." *PLoS One* 9.10 (2014): e110332.

Benevides, Larissa J., et al. "Fear-induced behavioural modifications in damselfishes can be diver-triggered." *Journal of Experimental Marine Biology and Ecology* 514 (2019): 34-40.

Bittencourt, L., et al. "Anthropogenic noise influences on marine soundscape variability across coastal areas." *Marine Pollution Bulletin* 160 (2020): 111648.

Bittencourt, L., et al. "Underwater noise pollution in a coastal tropical environment." *Marine pollution bulletin* 83.1 (2014): 331-33.

Bracciali, Claudia, et al. "Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*)." *PLoS one* 7.7 (2012): e40582.

Campbell, Daniel, et al. "Underwater soundscape pattern during high season of nautical tourism in Cabo Frio Island, Brazil." *Proceedings of Meetings on Acoustics* 5ENAL. Vol. 37. No. 1. Acoustical Society of America, 2019.

Ceccarelli, Daniela M., Geoffrey P. Jones, and Laurence J. McCook. "Effects of territorial damselfish on an algal-dominated coastal coral reef." *Coral Reefs* 24.4 (2005): 606-620.

Ceccarelli, Daniela M., Geoffrey P. Jones, and Laurence J. McCook."Territorial damselfish as determinants of structure of benthic communities on coral reefs." *Oceanogr Mar Biol* 39 (2001):355–389

Codarin, Antonio, et al. "Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy)." *Marine pollution bulletin* 58.12 (2009): 1880-1887.

Cole, Kathleen Sabina, ed. *Reproduction and sexuality in marine fishes: patterns and processes*. Univ of California Press, 2010.

Cooper, William E., and Daniel T. Blumstein, eds. *Escaping from predators: an integrative view of escape decisions*. Cambridge University Press, 2015.

Cordeiro, Cesar Augusto Marcelino Mendes, A. R. Harborne, and C. E. L. Ferreira. "Patterns of distribution and composition of sea urchin assemblages on Brazilian subtropical rocky reefs." *Marine Biology* 161.10 (2014): 2221-2232.

Cordeiro, Cesar Augusto Marcelino Mendes, Alastair R. Harborne, and Carlos Eduardo Leite Ferreira. "The biophysical controls of macroalgal growth on subtropical reefs." *Frontiers in Marine Science* (2020): 488.

Cox, Kieran, et al. "Sound the alarm: A meta-analysis on the effect of aquatic noise on fish behavior and physiology." *Global Change Biology* 24.7 (2018): 3105-3116.

Daros, Felipe Alexandre, et al. "Otolith fingerprints of the coral reef fish Stegastes fuscus in southeast Brazil: a useful tool for population and connectivity studies." *Regional Studies in Marine Science* 3 (2016): 262-272.

Duarte, Carlos M., et al. "The soundscape of the Anthropocene ocean." *Science* 371.6529 (2021): eaba4658.

Egner, Sarah A., and David A. Mann. "Auditory sensitivity of sergeant major damselfish *Abudefduf saxatilis* from post-settlement juvenile to adult." *Marine Ecology Progress Series* 285 (2005): 213-222.

Eurich, Jacob G., et al. "Experimental evaluation of the effect of a territorial damselfish on foraging behaviour of roving herbivores on coral reefs." *Journal of Experimental Marine Biology and Ecology* 506 (2018): 155-162.

Ferreira, Carlos Eduardo L., et al. "Herbivory by the dusky damselfish *Stegastes fuscus* (Cuvier, 1830) in a tropical rocky shore: effects on the benthic community." *Journal of Experimental Marine Biology and Ecology* 229.2 (1998): 241-264.

Ferreira, Carlos Eduardo L., José EA Gonçalves, and Ricardo Coutinho. "Community structure of fishes and habitat complexity on a tropical rocky shore." *Environmental biology of fishes* 61.4 (2001): 353-369.

Frederich, Bruno & Parmentier, E.. (2016). *Biology of Damselfishes*.

Freeman, Lauren A., and Simon E. Freeman. "Rapidly obtained ecosystem indicators from coral reef soundscapes." *Marine Ecology Progress Series* 561 (2016): 69-82.

Shannon, M.F. McKenna, L.M. Angeloni, K.R. Crooks, K.M. Fristrup, E. Brown, et al. A synthesis of two decades of research documenting the effects of noise on wildlife *Biological Reviews*, 91 (2016), pp. 982-1005

Gordon, Timothy AC, et al. "Habitat degradation negatively affects auditory settlement behavior of coral reef fishes." *Proceedings of the National Academy of Sciences* 115.20 (2018): 5193-5198.

Hata, Hiroki, and Daniela M. Ceccarelli. "Farming Behaviour of Territorial Damselfishes." *Biology of damselfishes*. CRC Press, 2016. 140-170.

Haviland-Howell, Genevieve, et al. "Recreational boating traffic: a chronic source of anthropogenic noise in the Wilmington, North Carolina Intracoastal Waterway." *The Journal of the Acoustical Society of America* 122.1 (2007): 151-160.

Hawkins, Anthony D., and Arthur N. Popper. "Effects of man-made sound on fishes." *Effects of anthropogenic noise on animals*. Springer, New York, NY, 2018. 145-177.

Hildebrand J.A. Anthropogenic and natural sources of ambient noise in the ocean. *Mar Ecol Prog Ser*. 2009; 395: 5–20. <https://doi.org/10.3354/meps08353>

Holmes, Lucy J., et al. "Juvenile damselfish are affected but desensitize to small motor boat noise." *Journal of Experimental Marine Biology and Ecology* 494 (2017): 63-68.

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B. C., ... Scheffer, M. (2017). Coral reefs in the Anthropocene. *Nature*, 546(7656), 82–90. <https://doi.org/10.1038/nature22901>

Jolivet, Aurélie, et al. "Validation of trophic and anthropic underwater noise as settlement trigger in blue mussels." *Scientific reports* 6.1 (2016): 1-8.

King, S. L., Schick, R. S., Donovan, C., Booth, C. G., Burgman, M., Thomas, L., & Harwood, J. (2015). An interim framework for assessing the population consequences of disturbance. *Methods in Ecology and Evolution*, 6, 1150– 1158.

Kunc, Hansjoerg P., and Rouven Schmidt. "The effects of anthropogenic noise on animals: a meta-analysis." *Biology Letters* 15.11 (2019): 20190649.

Kunc, Hansjoerg P., Kirsty Elizabeth McLaughlin, and Rouven Schmidt. "Aquatic noise pollution: implications for individuals, populations, and ecosystems." *Proceedings of the Royal Society B: Biological Sciences* 283.1836 (2016): 20160839.

Leduc, Antoine OHC, et al. "Land-based noise pollution impairs reef fish behavior: a case study with a Brazilian carnival." *Biological Conservation* 253 (2021): 108910.

Lillis, Ashlee, David B. Eggleston, and DelWayne R. Bohnenstiehl. "Oyster larvae settle in response to habitat-associated underwater sounds." *PloS one* 8.10 (2013): e79337.

Longo, G. O., and S. R. Floeter. "Comparison of remote video and diver's direct observations to quantify reef fishes feeding on benthos in coral and rocky reefs." *Journal of Fish Biology* 81.5 (2012): 1773-1780.

Longo, Guilherme O., Carlos Eduardo L. Ferreira, and Sergio R. Floeter. "Herbivory drives large-scale spatial variation in reef fish trophic interactions." *Ecology and evolution* 4.23 (2014): 4553-4566.

McCormick, Mark I., et al. "Boat noise impacts risk assessment in a coral reef fish but effects depend on engine type." *Scientific reports* 8.1 (2018): 1-11.

Mills, Suzanne C., et al. "Hormonal and behavioural effects of motorboat noise on wild coral reef fish." *Environmental Pollution* 262 (2020): 114250.

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecological Economics*, 29(2), 215–233.
[https://doi.org/10.1016/S0921-8009\(99\)00009-9](https://doi.org/10.1016/S0921-8009(99)00009-9)

Moberg, Fredrik, and Carl Folke. "Ecological goods and services of coral reef ecosystems." *Ecological economics* 29.2 (1999): 215-233.

Myrberg, A. A., and Spires, J. Y. (1980). "Hearing in damselfishes—An analysis of signal-detection among closely related species," *J. Comp. Physiol.* 140, 135–144.

Myrberg, Arthur A., and Juanita Y. Spires. "Sound discrimination by the bicolor damselfish, *Eupomacentrus partitus*." *Journal of experimental biology* 57.3 (1972): 727-735.

Myrberg Jr, Arthur A. "Ethology of the bicolor damselfish, *Eupomacentrus partitus* (Pisces: Pomacentridae): a comparative analysis of laboratory and field behaviour." *Animal Behaviour Monographs* 5 (1972): 197-IN2.

Nanninga, Gerrit B., et al. "Behavioural acclimation to cameras and observers in coral reef fishes." *Ethology* 123.10 (2017): 705-711.

Nedelec, S. L., Mills, S. C., Lecchini, D., Nedelec, B., Simpson, S. D., & Radford, A. N. (2016). Repeated exposure to noise increases tolerance in a coral reef fish. *Environmental pollution*, 216, 428-436.

Nedelec, Sophie L., et al. "Motorboat noise disrupts co-operative interspecific interactions." *Scientific reports* 7.1 (2017): 1-8.

Olivier, Damien, et al. "The cerato-mandibular ligament: a key functional trait for grazing in damselfishes (Pomacentridae)." *Frontiers in Zoology* 11.1 (2014): 1-14.

Pandolfi, J. M.; Jackson, J. B. C.; Baron, N.; Bradbury, R. H.; Guzman, H. M.; Hughes, T. P.; Kappel, C. V.; Micheli, F.; Ogden, J. C.; Possingham, H. P.; Sala, E. Are U.S. coral reefs on the slippery slope to slime? *Science*, 307: 1725–1726, 2005

Parmentier, Eric, et al. "Call properties and morphology of the sound-producing organ in *Ophidion rochei* (Ophidiidae)." *Journal of Experimental Biology* 213.18 (2010): 3230-3236.

Picciulin, Marta, et al. "In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a Marine Protected Area." *Journal of Experimental Marine Biology and Ecology* 386.1-2 (2010): 125-132.

Popper, Arthur N., and Anthony D. Hawkins. "An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes." *Journal of fish biology* 94.5 (2019): 692-713.

Popper, Arthur N. "Effects of anthropogenic sounds on fishes." *Fisheries* 28.10 (2003): 24-31.

Python Software Foundation (2022). Python Language Site: Documentation, 2020. Available in: <<https://www.python.org/doc/>>.

Radford, Craig A., et al. "Modelling a reef as an extended sound source increases the predicted range at which reef noise may be heard by fish larvae." *Marine Ecology Progress Series* 438 (2011): 167-174.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Sebastianutto, Linda, et al. "How boat noise affects an ecologically crucial behaviour: the case of territoriality in *Gobius cruentatus* (Gobiidae)." *Environmental biology of fishes* 92.2 (2011): 207-215.

Sertlek, H. Ozkan, Hans Slabbekoorn, and Michael A. Ainslie. "The contribution of shipping sound to the dutch underwater soundscape: Past, present, future." *Proceedings of Meetings on Acoustics* 5ENAF. Vol. 37. No. 1. Acoustical Society of America, 2019.

Simpson, Stephen D., et al. "Anthropogenic noise increases fish mortality by predation." *Nature communications* 7.1 (2016): 1-7.

Slabbekoorn, Hans, et al. "A noisy spring: the impact of globally rising underwater sound levels on fish." *Trends in ecology & evolution* 25.7 (2010): 419-427

Tyack, Peter L. "Implications for marine mammals of large-scale changes in the marine acoustic environment." *Journal of Mammalogy* 89.3 (2008): 549-558.

Valentin, J. L. "Analyse des paramètres hydrobiologiques dans la remontée de Cabo Frio (Brésil)." *Marine Biology* 82.3 (1984 a): 259-276.

Valentin, Jean L. "Spatial structure of the zooplankton community in the Cabo Frio region (Brazil) influenced by coastal upwelling." *Tropical Zooplankton*. Springer, Dordrecht, (1984 b). 183-199.

Vazzana, Mirella, et al. "Noise elicits hematological stress parameters in Mediterranean damselfish (*Chromis chromis*, perciformes): a mesocosm study." *Fish & shellfish immunology* 62 (2017): 147-152.

Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N., 2014. Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. *PLoS One* 9, e102946.

Weatherdon, Lauren V., et al. "Observed and projected impacts of climate change on marine fisheries, aquaculture, coastal tourism, and human health: an update." *Frontiers in Marine Science* (2016): 48.

Whitfield, A. K., and A. Becker. "Impacts of recreational motorboats on fishes: a review." *Marine pollution bulletin* 83.1 (2014): 24-31.

Worm, Boris, et al. "Impacts of biodiversity loss on ocean ecosystem services." *science* 314.5800 (2006): 787-790.

7. CONCLUSÕES GERAIS

Os dados apresentados no presente trabalho indicam que o ruído antropogênico gerado por embarcações de pequeno porte afeta negativamente o comportamento do peixe-donzel *Stegastes fuscus*. Quando submetidos ao som do playback, os indivíduos diminuíram significativamente suas taxas de forrageamento e aumentaram o seu tempo de refúgio. Ainda, as respostas ao tom (400Hz) foram similares, mas mais pronunciadas, às respostas do *playback*. Essa faixa de frequência foi escolhida por ser a faixa onde estão acontecendo as interações estudadas, havendo portanto uma sobreposição.

A espécie em estudo caracterizou-se como espécie bioindicadora para a poluição sonora de ruídos de baixa frequência, dada suas mudanças comportamentais frente à exposição ao ruído. Peixes recifais, assim como outros organismos marinhos, constituem um grupo importante de potenciais bioindicadores para esse tipo de impacto. Apesar disso, poucos trabalhos no Brasil utilizam espécies de peixes recifais como modelo ecológico para avaliar o impacto da poluição sonora.

A densidade e a frequência de embarcações é exponencialmente crescente dentro da RESEX, principalmente em épocas de alta temporada. Nossos resultados são importantes para auxiliar regras locais de manejo do tráfego de embarcações, além de auxiliar na elaboração de normativas marinhas.