# UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG INSTITUTO DE OCEANOGRAFIA PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

# ANÁLISE TEMPORAL DA ECOLOGIA DA PAISAGEM ACÚSTICA NA REGIÃO PORTUÁRIA DO ESTUÁRIO DA LAGOA DOS PATOS (BRASIL), COM ÊNFASE NO BOTO (Tursiops gephyreus) E NA MIRAGAIA (Pogonias courbina)

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Dissertação apresentada ao Programa de Pós-graduação em Oceanografia Biológica da Universidade Federal do Rio Grande – FURG, como requisito parcial à obtenção do título de MESTRE.

Orientador: Prof. Dr. Luciano Dalla Rosa

Coorientador: Pedro Friedrich Fruet

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#### ATA DE REUNIÃO, DE 13 DE ABRIL DE 2023

#### ATA 03/2023 - DEFESA DE DISSERTAÇÃO - Biól. ANDREA DE MOURA MILANELLI

Às treze horas e trinta minutos do dia 13 de abril do ano de dois mil e vinte e três, na Sala virtual Link: https://conferenciaweb.rnp.br/webconf/luciano-dalla-rosa-2, reuniu-se a Comissão Examinadora de Dissertação da Biól. ANDREA DE MOURA MILANELLI, composta pelos seguintes membros: Prof. Dr. Luciano Dalla Rosa - Orientador, Instituto de Oceanografia - FURG, Dr. Pedro Friedrich Fruet - Co-orientador - Museu Oceanográfico "Prof. Eliézer de C. Rios" - FURG, Prof. Dr. Alexandre Miranda Garcia, Instituto de Oceanografia - FURG, Prof. Dr. Stefan Cruz Weigert, Instituto de Oceanografia - FURG, Dra. Lis Bittencourt, Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro - UERJ. Título da Dissertação: "Análise temporal da ecologia da paisagem acústica na região portuária do Estuário da Lagoa Dos Patos (Brasil), com ênfase no boto (Tursiops gephyreus) e na miragaia (Pogonias courbina)". Dando início à reunião, o Coordenador em exercício do Programa de Pós-Graduação em Oceanografia Biológica - Prof. Dr. Luciano Dalla Rosa -agradeceu a presença de todos e fez a apresentação da Comissão Examinadora. Logo após, explicou que a candidata teria um tempo de 45 a 60 min. para explanação do tema, e cada membro da Comissão, um máximo de 30 min. para perguntas. Dando prosseguimento, passou a palavra à candidata que apresentou o tema e respondeu às perguntas formuladas. Após explanação, a Comissão aprovou o que segue: As sugestões de todos os membros da Comissão Examinadora, que seguem em pareceres em anexo, foram aceitas pelo orientador/candidata para incorporação na versão final. Foi atribuída a seguinte classificação à candidata: Prof. Dr. Alexandre Miranda Garcia - Classificação: Aprovada; Prof. Dr. Stefan Cruz Weigert - Classificação: Aprovada; Dra. Lis Bittencourt - Classificação: Aprovada. A candidata foi considerada APROVADA por UNANIMIDADE. Nada mais havendo a tratar, lavro a presente ata que após lida e aprovada, será assinada pela Comissão Examinadora, pela aluna e pela Coordenadora do PPGOB.



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<ul> <li>APÊNDICE 2 ACOUSTIC RECORDING OF THE SOUTHERN BLACK DRU (<i>POGONIAS COURBINA</i>) REPRODUCTIVE CHORUS IN THE PATE LAGOON ESTUARY, BRAZIL</li></ul>	<b>M</b> <b>OS</b> <b>73</b> <b>76</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>77</b> <b>78</b> <b>77</b> <b>77</b> <b>78</b> <b>77</b> <b>77</b> <b>78</b> <b>77</b> <b>78</b> <b>77</b> <b>78</b> <b>77</b> <b>79</b> <b>81</b> <b>83</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>87</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b>17</b> <b></b>

<b>APÊNDICE 3</b>	MATERIAL SUPLEMENTAR	
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#### RESUMO

O estuário da Lagoa dos Patos (ELP) é um ambiente chave no ciclo de vida de muitas espécies, com forte potencial de impacto por atividades humanas, e sua acústica não foi estudada até o momento. Portanto, este estudo visa caracterizar a paisagem acústica em um ponto específico da região e verificar sua variação temporal e sazonal, bem como verificar a influência da temperatura e salinidade nos níveis de pressão sonora (SPL) em três diferentes faixas de frequência ao longo do ano. Foram utilizados dois equipamentos autônomos para a coleta de dados instalados na Estação Naval do Rio Grande. As coletas abrangeram uma semana consecutiva por estação do ano e a análise dos arquivos foi feita manualmente no software Raven. As emissões sonoras do boto Tursiops gephyreus e dos peixes estiveram presentes em todo o período amostral, porém o pico de detecções ocorreu durante o verão e a primavera, respectivamente. Foi detectado o coro reprodutivo da miragaia (Pogonias courbina) durante a primavera, especialmente no período na noite, porém com um padrão diário no pico de emissão envolvendo o final da tarde e o crepúsculo vespertino. O ruído das embarcações foi detectado durante o ano inteiro, porém de forma mais intensa no verão. Modelos lineares generalizados (GLM) indicaram, para as três faixas de frequência do SPL, influência da estação do ano na composição da paisagem acústica do ELP com flutuações positivas para as estações mais quentes (verão > primavera > outono > inverno). A salinidade, por sua vez, apresentou uma relação levemente negativa com os valores de SPL nos três modelos, sugerindo uma diminuição nas frequências com o aumento da salinidade. O SPL de baixa frequência demonstrou ser o principal componente da paisagem acústica do ELP, e o modelo correspondente a este SPL foi o que teve a maior deviância explicada. Ao se comparar os resultados do SPL com as detecções manuais dos sons de baixa frequência (peixes e embarcações), sugere-se que as análises estão em concordância. O SPL de alta frequência apresentou algumas dissimilaridades com as detecções manuais dos sons de alta frequência (boto e embarcações) e teve o menor valor de deviância explicada no modelo com as covariáveis utilizadas. O presente estudo é pioneiro na região do ELP e demonstrou a utilidade de métodos acústicos para avaliação da ecologia local, além de alertar sobre a contribuição deste método para futuras medidas protetivas de espécies ameaçadas.

Palavras-chave: Monitoramento Acústico Passivo, Fatores Abióticos, Poluição Sonora, Sciaenidae, *Tursiops gephyreus* 

#### ABSTRACT

The Patos Lagoon estuary (PLE) is a key habitat in the life history of several species. The habitat presents high impact risk from the anthropogenic activity and its bioacoustics have not been previously assessed. This study aims to characterize the soundscape of a fixed point in the PLE habitat to investigate weekly and seasonal variation, besides evaluating the influence of temperature and salinity in the sound pressure levels (SPL) across three different frequencies. We deployed two autonomous recorders to collect data in the Naval Station of Rio Grande. We sampled for a week in each season and analyzed the archival data manually in Raven. The sounds produced by fish and the dolphin Tursiops gephyreus were present throughout the sampling period, with detections peaking in summer and spring. We found the reproductive chorus of the fish southern black drum (*Pogonias courbina*) in spring, mostly at night, exhibiting a diel pattern with peaks during late afternoon and dusk. We detected ship noise throughout the year with higher intensity during summer. Generalized Linear Models (GLM) suggested that season influenced the soundscape of the PLE across the three SPL frequencies, with positive variation in warmer seasons (summer > spring > fall > winter). Salinity, in its turn, showed a weak negative association with SPL values in the three models, suggesting that frequencies decrease as salinity increases. Low-frequency SPL was the main factor of the soundscape in the PLE, and the corresponding model had the highest explained variance. Our results agree between the SPL and manual detections of low-frequency sources (fish and vessels). High-frequency SPL presented discrepancies with the manual detection of high-frequency sources (dolphin and vessels) and was the model with the lowest explained variance. Our study is the first to characterize the soundscape of the Patos Lagoon estuary. Our findings have demonstrated the applicability of acoustic methods in evaluating the local ecology, besides shedding light to the contribution of this method to future protective measures of endangered species.

Keywords: Passive Acoustic Monitoring, Abiotic Factors, Sound Pollution, Sciaenidae, *Tursiops gephyreus* 

#### 1. INTRODUÇÃO

A habilidade de perceber, produzir e responder ao som no ambiente marinho é uma característica comum entre diversos animais que o habitam, incluindo invertebrados, peixes e cetáceos (Hatch et al., 2016). Dentre os invertebrados, os crustáceos produzem sons através de bolhas geradas pelas pinças a fim de atordoar a presa e defender o território (Vesluis et al., 2000) e sabe-se que camarões da família Penaeidae emitem sons de alta frequência com sua mandíbula durante o processo de alimentação (Smith & Tabrett 2013, Peixoto et al., 2020). Os peixes emitem sons de baixa frequência para atrair parceiros sexuais e para comunicação intraespecífica (Myrberg 1981) e sabe-se que, para a família Sciaenidae, a acústica também está vinculada à episódios de desova (Luczkovich et al., 2008, Tellechea et al., 2011a). Peixes da família Sciaenidae são os mais representativos em produção de som dentre os teleósteos (Chao 1986) e, de maneira geral, apresentam dois principais tipos de coro: perturbação, emitido por machos e fêmeas; e o vinculado à reprodução e desova, emitido somente por machos (Tellechea et al., 2010, Tellechea 2019). Os cetáceos, por sua vez, também possuem emissões sonoras com uma complexa variedade de tipos e funções. Os delfinídeos, por exemplo, modulam assobios, que são emissões tonais de banda estreita associadas às funções de interações sociais e coesão de grupo (Richardson et al., 1995, Janik e Slater 1998, Lammers et al., 2003). Além disso, emitem cliques de ecolocalização, associados à alimentação e navegação (Richardson et al., 1995, Lammers et al., 2003), bem como sons pulsados, referente à interação social (Blomqvist & Amundin 2004, Martin et al. 2019).

Essa interação bioacústica de diferentes espécies no espaço-tempo em um determinado ambiente compõe a comunidade acústica (Gasc et al., 2013) e seu arranjo é influenciado pela paisagem acústica (Forman & Godron 1986). O conceito de ecologia da paisagem acústica (EPA) é uma abordagem recente e integrativa dos componentes sonoros de um ambiente, ou seja, das fontes e características acústicas de todos os sons ambientais bióticos e abióticos presentes coletivamente em um determinado local e período (Pijanowski et al., 2011, International Organization for Standardization [ISO] 2017). A paisagem acústica é dividida em três componentes principais: geofonia, biofonia e antropofonia (Campbell et al., 2019). A geofonia engloba sons abióticos, como aqueles causados pela ação de ventos e ondas (Wilcock et al., 2014). A biofonia inclui sons bióticos, como vocalização de cetáceos (Au 1993, Bittencourt et al., 2016), coro de peixes

(Monczak et al., 2019a) e o estalar das pinças de crustáceos (Monczak et al., 2019b), enquanto a antropofonia é composta por sons antropogênicos como, por exemplo, o ruído gerado pelo tráfego de embarcações (Ward et al., 2019). A paisagem acústica, portanto, é altamente variável de acordo com as características físicas e biológicas de cada ambiente (Bittencourt et al., 2016, Campbell et al., 2019).

Considerando a relevância dos sons no ciclo de vida das espécies marinhas e os avanços tecnológicos e analíticos no campo de bioacústica, a EPA visa entender a dinâmica acústica dos ambientes, suas funcionalidades biológicas e como o som de origem antropogênica se relaciona com a biodiversidade (Haxel et al., 2013, Marley et al., 2016, Wang et al., 2019). Esta disciplina fornece resultados novos e promissores para a exploração da biodiversidade funcional (Sueur & Farina, 2015) e caracterização de ecossistemas marinhos (Harris et al., 2015). Estudos de EPA são possíveis graças ao desenvolvimento de equipamentos autônomos que podem registrar passivamente a atividade sonora do ambiente por longos períodos, podendo variar entre 15 dias (Campbell et al., 2019) a mais de 5 anos de amostragem (eg. Ward et al., 2019), a depender dos objetivos propostos. Estudos têm focado na caracterização geral dos ambientes, quantificando a contribuição específica de cada fonte sonora (Wang et al., 2019), avaliação do impacto da antropofonia na biofonia local (Haxel et al., 2013, Marley et al., 2016, Campbell et al., 2019, Ward et al., 2019); e variação sazonal da paisagem acústica (Bittencourt et al., 2016, Monczak et al., 2019b).

Essa variedade de dados permite abordar questões ecológicas e de conservação (Sueur et al., 2014), além de ser útil e passível de aplicação para programas que vislumbram coleta de dados à longo prazo. Estudos recentes têm utilizado os dados coletados pelo monitoramento acústico passivo, por exemplo, para colaborar com ações de gestão e monitoramento de peixes, visto que esta metodologia é capaz de definir os padrões espaço-temporal de diferentes espécies em um hábitat, bem como suas condições preferenciais de reprodução (di Iorio et al., 2020, Picciulin et al., 2020). Além disso, esta metodologia fornece dados de abundância relativa de diferentes espécies em um ambiente (Doser et al., 2021), incluindo peixes (Peck et al., 2021), cetáceos (Barlow et al., 2021, Kiehbadroudinezhad et al., 2021) e pinípedes (Amundin et al., 2022).

No entanto, estudos de EPA marinha no Brasil ainda são escassos e estão concentrados nas regiões sudeste e nordeste (Rossi-Santos 2015, Rossi-Santos & Oliveira

2016, Sánchez-Gendriz & Padovese 2016, Sánchez-Gendriz & Padovese 2017, Campbell et al., 2019, Rossi-Santos 2019, Bittencourt et al., 2020, Deconto et al., 2021). Estes estudos ilustram algumas das várias vertentes de análise da paisagem acústica, realizadas através de índices acústicos que sinalizam a biodiversidade de um ambiente como, por exemplo, a complexidade acústica (ACI) (Bittencourt et al., 2020), entropia média (Campbel et al., 2019) e nível de pressão sonora (SPL) (Sánchez-Gendriz & Padovese 2016), uma das métricas mais utilizadas para quantificar o som ambiente devido à sua simplicidade (Rychtáriková e Vermeir 2013, Martin et al., 2019). Para a região sul do Brasil, no entanto, não existem estudos que abordem a paisagem acústica, apenas um número reduzido de pesquisas sobre a bioacústica de determinadas espécies de cetáceos e peixes (e.g. Azevedo et al., 2007, Madureira & Costa 2010, Lima et al., 2020), e avaliação do impacto das embarcações em cetáceos (Pelegrini et al., 2020).

O estuário da Lagoa dos Patos (ELP), sul do Brasil, apresenta importância significativa no ciclo de vida de pelo menos três dos principais grupos de animais marinhos que contribuem para a biofonia de uma paisagem acústica (Putland et al., 2017): crustáceos, peixes e mamíferos marinhos. Este ambiente semi-fechado oferece abrigo, alimentação e/ou condições de crescimento para pelo menos 150 espécies de peixes e, dentre elas, exemplares da família Sciaenidae: corvina (Micropogonias furnieri), pescadinha-real (Macrodon atricauda), papa-terras (Menticirrhus americanus e M. littoralis), maria-luíza (Paralonchurus brasiliensis), pescada-olhuda (Cynoscion guatucupa), castanha (Umbrina canosai) e a miragaia (Pogonias courbina) (Fischer et al., 2011). Esta última, foi recentemente classificada como "Vulnerável" pela lista vermelha da IUCN (Haimovici et al., 2020). A miragaia é endêmica do Atlântico Sul e apresenta uma distribuição restrita que compreende o estado do Rio de Janeiro (Brasil), até o sul do Golfo San Matías (Argentina) (Menezes et al., 2003, Cousseau & Perrotta 2013, Azpelicueta et al., 2019). A principal ameaça à espécie é a sobrepesca, intensificada entre as décadas de 1950 e 1970 (Santos et al., 2016): no ELP, o estoque pesqueiro sofreu um forte declínio, não havendo registro da espécie na região nos anos de 2004, 2005, 2008, 2009 e 2010 (IBAMA, 2012).

O ELP também é um habitat essencial para a vida de uma espécie predador de topo, o boto (*Tursiops gephyreus* – Wickert et al., 2016). Espécie endêmica do Atlântico Sul Ocidental, este pequeno cetáceo ocorre apenas em águas costeiras entre a Patagônia,

Argentina (43°S) e o norte do Estado de Santa Catarina (27°S) no Brasil. Devido à reduzida abundância, fragmentação populacional, declínio histórico em algumas regiões de ocorrência e alta exposição a impactos antrópicos como consequência de sua distribuição estritamente costeira, esta espécie foi recentemente listada como EM PERIGO na lista Nacional de Espécies Ameaçadas de Extinção (Portaria MMA nº 148, 2022) e como VULNERÁVEL na lista vermelha da IUCN (Vermeulen et al., 2019).

O ELP e a região costeira adjacente são consideradas áreas de altíssima relevância para a conservação do boto, uma vez que abrigam a maior população registrada para a espécie (80-90 indivíduos – Fruet et al., 2015), atua como elo central de conectividade com populações adjacentes e concentra intensa atividade humana em um ambiente restrito (Fruet et al., 2014). A região é habitada pelos botos durante o ano inteiro, e as áreas preferencialmente ocupadas são a desembocadura do estuário e o primeiro quilômetro distante da costa adjacente em áreas próximas ao estuário (Di Tullio et al., 2015). A boca da barra do Rio Grande é caracterizada como uma área chave para alimentação da espécie (Di Tullio et al., 2015), cuja dieta é essencialmente piscívora e têm como destaque a corvina (*M. furnieri*), maria-luísa (*P. brasiliensis*) e o peixe-espada (*Trichiurus lepturus*) (Pinedo 1982, Secchi et al., 2016).

Além de ser uma área de grande importância ecológica, o ELP e áreas costeiras adjacentes também são muito importantes para o desenvolvimento socioeconômico das cidades do Rio Grande e de São José do Norte, uma vez que abriga a terceira maior estrutura portuária do país, o Porto do Rio Grande, e intensa atividade de pesca artesanal, industrial e semi industrial. Portanto, a região está exposta a uma série de ruídos antropogênicos que possivelmente variam na sua intensidade de acordo com a sazonalidade das atividades. Em relação às fontes contínuas de ruídos sonoros, destacase o tráfego diário de navios de grande porte, rebocadores e outras embarcações relacionadas às atividades portuárias (e.g., lanchas rápidas da Praticagem da Barra), assim como atividades ligadas ao cais do porto e tráfego de embarcações pesqueiras, intensificado nos meses de verão nas áreas estuarina e costeira adjacente (Di Tullio et al., 2015). Por outro lado, destacam-se também fontes poluidoras que não são contínuas, como as atividades de manutenção portuária (e.g., dragagens, manutenção e ampliação da infraestrutura) (Koehler & Asmus 2009) e a prática de turismo embarcado com uso de

jet-ski e pequenas embarcações rápidas durante o verão, as quais contribuem para o incremento das fontes de ruído na região.

Os efeitos da emissão destes ruídos sonoros sobre a biota marinha são relativamente bem estudados em algumas regiões, especialmente em relação a cetáceos costeiros (Nowacek et al., 2007, Bittencout et al., 2017, Middel & Verones 2017). Dentre os efeitos conhecidos, destaca-se que os ruídos sonoros no ambiente podem causar o mascaramento acústico e, consequentemente, variações nos parâmetros de emissões sonoras dos animais. Para o gênero *Tursiops*, por exemplo, já foram reportados modificações significativas na modulação da amplitude do assobio (Kragh et al., 2019) e o aumento na produção (Buckstaff et al., 2004) e frequência do assobio (Gospić & Picciulin 2016). Apesar de menos estudados, os peixes também são impactados pelo ruído antropogênico em diferentes estágios ao longo da vida, incluindo período de desova, cuidado parental, desenvolvimento e crescimento (Jong et al., 2020). A miragaia, por exemplo, diminui sua taxa de emissão sonora no período reprodutivo com a presença de embarcações (Ceraulo et al., 2021).

Para aumentar a compreensão de como as paisagens sonoras impactam a ecologia do habitat, é necessário realizar previamente a caracterização dos padrões acústicos no espaço e no tempo (Lillis et al., 2018). O presente estudo propõe caracterizar as emissões sonoras que compõem a região portuária do ELP, especificamente ao redor da Marinha do Brasil, e como elas variam temporalmente. Os objetivos específicos incluem: (1) determinar o padrão temporal do SPL em diferentes faixas de frequência nas quatro estações do ano, e avaliar como certos fatores (mês, período do dia, salinidade e temperatura) podem influenciar a composição da paisagem acústica; (2) identificar as fontes sonoras dominantes, bem como avaliar seu padrão sazonal e diário; (3) catalogar o ruído dos principais tipos de embarcações que utilizam o ELP para facilitar futuros monitoramentos de poluição sonora; (4) averiguar se a miragaia está utilizando o ELP para fins reprodutivos e estimar os horários preferenciais para a emissão do coro reprodutivo. Como até o momento não há estudos de paisagem acústica na região, esperase que os resultados deste trabalho gerem informações básicas importantes para várias áreas do conhecimento e que possam complementar pesquisas específicas em andamento e direcionar estudos futuros.

#### 2. MATERIAIS E MÉTODOS

#### 2.1 Área de estudo

A Lagoa dos Patos, localizada no Rio Grande do Sul, Brasil, apresenta uma área de 10.360 km<sup>2</sup> e inclui uma região estuarina que ocupa cerca de 10% deste total (Asmus 1998) (Fig.1). O estuário é caracterizado por baías costeiras com profundidade geralmente inferiores a 2 metros, e por um canal artificial com aproximadamente 20 metros de profundidade utilizado para a navegação (Asmus 1998).

A comunicação entre esta região estuarina e a área marinha adjacente é dada por um canal único, delimitado e mantido artificialmente pelos molhes da Barra do Rio Grande, que apresenta 4 km de extensão (Chao et al., 1985). As marés e a salinidade são controladas pelo vento, principal fator para a entrada e saída de massas d'água (Garcia 1998). Durante a predominância de ventos NE ocorre o aporte de água doce e, portanto, o estuário encontra-se com uma salinidade mais baixa. Em contrapartida, em situações de predominância de ventos SE, ocorre uma intensa salinização (Costa et al., 1988).

#### 2.2 Coleta de dados

#### 2.2.1 Dados acústicos e ambientais no ELP

Para a coleta de dados acústicos, foram utilizados dois equipamentos autônomos, um gravador digital modelo SoundTrap ST300 HF (*Ocean Instruments NZ*, resposta de frequência 0.01 - 512 kHz e calibração '*end-to-end*' 176.2 dB re:1 µPa V<sup>-1</sup>) e um amostrador modelo F-POD (*Chelonia Ltd. UK*), instalados na Estação Naval do Rio Grande (ENRG) (Fig.1). Os equipamentos foram fundeados a aproximadamente 6 metros de profundidade, fixados a um cabo através de abraçadeiras de nylon 8mm (Fig. 2). As coletas foram programadas para abranger as quatro estações do ano entre setembro de 2020 a agosto de 2021, e as gravações ocorreram continuamente ao longo de uma semana (7 dias) por estação. A determinação do período amostral concentrou-se em selecionar os meses que compreendessem o meio de cada estação: novembro (primavera), fevereiro (verão), maio (outono) e agosto (inverno).

Os dados coletados pelo SoundTrap foram gerados de forma contínua (24 horas), divididos automaticamente em arquivos de 10 minutos sequenciais, com taxa de amostragem de 48kHz. O F-POD foi configurado para uma taxa amostral de 160 kHz e resolução de 2 milissegundos. Os dados ambientais de temperatura e salinidade foram coletados simultaneamente, de forma que no mesmo cabo e na mesma profundidade, estava instalado um CT (*SeaBird SBE 37-SM*) programado para registrar os dados a cada 15 minutos (Fig. 2).



Figura 1. Mapa da área de estudo no estuário da Lagoa dos Patos (ELP). Sinalizado em vermelho encontrase o ponto de coleta na Estação Naval do Rio Grande (ENRG).



Figura 2. Processo de instalação dos equipamentos (1: F-POD, 2: SoundTrap, 3: CT) para coleta de dados acústicos e ambientais na Estação Naval do Rio Grande (ENRG).

#### 2.2.2 Catálogo acústico das embarcações

A fim de iniciar a caracterização da antropofonia do ELP e facilitar a análise de dados da composição da paisagem acústica, foi criado um catálogo dos sons produzidos pelas embarcações que transitavam regularmente em frente ao local de instalação do SoundTrap (Material Suplementar). Este catálogo foi criado durante três saídas de campo realizadas especificamente para este fim, entre outubro e dezembro de 2021. Enquanto os dados acústicos eram coletados pelo SoundTrap, informações sobre as embarcações que trafegavam em frente ao local de instalação eram armazenadas: registro fotográfico, horário e distância do equipamento. Os materiais de coleta foram, respectivamente, uma câmera NIKON D300 com lente sigma 300mm fixa, relógio Kalenji W200 sincronizado com o SoundTrap, e uma trena a laser Bushnell Bone Collector – 6x24mm (Fig. 3).



Figura 3. Coleta de dados para o catálogo acústico das embarcações presentes no estuário da Lagoa dos Patos.

As informações técnicas das embarcações, como comprimento de fora a fora e capacidade do motor foram obtidas após a análise das fotos tiradas em campo. O Diagnóstico do Meio Socioeconômico e Etnoecológico (Golçalves, 2021) fornece dados específicos para cada frota pesqueira, e o portal *Marine Traffic* para embarcações de grande porte. A classificação das embarcações em pesca "industrial", "semi-industrial" e "artesanal" foi realizada a partir da verificação do material do casco, presença ou ausência

de casaria e do rastreador exigido pelo Programa Nacional de Rastreamento de Embarcações Pesqueiras por Satélite (PREPS) (Golçalves 2021).

#### 2.2.3 Registro acústico da miragaia (Pogonias courbina) em cativeiro

Com o objetivo de identificar a vocalização da miragaia no ELP, foram realizados esforços para o registro acústico da espécie em cativeiro na Estação Marinha de Aquicultura da Universidade Federal do Rio Grande (EMA-FURG), situada na Praia do Cassino, Rio Grande, RS. Foi realizada uma coleta no período reprodutivo da espécie (Machi et al., 2002), em novembro de 2021, que ocorreu intermitentemente ao longo de 7 dias. A gravação foi realizada pelo gravador autônomo SoundTrap ST300 HF, que estava acoplado a uma poita e presa na lateral do tanque (Fig. 4) com as mesmas configurações da coleta na ENRG.



Figura 4. Tanque de cultivo da miragaia (*Pogonias courbina*) na Estação Marinha de Aquacultura da Universidade Federal do Rio Grande (EMA – FURG) com o SoundTrap ST300HF instalada para a coleta de dados acústicos.

Foram gravados simultaneamente 14 indivíduos, mantidos em um tanque de cultivo de 16m<sup>3</sup> que continha uma bomba de aeração *Jacuzzi 1c 1LQ-M*, salinidade média

de 32,6  $\pm$  1,3 psu e temperatura média de 24,8  $\pm$  1,1°C. O comprimento médio dos indivíduos era de 51,5  $\pm$  7,5 cm, sendo ao menos 8 fêmeas e 4 machos.

Esta etapa da coleta de dados foi uma parceria com o Projeto Miragaia da FURG, em que os animais já estavam sendo cultivados em condições pré-determinadas para reprodução aprovadas pelo Comitê de Ética no Uso dos Animais (CEUA). Foi uma coleta oportunista e, portanto, nenhuma requisição extra foi realizada para o registro acústico da espécie.

#### 2.3 Análise de dados

#### 2.3.1 Identificação e quantificação das fontes sonoras

Os arquivos de áudio (.wav) gerados pelo SoundTrap foram analisados manualmente através do *software* Raven Pro 1.5 (*Cornell Laboratory of Ornithology, NY, USA*) com FFT de 1024 pontos e sobreposição de 75% (Rice et al., 2017). A contagem de cliques foi realizada através do *software* F-POD.exe (*Chelonia Inc., version 1.0*). Para cada áudio de 10 minutos, a quantificação dos sons biológicos foi baseada em diferentes categorias de acordo com o grupo taxonômico e o tipo de vocalização. Para análise de peixes, foram criadas 6 categorias (adaptado de Tellechea et al., 2011b) de acordo com o número de pulsos detectados (0 = sem detecção; 1 = 1 a 40; 2 = 41 a 100; 3 = 101 a 300; 4 = 301 a 500; 5 = > 500). As categorias para análise de botos variaram de acordo com o tipo de vocalização e foram definidas de acordo com o que melhor representava a frequência de emissão local: assobios (0 = sem detecção, 1 = 1 a 10; 2 = 11 a 40; 3 = 41 a 100; 4 > 100); sons pulsados (0 = sem detecção, 1 = 1 a 10; 2 = > 10); e cliques ( 0 = sem detecção, 1 = 1 a 100; 4 > 100). Os sons das embarcações foram contados individualmente.

#### 2.3.2 Nível de pressão sonora (SPL)

Os arquivos de áudio (.wav) foram separados de acordo com a estação do ano, e este conjunto de dados foi analisado através do PAMGuide (Merchant et al., 2015) no *software* MATLAB 7.11.0 (*The Mathworks, Natick, MA, USA*). O nível de pressão sonora (SPL) foi calculado para diferentes faixas de frequências baseadas nos parâmetros de frequências máxima e mínima dos assobios do boto *Tursiops gephyreurs* (Lima et al. 2020): banda larga (20 – 24000 Hz), baixa (20-1800Hz) e alta (1800-24000Hz) através

da análise "Broadband" (Merchant et al., 2015), configurado com janela Hann, sobreposição de 50% e 1 segundo de comprimento (Bittencourt et al., 2020). Os dados de calibração foram inseridos na configuração "end-to-end" de acordo com o fabricante. A análise do SPL de baixa frequência inclui somente pulsos de peixes (Bertucci et al., 2016) e ruído antropogênico (Monczak et al., 2019b), enquanto a de alta frequência abrange vocalizações do boto (Lima et al., 2020) e ruído antropogênico de alta frequência (Monczak et al., 2019b). A análise de banda larga, por sua vez, engloba todos os sons biológicos e antropogênicos.

#### 2.3.3 Catálogo acústico das embarcações

As análises foram realizadas através do *software* MATLAB 7.11.0 (*The Mathworks, Natick, MA, USA*) utilizando a rotina PAMGuide (Merchant et al., 2015). O espectrograma foi criado a partir da análise de Power Spectrum, configurado com janela Hann, sobreposição de 50% e a frequência em escala linear, enquanto o cálculo do SPL seguiu as configurações previamente utilizadas na análise da composição da paisagem acústica. A fim de uma melhor visualização do ruído focal, o áudio de 10 minutos gerado foi recortado para a hora exata da passagem da embarcação, portanto, o tempo de duração analisado não foi o mesmo entre as embarcações catalogadas.

#### 2.3.4 Parâmetros acústicos da miragaia (Pogonias courbina)

Para mensurar os pulsos sonoros emitidos pela miragaia em cativeiro e comparálos com os pulsos encontrados no ELP, foram analisados dois parâmetros acústicos comumente utilizados para identificação de peixes: duração e frequência dominante (Tellechea et al., 2011a, Tellechea & Norbis 2012, Tellechea 2019). A análise foi realizada através do *software* Raven Pro 1.5 (*Cornell Laboratory of Ornithology, NY, USA*), com FFT de 1024 pontos e sobreposição de 75% (Rice et al., 2017). Somente pulsos em que o início e o fim estavam visivelmente bem definidos foram analisados (Fig.5).



Figura 5. Espectrograma com pulsos sonoros da miragaia (*Pogonias courbina*) em cativeiro (A) e no estuário da Lagoa dos Patos (B) gerados pelo *software* Raven Pro 1.5. O coro reprodutivo está destacado em amarelo.

#### 2.3.5 Análise estatística

Modelos lineares generalizados (GLMs) (Monczak et al., 2019b) foram utilizados para relacionar os valores de SPL com variáveis ambientais e temporais. Foram ajustados modelos individuais para cada SPL (banda larga, baixa e alta) considerando temperatura e salinidade como covariáveis contínuas e período do dia e estação do ano como fatores. A variável resposta foi modelada seguindo uma distribuição Gamma com função de ligação logarítmica. Com base numa análise exploratória dos dados para identificação de potenciais *outliers* e outros problemas nos dados, a temperatura não foi incluída nos modelos por estar fortemente correlacionada com a estação do ano, evitando assim problemas com a colinearidade (Zuur et al., 2010). A construção do modelo foi realizada no *software* R e baseada na métrica do critério de informação de Akaike (AIC). Dentre os modelos gerados, optou-se por aqueles com o menor valor.

Para a análise comparativa dos SPL entre as estações do ano e dentro de uma mesma estação, bem como a taxa de emissão sonora de peixes e botos ao longo do dia e entre as estações do ano, foi utilizado o teste não-paramétrico de Kruskal-Wallis. Aos resultados que apresentaram diferença significativa (p < 0,05), foi aplicado o teste posthoc de Nemenyi para verificar os grupos diferentes entre si (Lyon et al. 2019).

Para a comparação entre os parâmetros acústicos da miragaia (duração e frequência dominante) em vida livre e em cativeiro foi utilizado o teste não-paramétrico Mann-Whitney.

#### **3.** SÍNTESE DOS RESULTADOS

#### **3.1** Apêndice 1 – Artigo intitulado "Temporal Patterns in the

Soundscape of the Patos Lagoon estuary, Brazil"

- Os peixes da família Sciaenidae tiveram a maior taxa de detecção na primavera e a menor no outono. Contudo, os picos de emissão sonora ao longo do dia variaram conforme as estações do ano. Na primavera e no outono as detecções foram constantes ao longo do dia e não apresentaram diferenças estatisticamente significativas entre os períodos (Kruskal-Wallis p > 0,05). No verão não foi possível analisar os períodos da tarde e do crepúsculo vespertino devido à alta poluição sonora proveniente da ENRG, cuja frequência mascarou os pulsos emitidos pelo grupo. No inverno, houve diferença significativa somente no período da tarde, que mostrou ser o período com a menor taxa de emissão sonora (Kruskal-Wallis e Nemenyi p < 0,05).</li>
- O boto *Tursiops gephyreus* foi detectado durante todas as estações do ano, porém a análise das emissões sonoras foi dividida nos três tipos de vocalização da espécie: assobios, *cliques* e sons pulsados. Os assobios tiveram maior detecção no verão e menor no inverno (Kruskal-Wallis e Nemenyi p < 0,05), e não houve diferença entre o outono e a primavera (Kruskal-Wallis p > 0,05). Já os picos de emissão sonora por período do dia variaram entre as estações. No verão e no inverno não houve diferenças estatisticamente significativas da taxa de emissão entre os períodos (Kruskal-Wallis p > 0,05). Já no outono, nota-se um padrão bem definido durante os períodos da noite e madrugada, em que os picos de emissão sonora são maiores quando comparados com os outros períodos (Kruskal-Wallis e Nemenyi p < 0,05). Na primavera, ressalta-</li>

se uma diferença significativa somente no período de crepúsculo vespertino (Kruskal-Wallis e Nemenyi p < 0,05), que apresentou a menor taxa de emissão. Os cliques foram encontrados majoritariamente no verão, seguido pelo outono, inverno e primavera (Kruskal-Wallis e Nemenyi p < 0,05). No entanto, para nenhuma das estações ao longo do ano foi possível definir um padrão nos picos de emissão sonora entre os períodos do dia (Kruskal-Wallis p > 0,05). O som pulsado, assim como as demais vocalizações do boto, também foi detectado principalmente durante o verão, seguido pelo outono (Kruskal-Wallis e Nemenyi p < 0,05). Os picos de emissão durante o verão, inverno e primavera não mostraram diferenças estatisticamente significativas entre os períodos do dia (Kruskal-Wallis p > 0,05). Durante o outono, foi observado um padrão bem definido nos períodos da noite e madrugada (Kruskal-Wallis e Nemenyi p < 0,05).

- O maior número de embarcações foi detectado durante o outono, seguido pelo verão (Kruskal-Wallis e Nemenyi p < 0,05). No inverno e na primavera as detecções foram menores e não apresentaram diferenças estatisticamente significativas entre elas. Com exceção da primavera, que não apresentou diferença significativa na concentração de embarcações por período do dia (Kruskal-Wallis p > 0,05), todas as estações apresentaram maior intensidade nos períodos da manhã e tarde (Kruskal-Wallis e Nemenyi p < 0,05).</li>
- As análises comparativas entre as estações ao longo do ano demonstraram que a primavera e o verão não apresentam diferenças estatisticamente significativas nos SPL de banda larga e de baixa frequência (Kruskal-Wallis p > 0,05), bem como foram os períodos de maior valor de ambos os SPL, seguido do outono e do inverno (Kruskal-Wallis e Nemenyi p < 0,05). O SPL de alta frequência, por sua vez, apresentou maior intensidade na primavera, seguido do verão, outono e inverno (Kruskal-Wallis e Nemenyi p < 0,05).</li>
- Os modelos selecionados para as três frequências de SPL incluíram as mesmas variáveis (estação do ano, período do dia e salinidade) e explicaram 57,6%, 67,4% e 69,1% da variância nos valores de SPL de alta, larga e baixa frequência, respectivamente. Nos três modelos, os valores de SPL foram significativamente maiores no verão, primavera e outono, nessa ordem, em

relação ao inverno. A salinidade, por sua vez, apresentou relação negativa com os valores de SPL nos três modelos, sugerindo uma diminuição dos valores de SPL com o aumento da salinidade. Com relação ao período do dia, os modelos também apresentaram diferenças significativas. O SPL de banda larga apresentou respostas significativas em todos os períodos em relação à manhã, porém com sinal negativo nos períodos da 'Madrugada' e 'Crepúsculo Matutino' e positivo nos demais. O SPL de baixa frequência apresentou valores positivos significativos para os períodos 'Tarde', 'Crepúsculo Vespertino' e 'Noite' em relação à manhã. O SPL de alta frequência, por sua vez, teve os períodos 'Noite', 'Madrugada' e 'Crepúsculo Matutino' com valores significativamente menores em relação ao período da manhã, e o período 'Tarde' com valores significativamente maiores. De maneira geral, o período 'Tarde' apresentou valores significativamente maiores que o período da manhã para os três SPLs.

# **3.2** Apêndice 2 – Artigo intitulado "Acoustic recording of the southern black drum (*Pogonias courbina*) reproductive chorus in the Patos Lagoon estuary, Brazil"

- Não houve diferença estatística entre os parâmetros acústicos (duração e frequência dominante) da miragaia, *Pogonias courbina*, coletados em campo e em cativeiro (Mann-Whitney p > 0,05). Esta similaridade indica que as emissões sonoras encontradas no ELP e na EMA foram emitidas pela mesma espécie.
- No ELP, a duração do pulso (ms) foi de 208 ± 31,9 e a frequência dominante (Hz) 116 ± 46,6. Na EMA, os valores foram 238,5 ± 37,4 e 149 ± 42,1, respectivamente.
- A detecção do coro reprodutivo da miragaia ocorreu principalmente no período da noite. No entanto, o intervalo de maior intensidade de emissão sonora ocorreu no final da tarde e crepúsculo vespertino, entre as 18 e 20 horas.

#### **3.3** Apêndice 3 – Material Suplementar

Foram registrados seis tipos de embarcações: lanchas de passeio e da praticagem da Barra, navio cargueiro, e barcos de pesca artesanal, semi industrial e industrial. Devido ao alto tráfego na região, as embarcações de pesca artesanal foram a categoria com maior número de registros e, consequentemente, a mais bem explorada em relação à estimativa de distância de captação sonora pelo SoundTrap. Os resultados sugerem que, para embarcações com essas características e nas condições físico-químicas da água no momento da gravação, o som seja captado até aproximadamente 300 metros. As outras embarcações presentes no catálogo tiveram sua assinatura acústica bem registrada e todas estavam dentro do raio de 300 metros.

#### 4. CONCLUSÕES PRINCIPAIS

Este é um estudo inédito abordando a paisagem acústica no estuário da Lagoa dos Patos e engloba o primeiro registro do coro reprodutivo da miragaia (*Pogonias courbina*) na região. Os resultados corroboram com os padrões temporais de uso de área e ecologia do boto *Tursiops gephyreus* previamente determinados na região, bem como o padrão de vocalização de peixes em outras localidades. Esta metodologia tem a vantagem da amostragem contínua inalcançável por métodos tradicionais e, dessa forma, este estudo mostra a extensa gama de possibilidades para a utilização do monitoramento acústico passivo na caracterização do habitat, bem como para ações de monitoramento e gestão de espécies ameaçadas.

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## **APÊNDICE 1**

## TEMPORAL PATTERNS IN THE SOUNDSCAPE IN THE PORT AREA OF THE PATOS LAGOON ESTUARY, BRAZIL

Milanelli AM, Rossi-Santos M, Fruet PF, Assumpção R, Cavalcanti AM, Dalla Rosa L

(Manuscrito submetido ao periódico Estuarine, Coastal and Shelf Science)

#### 1 TEMPORAL PATTERNS IN THE SOUNDSCAPE IN THE PORT AREA OF THE 2 PATOS LAGOON ESTUARY, BRAZIL

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25 Andrea Milanelli: conceptualization, methodology, data collection, data analysis, data 26 interpretation, statistical analysis, article writing, review and editing. Marcos Rossi-27 Santos: conceptualization, methodology, article review and editing. Pedro Friedrich 28 Fruet: conceptualization, resources, funding acquisitions, article review and editing. 29 Ricardo Assumpção: data collection, article review. Augusto Muniz Cavalcanti: data 30 collection, article review. Luciano Dalla Rosa: supervision, conceptualization, 31 resources, funding acquisition, data interpretation, statistical analysis, article review and 32 editing.

### ABSTRACT

34 Soundscape ecology allows, through environmental sound capture, the 35 determination of the area use of certain species, estimation of population abundance, 36 verification of the health of the habitat and assistance in management and monitoring 37 processes. The Patos Lagoon estuary (PLE) is a key environment in the life cycle of many 38 species, with strong potential for impact by human activities, and its acoustics have not 39 been studied thus far. This study aims to characterize the soundscape of the region and 40 verify its temporal and seasonal variation, and to verify the influence of temperature and 41 salinity on sound pressure levels (SPL) in three different frequency bands throughout the 42 year. Two autonomous devices were used for data collection, a SoundTrap digital 43 recorder (Ocean Instruments, NZ) and an F-POD sampler (Chelonia Ltd. UK). Data 44 collection occurred between September 2020 and August 2021 and covered one 45 consecutive week per season. The analysis of the files (.wav) was performed manually using Raven software. Sound emissions by the Lahille's bottlenose dolphin Tursiops 46 47 truncatus gephyreus and the pulses emitted by fish were present throughout the sampling period, but peak detections occurred during the summer and spring, respectively. The 48 49 boats were detected throughout the year but more intensely in the summer. Generalized 50 linear models (GLMs) indicated, for the three SPL frequency bands, the influence of the 51 season on the composition of the PLE soundscape with positive fluctuations for the 52 warmer seasons (summer > spring > autumn > winter). Salinity, in turn, showed a slightly 53 negative relationship with the SPL values in the three models, suggesting a decrease in 54 frequencies with increasing salinity. Our results also show that the low frequency SPL 55 was the main component of the ELP soundscape. In addition, the GLM for this SPL had 56 the highest  $R^2$  value and its outputs compared well with the manual detections of low-57 frequency sounds (fish and boats).

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## **GRAPHICAL ABSTRACT**



- 69 Key Words: Acoustic Measurement, Abiotic Factors, Underwater Noise, Bioacoustics,
- 70 Sciaenidae, Tursiops truncatus gephyreus

### 1. INTRODUCTION

73 The ability to perceive, produce and respond to sound in the marine environment 74 is a common feature among several species that inhabit it, including invertebrates, fish 75 and cetaceans (Hatch et al. 2016). Among invertebrates, crustaceans produce sounds 76 through bubbles generated by the pincers to stun the prey and defend the territory (Vesluis 77 et al. 2000). Also, shrimp of the Penaeidae family emit high frequency sounds with their 78 mandible during the feeding process (Smith & Tabrett 2013, Peixoto et al. 2020). Fish 79 emit low-frequency sounds to attract sexual partners and for intraspecific communication 80 (Myrberg 1981), and it is known that, for the family Sciaenidae, acoustics are also linked 81 to spawning episodes (Luczkovich et al. 2008, Tellechea et al. 2011a). Sciaenidae fish 82 are the most representative in sound production among teleosts (Chao 1986) and, in 83 general, have two main types of choruses: disturbance, emitted by males and females, and 84 advertisement, linked to reproduction and spawning, emitted only by males (Tellechea et 85 al. 2010, Tellechea 2019). Cetaceans, in turn, also have sound emissions with a complex 86 variety of types and functions. For example, delphinids modulate whistles, which are 87 tonal emissions associated with social interactions and group cohesion (Richardson et al. 88 1995, Janik & Slater 1998, Lammers et al. 2003). In addition, they emit echolocation 89 clicks associated with feeding and navigation (Richardson et al. 1995, Lammers et al. 90 2003), as well as burst pulse sounds, also related to social interactions (Blomqvist & 91 Amundin 2004, Martin et al. 2019).

92 This bioacoustic interaction in space-time composes the acoustic community 93 (Gasc et al. 2013), and its arrangement is influenced by the soundscape (Forman & 94 Godron 1986). Soundscape ecology is a recent and integrative approach to the sound 95 components of an environment, i.e., the acoustic sources and characteristics of all biotic 96 and abiotic environmental sounds collectively present in each location and period 97 (Pijanowski et al. 2011, International Organization for Standardization [ISO] 2017). The 98 soundscape is divided into three main components: geophony, biophony and 99 anthropophony (Pijanowski et al. 2011, Campbell et al. 2019). Geophony encompasses 100 abiotic sounds, such as those caused by the action of winds and waves (Wilcock et al. 101 2014). Biophony includes biotic sounds, such as cetacean vocalization (Au 1993, 102 Bittencourt et al. 2016), fish chorus (Monczak et al. 2019a) and the snapping of crustacean 103 pincers (Monczak et al. 2019b). Anthropophony is summarized by anthropogenic sounds, such as the noise generated by boat traffic (Ward et al. 2019). The soundscape is therefore
highly variable according to the physical and biological characteristics of each
environment (Bittencourt et al. 2016, Campbell et al. 2019).

107 Soundscape ecology studies are possible due to the development of autonomous 108 equipment that can passively record environmental sound activity for long periods, 109 ranging from 15 days (Campbell et al. 2019) to more than 5 years of sampling (Ward et 110 al. 2019) depending on the proposed objectives. This technique is known as passive 111 acoustic monitoring (PAM), and it allows the quantification of the specific contribution 112 of each sound source (Wang et al. 2019), the evaluation of the impact of anthropophony 113 on local biophony (Campbell et al. 2019, Ward et al. 2019, Ward et al. 2019), and the 114 verification of the seasonal variation in the soundscape (Monczak et al. 2019b). This 115 variety of information, in turn, makes it possible to address ecological and conservation 116 issues (Sueur et al. 2014), in addition to being useful and susceptible to application by 117 programs that envision long-term data collection.

118 The Patos Lagoon estuary (PLE), in southern Brazil, is of significant importance 119 in the life cycle of at least three of the main groups of marine animals that contribute to 120 the biophony of a soundscape (Putland et al. 2017): crustaceans, fish and marine 121 mammals. This semi enclosed environment is considered the largest natural breeding 122 ground for pink shrimp (Farfantepenaeus paulensis) (Valentini et al. 1991), in addition 123 to offering shelter, food and/or growth conditions for at least 150 fish species. Among 124 them stand out specimens of the Sciaenidae family: whitemouth croaker (Micropogonias 125 furnieri), southern king weakfish (Macrodon atricauda), southern kingcroacker 126 (Menticirrhus americanus), banded croaker (Paralonchurus brasiliensis), stripped 127 weakfish (Cynoscion guatucupa), Argentine croaker (Umbrina canosai) and southern 128 black drum (Pogonias courbina) (Fischer et al. 2011).

The PLE is also an essential habitat for a top predator species, the Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*). Endemic subspecies of the Western South Atlantic, this small cetacean occurs only in coastal waters between Patagonia, Argentina (43°S) and northern Santa Catarina State (27°S), Brazil. The dolphins inhabit the PLE region year-round and are found mainly in the estuary mouth and the first kilometer away from the adjacent coast in areas close to the estuary (Di Tullio et al. 2015). The estuary mouth is a key feeding area for the species (Di Tullio et al. 2015), whose diet 136 is essentially piscivorous and consists mainly of the whitemouth croaker (M. furnieri),

137 banded croaker (*P. brasiliensis*), southern kingcroacker (*M. americanas*), mullet (*Mugil* 

138 liza) and cutlassfish (Trichiurus lepturus) (Pinedo 1982, Secchi et al. 2016, Campos-

139 Rangel et al. 2021).

140 The PLE is also very important for the development and maintenance of the cities 141 of Rio Grande and São José do Norte, as it is home to the Port of Rio Grande and intense 142 artisanal and semi-industrial fishing activity. Therefore, the region is exposed to a series 143 of anthropogenic noises that possibly vary in intensity according to the seasonality of 144 activities. Regarding continuous sources of sound noise, stands out the daily traffic of 145 large ships, tugboats and other boats related to port activities (e.g., fast boats from the 146 port pilotage) as well as activities related to the port piers and the fishing boat traffic, the 147 latter intensified in the summer months in the estuarine and adjacent coastal areas (Di 148 Tullio et al. 2015). On the other hand, pollution sources that are not continuous are also 149 noteworthy, such as port maintenance activities (e.g., dredging, maintenance and 150 expansion of infrastructure) (Koehler & Asmus 2009) and the use of leisure craft boats 151 and jet ski during the summer, which contribute to the increase in noise sources in the 152 region.

The effects of these noise emissions on marine biota are relatively well studied in some regions, especially regarding coastal cetaceans (Nowacek et al. 2007, Bittencout et al. 2017, Middel & Verones 2017). Among the known effects, environmental noise can cause acoustic masking and, consequently, variations in the sound emission parameters of animals, a fact that has already been reported for cetaceans of the genus *Tursiops* (e.g., Buckstaff et al. 2004, Gospić & Picciulin 2016, Kragh et al. 2019) and fish (Jong et al. 2020, Ceraulo et al. 2021).

160 To increase the understanding of how soundscapes impact the ecology of the 161 habitat, it is necessary to first characterize the acoustic patterns in space and time (Lillis 162 et al. 2018). One of the main metrics used to quantify ambient sound is the sound pressure 163 level (SPL) (Martin et al. 2019), which is widely used due to its simplicity (Rychtáriková 164 and Vermeir 2013). Therefore, this study proposes to characterize the sound emissions 165 that compose the PLE and how they vary over time. The specific objectives are: (1) 166 determining the temporal pattern of the SPL in different frequency bands in the four 167 seasons of the year and evaluating how certain factors (month, time of day, salinity and

temperature) can influence the composition of the soundscape and (2) identifying thedominant sound sources and assessing their seasonal and daily patterns.

170

## 1712.MATERIALS AND METHODS

172

## 2.1 Study area

The Patos Lagoon, located in Rio Grande do Sul, Brazil, has an area of 10,360 km<sup>2</sup> and includes an estuarine region that occupies approximately 10% of this total (Asmus 1998) (Fig. 1). The estuary is characterized by coastal bays with depths generally less than 2 meters and by an artificial channel with approximately 20 meters depth used for navigation (Asmus 1998). The bottom sediment is composed of sandy-muddy banks and edges (Bemvenuti 1998).

179 The communication between this estuarine region and the adjacent marine area is 180 provided by a single artificial channel delimited and maintained by the jetties of Barra do Rio Grande, which is 4 km long (Chao et al. 1985). The salinity and tides are controlled 181 182 by the wind and continental discharges, the main factors for the entry and exit of water 183 bodies (Garcia 1998, Kjerfve et al. 2001, Seeliger and Odebrecht 2010). During the 184 predominance of NE winds, freshwater inflows occur, and therefore, the estuary has a lower salinity. In contrast, with the predominance of SE winds, intense salinization occurs 185 186 (Costa et al. 1998).

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188

## 2.2 Data collection

189

## 2.2.1 Acoustic and environmental data

190 For the collection of acoustic data, two autonomous equipment were used for data 191 collection: a digital recorder model SoundTrap ST300 HF (Ocean Instruments NZ, 192 frequency response 0.01 - 512 kHz and end-to-end calibration of 176.2 dB re: 1  $\mu$ Pa V<sup>-</sup> 193 <sup>1</sup>) and an F-POD model sampler (Chelonia Ltd. UK), installed at the Naval Station of Rio 194 Grande (NSRG) (Fig. 1). The rigs were anchored at a depth of approximately 6 meters 195 and fixed to a cable using 8 mm nylon cable ties. Recordings were made intermittently 196 over one week (7 days) in each season between September 2020 and August 2021. The 197 sampling period focused on selecting the months that comprised the middle of each 198 season: November (spring), February (summer), May (autumn) and August (winter).

The data collected by the SoundTrap were generated continuously (24 hours) and automatically divided into sequential 10-minute files in a frequency range of 48 kHz. The F-POD was configured in a frequency range of 160 kHz and resolution of 2 milliseconds. The temperature and salinity data were collected simultaneously by a CT (SeaBird SBE 37-SM) installed on the same cable and at the same depth, programmed to record data every 15 minutes (Fig. 1).



205

Figure 1. Map of the study area in the Patos Lagoon estuary (PLE). The data collection point at the Naval Station of Rio Grande is marked in black.

208

209 **2.3 Data analysis** 

## 210 2.3.1 Identification and quantification of sound sources

The clicks emitted by the dolphins were counted using the F-POD software (Chelonia Inc., version 1.0). The audio files (.wav) generated by the SoundTrap were manually analyzed using Raven Pro 1.5 software (Cornell Laboratory of Ornithology, NY, USA) with an FFT of 1024 points and 75% overlap (Rice et al. 2017). The manual biophony analysis included only fish and Lahille's bottlenose dolphin vocalizations, not including potential sound emissions from crustaceans present in the region. The audio considered valid for the quantification of biological sounds could not contain more than 5 minutes of noise, compromising the specific frequency range for each taxonomic group.

219 Therefore, the number of audios analyzed varied according to the group (Tab. 1).

220 Fish vocalizations detected during the analysis were grouped into the Sciaenidae 221 family without species identification (Tab. 1). Species such as whitemouth croaker 222 (Tellechea 2010), striped weakfish (Tellechea 2012), southern king weakfish (Tellechea 223 2019), Argentine croaker (Tellechea et al. 2017) and southern black drum (Macchi et al. 224 2003, Tellechea et al. 2011a) already have previous records of their sound emissions in 225 other regions. However, in our study, it was not possible to identify the emissions at the 226 species level due to the similarity between the sound pulses. And although the southern 227 black drum exhibits distinct pulse patterns during its reproductive period (Tellechea et al. 228 2011b), these were not analyzed separately in this study.

229

Table 1. Number of analyzed audios and percentage according to taxonomic group by season. The difference between the total n (1009) per station and the amount of audio analyzed for each group is due to the discarding of audio with anthropogenic noise in the respective frequency bands.

	Spring n=1009	Summer n=1009	Autumn n=1009	Winter n=1009
Sciaenidae spp.	631 (62.5%)	528 (52.3%)	821 (81.3%)	636 (63%)
T. t. gephyreus	930 (92.1%)	928 (91.9%)	924 (91.5%)	963 (95.4%)

233

234 For each audio, the quantification of biological sounds was based on different categories according to the taxonomic group and the type of vocalization. For fish 235 236 analysis, 6 categories were created (adapted from Tellechea et al. 2011a) according to the number of detected pulses (0 = no detection, 1 = 1 to 40, 2 = 41 to 100, 3 = 101 to 300, 4237 238 = 301 to 500, 5 = > 500). The categories for the dolphin analysis varied according to the 239 type of vocalization and were defined according to what best represented the frequency 240 of local emission: whistles (0 = no detection, 1 = 1 to 10; 2 = 11 to 40; 3 = 41 to 100; 4 > 100241 100); burst pulse sounds (0 = no detection, 1 = 1 to 10; 2 = > 10); and clicks (0 = no detection, 1 = 1 to 100, 2 = 101 to 500, 3 = 501 to 1000, 4 > 1000). The sounds of the 242 243 boats were counted individually.

To obtain the interval with the highest vocalization of the species, the time of day was classified into six periods according to the solar calendar of the city of Rio Grande, RS, Brazil: dawn, morning twilight, morning, afternoon, afternoon twilight and night (Tab. 2).

	Dawn	Morning twilight	Morning	Afternoon	Evening twilight	Night
Spring	0 – 3 a.m.	3 – 5 a.m.	5 – 12 a.m.	12 – 19 p.m.	19 – 20 p.m.	20 p.m. – 0 a.m.
Summer	0 - 4 a.m.	4 – 6 a.m.	6 – 12 a.m.	12 – 19 p.m.	19 – 20 p.m.	20 p.m. – 0 a.m.

7 - 12 a.m.

7 – 12 a.m.

12 – 17 p.m.

12 – 18 p.m.

17 – 18 p.m.

18 – 19 p.m.

Table 2. Periods of the day defined according to the solar calendar in Rio Grande, RS, Brazil.

250 251 Autumn

Winter

### 2.3.2 Sound pressure level (SPL)

5 – 7 a.m.

5 - 7 a.m.

0 - 5 a.m.

0 - 5 a.m.

252 Measurements of sound pressure level (SPL) in audio files (.wav) generated by 253 the SoundTrap were obtained using PAMGuide (Merchant et al. 2015) in MATLAB 254 7.11.0 software (The Mathworks, Natick, MA, USA). The sound pressure level (SPL) 255 was calculated for different frequency ranges based on the parameters of maximum and 256 minimum frequency of the whistles of the Lahille's bottlenose dolphin T. t. gephyreurs 257 (Lima et al. 2020): broadband (20 – 24000 Hz), low-frequency band (20- 1800 Hz) and high-frequency band (1800-24000 Hz) through "Broadband" analysis (Merchant et al. 258 259 2015), configured with a Hann window, 50% overlap and 1 second in length (Bittencourt 260 et al. 2020). Calibration data were entered according to the SoundTrap manufacturer. The 261 low-frequency SPL analysis included only fish pulses (Bertucci et al. 2016) and 262 anthropogenic noise (Monczak et al. 2019b), whereas the high-frequency analysis 263 included dolphin vocalizations (Lima et al. 2020) and high-frequency anthropogenic noise (Monczak et al. 2019b). Broadband analysis, in turn, encompassed all biological 264 265 and anthropogenic sounds.

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### 2.3.3 Statistical analyses

The non-parametric Kruskal–Wallis test was used for the comparative analysis of SPL between seasons and within the same season, as well as the sound emission rate of fish and Lahille's bottlenose dolphin throughout the day and between seasons. The posthoc Nemenyi test was applied to the results that showed a significant difference (p < 0.05) to verify the different groups from each other (Lyon et al. 2019).

273 Generalized linear models (GLMs) were used to relate SPL values to 274 environmental and temporal variables (e.g., Monczak et al. 2019b). Individual models

18 p.m. – 0 a.m.

19 p.m. – 0 a.m.

275 were fitted for each SPL (broadband, low and high) considering temperature and salinity 276 as continuous covariates and time of day and season of the year as factors. The response 277 variable was modeled following a Gamma distribution with a logarithmic link function. 278 Based on an exploratory analysis of the data to identify potential outliers and other 279 problems in the data, temperature was not included in the models because it was strongly 280 correlated with season, thus avoiding problems with collinearity (e.g., Zuur et al. 2010). 281 Models were fitted using R software and model selection was based on the lowest AIC 282 (Akaike 1973).

283

285

284 **3.** 

# 3. **RESULTS**

## **3.1** Identification and quantification of sound sources

A total of 4036 10-minute audios were analyzed, with each season containing 1009 files. Of all the audios manually analyzed, 1420 files (35%) were not viable in the analysis of biological sounds due to noise pollution from unknown sources. Of this partial, 34.2% was recorded in summer, 26.2% in winter, 26.6% in spring and 13.2% in autumn (Fig. S1). This noise was mostly of low frequency and is suspected to be from the NSRG. Boats were detected in 2004 audio files (49.6%), with 28.4% recorded in autumn, 25.9% in spring, 24.3% in summer and 21.4% in winter (Fig. S1).

- 293
- 294 295

### **3.2** Soundscape composition

3.2.1 Biophony

The biophony recorded to characterize the soundscape of the Patos Lagoon estuary in the NSRG region is represented in Figure S2 and included the vocalizations of fish from the Sciaenidae family and the Lahille's bottlenose dolphin *T. t. gephyreus*, whose sound emission analysis was divided into the three types of vocalizations of the species: whistles, clicks and burst pulse sounds.

Fish of the family Sciaenidae had the highest detection rate in spring (p < 0.05) and the lowest in autumn (p < 0.05), and there was no difference between winter and summer (p > 0.05) (Fig. 2). However, sound emission peaks throughout the day varied according to the seasons (Fig. 3). In spring and autumn, detections were constant throughout the day and showed no significant difference between periods (p > 0.05). In winter, a significant difference was found only in the afternoon, which proved to be the 307 period with the lowest sound emission rate (p < 0.05). In the summer, this comparison 308 was not possible due to the high noise pollution from the NSRG, whose frequency masked 309 the pulses emitted by the group (data in gray - Fig. 3) in the afternoon and evening 310 twilight.





313 Figure 2. Boxplots for comparison of the distribution of the sounds emitted by Lahille's bottlenose dolphin 314 T. t. gephyreus (A - whistle, B - click, and C -burst pulse sounds), Sciaenidae fish (D) and the number of boat detections (E) in the Patos Lagoon estuary during the four seasons of the year (x-axis). Biological 315 316 sounds were quantified in the categories (y-axis) specific to each group. Fish: 0 (no detection), 1 (1 to 40), 317 2 (41 to 100), 3 (101 to 300), 4 (301 to 500) and 5 (>500). Whistles: 0 (no detection), 1 (1 to 10), 2 (11 to 318 40), 3 (41 to 100), 4 (> 100). Burst pulse: 0 (no detection), 1 (1 to 10), 2 (> 10). Clicks: 0 (no detection), 1 319 (1 to 100), 2 (101 to 500), 3 (501 to 1000), 4 (> 1000). Boat noise was quantified by the sum per hour of 320 day. Different letters indicate a significant difference (p < 0.05) between the frequency of sound emission 321 in the different seasons throughout the year.



Figure 3. Distribution of vocalization detections of Sciaenidae fish by season quantified by the average of the categories of audio analyzed per hour (0: no detection, 1: 1 to 40, 2: 41 to 100, 3: 101 to 300, 4: 301 to 500 and 5: > 500) over the sampled days. The data represented in gray were not analyzed due to masking caused by anthropogenic noise. Note the difference in scales between the images.

328

329 The Lahille's bottlenose dolphin was detected during all seasons of the year at 330 the study site. Among the types of sound emissions used by the species during this study, 331 whistles were the most common (47% of detections), followed by clicks (38%) and burst 332 pulse sounds (15%). The whistles had higher detection in summer and lower detection in winter (p < 0.05), and there was no difference between autumn and spring (p > 0.05) (Fig. 333 334 2). The peaks of whistle emission by time of day varied between seasons (Fig. 4). In 335 summer and winter, there were no significant differences in the emission rate between the 336 periods (p > 0.05). In autumn, there was a well-defined pattern during the night and dawn 337 periods, in which the sound emission peaks were higher than those in the other periods (p 338 < 0.05). In spring, a significant difference was observed only in the afternoon twilight 339 period, which presented the lowest emission rate (p < 0.05) (Fig. 4).



341

Figure 4. Distribution of the whistle detection of the Lahille's bottlenose dolphin *T. t. gephyreus* by season quantified by the average of the categories of audio analyzed per hour (0: no detection, 1: 1 to 10, 2: 11 to 40) along the sampled days. The data represented in gray were not analyzed due to masking caused by anthropic noise. Note the different scales between the images.

347 Clicks were found mostly in summer, followed by autumn, winter and spring 348 (Fig. 2). However, it was not possible to define a pattern in sound emission peaks for 349 clicks among the periods of the day for any of the seasons (p > 0.05) (Fig. 5). The burst 350 pulse sounds were also detected mainly during the summer, followed by autumn (Fig. 2). 351 The burst pulse sounds emission peaks during the summer, winter and spring did not show significant differences between the periods of the day (p > 0.05), whereas during the 352 353 autumn, there was a well-defined pattern in the periods of the night and dawn (p < 0.05) 354 (Fig. 6).





Figure 5. Distribution of click detections of the Lahille's bottlenose dolphin *T. t. gephyreus* by season quantified by the average of the audio categories analyzed per hour (0: no detection, 1: 1 to 100, 2: 101 to 500, 3: 500 to 1000, and 4: >1000) over the sampled days. Note the different scales between the images.



361

Figure 6. Distribution of burst pulse sounds detections of the Lahille's bottlenose dolphin *T. t. gephyreus* by season quantified by the average of the audio categories analyzed per hour (0: no detection, 1: 1 to 10, 2: >10) along the sampled days. The data represented in gray were not analyzed due to masking caused by anthropogenic noise. Note the different scales between the images.

366

367 *3.2.2 Anthropophony* 

368 The largest number of boats was detected during the autumn, followed by the 369 summer. In winter and spring, the detections were lower and there was no significant 370 difference between them (Fig. 2). Except for spring, which showed no significant 371 difference in the concentration of boats by time of day (p > 0.05), all seasons showed





373

Figure 7. Distribution of boat detections by season quantified by the number of boats per hour over the sampled days. Note the different scales between the images.

376

377

## **3.3** Temporal pattern of sound pressure level (SPL)

378 The comparative analyses between the seasons of the year showed that the 379 broadband and low-frequency SPLs did not differ significantly between spring and 380 summer, which are the periods with the highest values of both SPLs, followed by autumn 381 and winter (Fig. 8). The high-frequency SPL, in turn, showed greater intensity in spring, 382 followed by summer, autumn and winter (p < 0.05). When comparing SPLs within each 383 season, the broadband and low-frequency SPLs did not differ significantly at any period 384 of the year. In contrast, the high-frequency SPL differed in all seasons (Fig. 8). Figure S3 385 shows the variability of the SPL values within each season of the year. The significant 386 number of outliers is noteworthy, especially in the high-frequency SPL during spring and 387 summer.



389

Figure 8. Temporal composition of the soundscape in the Patos Lagoon estuary, quantified by the average sound pressure levels (SPL) of broadband (20 to 24000 Hz), low (20 to 1800 Hz) and high (1800 to 24000 Hz) frequencies with the standard errors. Different capital letters indicate significant differences (p < 0.05) between the SPLs in the different seasons throughout the year. Different lowercase letters indicate a significant difference (p < 0.05) between the SPLs at the same station. The mean values of salinity and water temperature during the sampling period (y-2 axis) were also plotted.

### 397

### **3.4** Influence of covariates on SPL frequency values

398 The models selected for the three SPL frequencies included the same variables 399 (season of the year, time of day and salinity) and explained 57.6%, 67.4% and 69.1% of 400 the variance in the high frequency, broadband and low frequency SPL values, respectively 401 (Tab. 3). In the three models, the SPL values were significantly higher in the summer, 402 spring and autumn, in that order, compared to the winter (Tab. 3), confirming the results 403 evidenced in the previous section (Fig. S3) of an increase in the values of SPL in the 404 warmer seasons. Salinity, in turn, showed a negative relationship with SPL values in the 405 three models, suggesting a decrease in SPL values with increasing salinity (Table 3).

Regarding the time of day, the models also showed significant differences. The broadband SPL showed significant responses in all periods compared to the 'Morning' but with a negative sign in the periods of 'Dawn' and 'Twilight Morning' and a positive sign in the other periods. The low-frequency SPL showed significant positive values for the periods 'Afternoon', 'Twilight' and 'Night' in relation to the 'Morning'. The highfrequency SPL, in turn, had significantly lower values for the 'Night', 'Dawn' and 'Twilight' periods compared to the morning period, and the 'Afternoon' period had

- 413 significantly higher values. In general, the 'Afternoon' period showed significantly higher
- 414 values than the 'Morning' period for the three SPLs.
- 415

Table 3. Results of the GLMs for the SPL in the three frequency bands (low – 20 to 1800 Hz, high – 1800 to 24000 Hz, and broadband 20 to 24000 Hz).

SPL"frequency" ~ season + time of day + salinity, Family= Gamma (link = "log")								
	Low SPL		Broadband SPL		High SPL			
	Estimate	p-value	Estimate	p-value	Estimate	p-value		
Intercept	4,6444	< 2e-16	4,6569	< 2e-16	4,5868	< 2e-16		
Season: Autumn	0,1129	< 2e-16	0,1116	< 2e-16	0,1291	< 2e-16		
Season: Spring	0,1500	< 2e-16	0,1449	< 2e-16	0,1394	< 2e-16		
Season: Summer	0,1801	< 2e-16	0,1744	< 2e-16	0,1496	< 2e-16		
Period: Dawn	-0,0035	0,103	-0,0078	0,0003	-0,0359	< 2e-16		
Period: Early Twilight	-0,0038	0,169	-0,0065	0,0193	-0,0261	< 2e-16		
Period: Afternoon	0,0284	< 2e-16	0,0258	< 2e-16	0,0078	0,000442		
Period: Evening Twilight	0,0358	< 2e-16	0,0322	< 2e-16	0,0005	0,8964		
Period: Night	0,0125	4,04e-09	0,0094	9,20e-06	-0,0218	< 2e-16		
Salinity	-0,0011	< 2e-16	-0,0011	< 2e-16	-0,0017	< 2e-16		
Deviation explained	69,08%		67,40%		57,62%			

## 4. **DISCUSSION**

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# 4.1 Temporal composition of the soundscape

The temporal composition of biological and anthropogenic sounds in the PLE exemplifies the complexity of the region, which is characteristic of estuarine soundscapes (Monczak et al. 2019b). The PLE is a region with a strong seasonal influence of the wind regime (Costa et al. 1998, Garcia 1998) and continental discharges (Kjerfve et al. 2001) that determine conditioning factors (e.g., salinity level) for the dynamics of the marine and estuarine biota (Seeliger and Odebrecht 2010), including fish and dolphins.

The distribution of sound pulses emitted by fish of the Sciaenidae family in the PLE throughout the year showed a greater amount of emissions in spring, followed by summer and winter. These periods coincide with the reproductive period of the five species cited above: whitemouth croaker (spring and summer) (Acha et al. 1999, Macchi et al. 2003), southern king weakfish (spring and summer) (Vizziano & Berois 1990), Argentine croaker (winter and spring) (Haimovici & Cousin 1989), stripped weakfish (peak in early autumn and spring) (Vieira & Haimovici 1997) and southern black drum
(spring) (Tellechea et al. 2011a). Spring, therefore, is an integral (or at least partial) part
of the reproductive season of these species, justifying the high incidence of vocalizations
in this period.

437 These fish species are estuarine-opportunistic (southern king weakfish, Argentine 438 croaker and stripped weakfish) and estuarine-dependent (southern black drum and 439 whitemouth croaker) and, therefore, do not usually use the estuarine region for spawning 440 events (Fischer et al. 2011). However, some factors corroborate the eventual occurrence 441 of these species in the PLE during the reproductive period. For example, in September 442 2020 (beginning of data collection), the La Niña phenomenon was underway in the 443 equatorial Pacific Ocean, which is associated with periods of drought in southern Brazil 444 (Grimm et al. 1998). During the predominance of dry periods, the entry of salt water into 445 the estuary is more intense and favors the entry of marine species into the region, 446 especially those considered estuarine-dependent (Garcia et al. 2001). Strong evidence is 447 demonstrated by the occurrence of reproductive aggregations of the southern black drum 448 in the PLE as reported from incidental captures (Santos et al. 2016).

449 The conditions during La Niña periods also influence the use of the estuarine 450 region by the Lahille's bottlenose dolphin, whose distribution is related to the distribution 451 of prey and, consequently, to environmental factors such as salinity and temperature (Di 452 Tullio et al. 2015). Click emissions by this species are strongly correlated with foraging 453 activities (Richardson et al. 1995, Lammers et al. 2003), and in the present study, their 454 peak emission throughout the year coincided with the highest salinity values (Figures 2 455 and 8). This result corroborates previous studies in the area showing that salinity is 456 positively correlated with prey availability (Di Tullio et al. 2015) and, consequently, 457 affects dolphin distribution. The cutlassfish (T. lepturus), one of the main prey items of 458 the Lahille's bottlenose dolphin and whose relative importance in the diet has increased 459 in recent years (Secchi et al. 2016), has its density in estuaries strongly related to the 460 entrance of water from the subtropical continental shelf (Pereira 1989, Lima et al. 1996) 461 because it is an opportunistic estuarine marine species (Fischer 2011). On the other hand, 462 this study detected a contrast between click emissions by the Lahille's bottlenose dolphin 463 and fish pulses during spring, a period that represented the lowest and highest emission 464 rates per group, respectively. A similar result was found in a soundscape study involving

465 *Tursiops truncatus* in the USA (Marian et al. 2020), which seems to reinforce the 466 hypothesis that bottlenose dolphins also use passive listening to detect their prey (McCabe 467 et al. 2010).

468 The three types of sounds emitted by the Lahille's bottlenose dolphin were 469 detected mostly in the summer, a period that represents the peak of births of the resident 470 population in the PLE. Studies conducted with dolphin populations in the wild and in 471 captivity show that whistles and burst pulses sounds play an important role in social 472 functions, especially the increase in emission rates in the reproductive period. Whistles 473 are emitted by mothers before birth and during the first months of the pups' life (Mann & 474 Smuts 1998, Mello & Amundin 2005, Fripp & Tyack 2008, Ames et al. 2019), while 475 burst pulses sounds have their emission rate influenced by group composition, being 476 higher between males and females than between males and males (Lopez 2012, Lopez 477 2022). The Lahille's bottlenose dolphin population residing in the PLE is predominantly 478 composed of adult females (2 females: 1 male), which find predictable and easily 479 accessible protection and food in this environment, helping to meet the energy 480 requirements of lactating females (Fruet et al. 2015). This could explain the greater 481 detection of these sound types in the PLE during the summer, as well as the high rate of 482 whistle emission in the spring (when starts the reproductive season of the Lahille's 483 bottlenose dolphin in the PLE). Additionally, it is noteworthy that although the burst pulse 484 sounds occurred more intensely in the summer, this type of vocalization was the least 485 detected, contrary to what was found in other regions (Herman and Tayolga 1980, 486 Herzing 2000, Monczak et al. 2019b, Marian et al. 2021, Lopez 2022).

487 Regarding anthropophony, we found that the periods of greatest intensity of boat traffic occurred during the autumn and summer, especially in the morning and afternoon, 488 489 coinciding with the reproductive period of the whitemouth croaker (M. furnieri) and the southern king weakfish (M. atricauda). The impacts of anthropogenic noise on biota are 490 491 widely discussed in the aquatic environment, both for fish (Smott et al. 2018, Jong et al. 492 2020, Ceraulo et al. 2021) and for cetaceans (Bittencourt et al. 2017, Middel & Verones 493 2017, Kragh et al. 2019, Rossi-Santos 2019). For PLE dependent fish species, evaluation 494 of the dimensions of this impact will depend on future studies, as there are many gaps in 495 the bioacoustics research of this group in the region. In this context and considering the 496 superposition of anthropic noise with the sound pulses emitted by fish during the

497 reproductive period, there is a need to evaluate the impact of noise pollution on fish498 species in the PLE.

499 The use of passive acoustic monitoring (PAM) can help in the detection of 500 preferred areas and periods for fish reproduction and, consequently, serve as a tool for the 501 conservation of the group (Rowell et al. 2020). Considering that the PLE is an area of 502 intense fishing activity, the Ministry of Agriculture, Livestock and Food Supply 503 determined, based on the MMA/SEAP Joint Normative Instruction, specific fishing 504 periods for each commercially valuable species: mullet, whitemouth croaker, white sea 505 catfish (Genidens barbus) and pink shrimp (Farfantepenaeus paulensis) (INC 2004). 506 However, illegal fishing events are very recurrent even after the determination of more 507 effective methods of inspection in the PLE (Costa et al. 2018), indicating a notorious flaw 508 in the monitoring system. Considering the financial difficulty and the difficulty of 509 covering the entire region by traditional methods, the PAM becomes an alternative to 510 assist inspection because it can facilitate the detection of areas of great importance in the 511 species cycle, as well as define its seasonal and daily distribution pattern. PAM is a 512 technique that requires less effort, has a lower cost in the medium to long term, and can 513 cover a larger area during all periods of the day, depending on the number and distribution 514 of equipment.

515 For the population of Lahille's bottlenose dolphins residing in the PLE, as for the 516 fish, there is no focal study on the impact of anthropogenic noise on individuals in the 517 region. In this study, the daily pattern of burst pulse sound emissions and whistles (night 518 and dawn) in autumn contrasted with the pattern of anthropogenic noise (morning and 519 afternoon). However, in summer, winter and spring, the periods of Lahille's bottlenose 520 dolphin sound emission and anthropogenic noise overlapped. Despite the contrast found 521 in autumn, the sound emission pattern of these vocalizations is concentrated at night and 522 dawn, even in less noisy places (Marian et al. 2020, Gauger et al. 2022). However, the 523 high whistle rate in summer and spring parallel to the overlap with anthropic noise 524 throughout the day may indicate a disturbance in the vocalization rate (Buckstaff 2004).

525 The threat of noise pollution also involves behavioral changes that can harm the 526 survival of the species. For example, changes were detected in the feeding, resting and 527 socialization behavior of a population of *Tursiops truncatus* in Turkey in response to the 528 presence of boats in one of the busiest waterways in the world (Bas et al. 2017). In 529 contrast, no significant impacts of dredging events on the occurrence and habitat use of 530 Tursiops aduncus in an urbanized estuary in Australia were identified over 14 years 531 (Bossley et al. 2022). This range of responses of cetaceans to noise in different regions 532 highlights the need for a study focused on the impact of anthropogenic noise on the 533 Lahille's bottlenose dolphin population residing in the PLE, as it is a species that was 534 recently listed as "Endangered" by the Brazil National List of Endangered Species (MMA Ordinance n° 148) and as "Vulnerable" on the IUCN red list (Vermeulen et al. 2019). In 535 536 addition, the peak of detected boats occurred during autumn and summer, seasons in 537 which the PLE is most used for foraging and reproduction of the species.

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## 4.2 Temporal pattern of Sound Pressure Level

540 Due to its similarity to the broadband SPL in all seasons, we assumed that the 541 low-frequency SPL is the main component of the soundscape in the PLE. The low-542 frequency SPL had its highest intensity in the summer and spring, according to our 543 manual detection analyses. Together, these seasons encompassed the highest intensity of 544 fish vocalization, a high concentration of boats and low-frequency anthropogenic noise 545 of unknown origin. In addition, the low-frequency SPL showed positive coefficients for 546 the afternoon, evening twilight and night compared to the morning, coinciding with the 547 periods of higher intensity of low-frequency noise from the NSRG.

548 Results of the high-frequency SPL did not coincide with those from the manual 549 detection analyses, since its highest values occurred during the spring and, in this season, 550 dolphins and boats were not recorded with great intensity when compared to the summer 551 and autumn. However, this divergence between the analyses may be related to the 552 ineffectiveness of the detector at high frequencies or to the lack of boat identification 553 through their acoustic signatures and SoundTrap distance estimates, factors that influence 554 the intensity of sound energy (Kline et al. 2020). On the other hand, the results of the 555 high-frequency SPL agree with those from the statistical model applied, in which the 556 periods of night, dawn and early twilight have negative coefficients in relation to the 557 morning period and represent the periods of lower intensity of boat traffic.

These results suggest that anthropophony has a greater contribution to SPL values than biophony, corroborating a study conducted in a coastal region of Canada, in which boats were the main contributors to SPL values in the frequency range from 50 to 1000 561 Hz (Halliday et al. 2020). In contrast, in the Tagus estuary in Portugal, the highest values 562 for the SPL from 20 to 2000 Hz were found in the spring and were associated with the 563 intense chorus produced by fish in the region (Vieira et al. 2021). In this context, it is 564 noteworthy that the composition of the high-frequency SPL may also include sounds from 565 crustaceans that use the PLE as part of their life cycle (Valentini et al. 1991), as this group 566 is commonly one of the main components of a soundscape (Putland et al. 2017) but was 567 not addressed in this study. The complexity of coupling high-frequency SPL components 568 to the reality of manual analysis of the sounds collected in the PLE is perhaps represented 569 in the fit of the corresponding GLM, which had the lowest explained deviation among the 570 three models.

571 Additionally, the negative influence of salinity in the models applied to all SPLs 572 suggests the predominance of anthropogenic noise in the soundscape of the PLE when 573 calculated by this metric. As the SPL measurement encompasses the entire predetermined 574 frequency range (including boats), its result includes sounds of greater sound intensity, 575 and consequently, many biological sounds were masked. However, for both frequency 576 bands analyzed, the results varied between 80 and 120 dB, coinciding with estuarine 577 regions with intense boat traffic in the USA (Monczack et al. 2019) and Portugal (Vieira 578 et al. 2021) and in coastal regions of Canada (Halliday et al. 2020).

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## 4.3 Limitations and perspectives

581 This study was performed under some restrictions and limitations that hindered 582 the interpretation of some results. Despite the understanding that the NSRG noise is part 583 of the sampling site soundscape, the analysis of the daily and seasonal sound patterns of fish was impaired by the intense masking of the sound pulses emitted by this group, 584 585 especially in summer. Consequently, future studies aiming to define area use and 586 preferred times of sound emission by fish should be conducted in areas less affected by 587 anthropogenic noise. Additionally, aiming at the species-specific identification of fish in 588 the PLE region, we recommend conducting studies in captivity to enable comparing the 589 sound pulses found in the two environments. This combined information is of great value 590 in providing data for potential protective and monitoring measures for threatened species 591 in the region, which are experiencing an intense social conflict between fisheries and 592 conservation.

593 Finally, this study highlighted a large gap regarding the possible impacts of 594 anthropogenic noise on the PLE biota. This is a growing and unexplored field of 595 bioacoustics in the region. Considering the intense boat traffic and the dependence on this 596 area by several species of fish and the Lahille's bottlenose dolphin for their vital activities, 597 this methodology has great potential for future studies. In this context, evidence shows 598 that the metrics of peak sound pressure level and sound exposure level are the most 599 appropriate (Southall et al. 2007, Martin et al. 2019, Southall et al. 2019). Additionally, 600 studies focused on determining the acoustic signature of boats are also required in the 601 region to contribute to the monitoring of fisheries, including any illegal fishing activity 602 (Kline et al. 2020).

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## 5. CONCLUSION

605 The detection of fish and Lahille's bottlenose dolphin T. t. gephyreus vocalizations through manual analysis of the spectrograms coincided with the 606 607 reproductive and foraging pattern of all these species in the region, attesting to the strong 608 seasonal and daily influence of these sound emissions in the Patos Lagoon estuary. Based 609 on this, this methodology proved to be an applicable tool for the analysis of the ecology 610 of aquatic biota since its results coincided with previous studies of behavior and 611 distribution. In parallel, the SPL metric complemented the general panoram of the 612 soundscape of the area, but was strongly influenced by anthropogenic noise, which 613 predominated over the biological sounds in the sampled area.

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### **APÊNDICE 2**

## ACOUSTIC RECORDING OF THE SOUTHERN BLACK DRUM (*POGONIAS COURBINA*) REPRODUCTIVE CHORUS IN THE PATOS LAGOON ESTUARY, BRAZIL

Milanelli AM, Kuroski F, Assumpção R, Rossi-Santos M, Fruet PF, Sampaio LAN, Dalla Rosa L

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# ACOUSTIC RECORDING OF THE SOUTHERN BLACK DRUM (*POGONIAS COURBINA*) REPRODUCTIVE CHORUS IN THE PATOS LAGOON ESTUARY, BRAZIL

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#### ABSTRACT

The southern black drum (*Pogonias courbina*) is a species vulnerable to extinction that uses acoustics for reproductive purposes. The Patos Lagoon estuary (PLE), in southern Brazil, is essential for its life cycle. In this study, we detected the reproductive chorus of the species in the region through passive acoustic monitoring (PAM). The presence of chorus in the wild was confirmed by a comparative analysis of recordings in captivity using the parameters of duration and dominant frequency. In the PLE, the mean pulse duration (ms) was  $208 \pm 31.9$ , and the mean dominant frequency (Hz) was  $116 \pm 46.6$ . In captivity, the values were  $238.5 \pm 37.4$  and  $149 \pm 42.1$ , respectively, and showed no significant difference. In the PLE, sampling of acoustic and environmental data (temperature and salinity) was performed synchronously. The detection of the reproductive chorus of the southern black drum in the PLE occurred only in spring, especially at night. However, the interval with the highest sound emission intensity occurred in the late afternoon and afternoon twilight. As the PAM makes it possible to detect preferred spawning areas, its use in future protective e measures of the species is suggested.

Key Words: Advertisement call, Spawning, Southern black drum, Diel pattern

#### **1. INTRODUCTION**

Fish emit low-frequency sounds to attract sexual partners and for intraspecific communication (Myrberg 1981). It is also known that for the Sciaenidae family acoustics is also linked to spawning episodes (Luczkovich et al. 2008, Tellechea et al. 2011a). Sciaenidae fish present two main choruses that may be associated with harassment (disturbance calls), emitted by males and females, or spawning events (advertisement call), emitted only by males (Tellechea et al. 2010, Tellechea 2019).

The Patos Lagoon estuary (PLE), in southern Brazil, offers shelter, food or growth conditions for at least 150 species of fish, including several of the *Sciaenidae* family: withemouth croaker (*Micropogonias furnieri*), southern king weakfish (*Macrodon atricauda*), southern kingcroacker (*Menticirrhus americanus*), banded croaker (*Paralonchurus brasiliensis*), stripped weakfish (*Cynoscion guatucupa*), Argentine croaker (*Umbrina canosai*) and southern black drum (*Pogonias courbina*) (Fischer et al. 2011). Southern black drum was recently classified as "vulnerable" by the IUCN red list, mainly due to overexploitation (Haimovici et al. 2020).

The southern black drum is endemic to the western South Atlantic Ocean and has a restricted distribution that ranges from the state of Rio de Janeiro (Brazil) to the southern Gulf of San Matías (Argentina) (Menezes et al. 2003, Cousseau and Perrotta 2013, Azpelicueta et al. 2019). The main threat to the species is overfishing, which intensified between the 1950s and 1970s (Santos et al. 2016). In the PLE, the fishing stock suffered a strong decline, with no record of the species landing in the region in 2004, 2005, 2008, 2009 and 2010 (IBAMA 2012). Currently, despite some efforts to protect the southern black drum, such as its classification in the National List of Endangered Species and the repopulation of the PLE population, the population *status* in the PLE is decreasing (Haimovici et al. 2020). Reproductive parameters such as fecundity, age, and duration of first maturation of the species decreased compared to specimens analyzed before overfishing (Santos and Velasco 2021). Therefore, conservation efforts for the species are urgent and necessary.

As the PLE is a key region for the southern black drum's life cycle, our objective was to use passive acoustic monitoring (PAM) to detect potential reproductive choirs of the species. The specific objectives were (1) to characterize, in captivity, the southern black drum chorus during its reproductive period and (2) to use a series of acoustic recordings obtained at the PLE to search for possible detections of the species in the wild and to estimate its preferred times of sound emission. This is a pioneer study in the region and may contribute to future management and protection measures of the species.

#### 2. MATERIALS AND METHODS

#### 2.1 Study area

The Patos Lagoon estuary is a region of approximately 1000km<sup>2</sup> that connects with the adjacent coastal area through a single channel, delimited and maintained by the jetties of Barra do Rio Grande (Seeliger and Odebrecht, 2010) (Fig. 1). The seasonal variability of river discharge and salinization in the region is controlled by the wind regime (Stech and Lorenzetti 1992, Tomazelli 1993). While winds from the NE quadrant favors the discharge of fresh water and, therefore, reduce the salinity of the estuary, winds from the southern quadrant promote the entry of marine water into the region and result in a more saline environment (Costa et al., 1998).



Figure 1. Map of the study area in the Patos Lagoon estuary (PLE). The sampling site at the Naval Station of Rio Grande (ENRG) is marked as a red dot.

#### 2.2 Data collection

#### 2.2.1 Acoustic and environmental data in the Patos Lagoon estuary

Data was obtained with an autonomous digital recording system, model SoundTrap ST300 HF (*Ocean Instruments NZ*). The unit was installed at the Naval Station of Rio Grande (ENRG) (Fig. 1) at a depth of 6 meters together with a salinity and temperature sampler (SeaBird SBE 37-SM), and it was configured to continuously sample data in 10-minute sequential audios over a frequency range of 48 kHz. The acoustic and environmental data were collected simultaneously between November 2020 and August 2021, covering 7 days per season. 2.2.2 Acoustic record of the southern black drum (Pogonias courbina) in captivity

To identify the southern black drum vocalization in the PLE, efforts were made to acoustically record the species in captivity at the Marine Aquaculture Station of the Federal University of Rio Grande (EMA-FURG), located in Praia do Cassino, Rio Grande, RS. A collection was performed during the reproductive period of the species (Machi et al., 2003) in November 2021, which occurred intermittently over 7 days. The recording was performed by an autonomous SoundTrap ST300 HF recorder, which was attached to a well and attached to the side of the tank with the same settings as the ENRG collection.

Fourteen individuals were simultaneously recorded and kept in a 16 m<sup>3</sup> culture tank containing a recirculation pump (*Jacuzzi* 1.5 1LQ-M), Skimmer, mechanical biological filter (Bead Filter) and settling tank. The mean length of the individuals was  $51.5 \pm 7.5$  cm, with at least 8 females and 4 males. The mean salinity and water temperature during sampling were  $32.6 \pm 1.3$  psu and  $24.8 \pm 1.1^{\circ}$ C, respectively. This stage of data collection was performed in partnership with the Southern black drum Project of FURG, which maintains wild specimens collected in 2019 in its laboratory for research and reproduction purposes. The Southern black drum Project is authorized by the Committee for Ethics in the Use of Animals (CEUA-FURG).

#### 2.3 Data analysis

Two acoustic parameters commonly used for fish sound analysis were used to measure the sound pulses emitted by the southern black drum in captivity and compare them with the fish pulses recorded in the PLE: duration and dominant frequency (Tellechea et al., 2011a; Tellechea and Norbis 2012, Tellechea 2019). Only pulses in which the beginning and end were visibly well defined and that were at least 10 dB louder than the background noise were analyzed (Fig. 2).



Figure 2. Spectrogram with sound pulses of the southern black drum (*Pogonias courbina*) in captivity (A) and in the estuary of Patos Lagoon (B) generated by the Raven Pro 1.5 software. The advertisement call is highlighted in yellow.

The analyses were performed manually using the software Raven Pro 1.5 (*Cornell Laboratory of Ornithology, NY, USA*) with a Hann window, FFT of 1024 points and 75% overlap (Rice et al., 2017). A total of 4,036 10-minute audios generated in the PLE were analyzed to estimate the presence of the chorus in the wild, and for each audio, the quantification of biological sounds was based on 6 categories (adapted from Tellechea et al., 2011a) according to the number of pulses detected (0 = no detection; 1 = 1 to 40; 2 =

41 to 100; 3 = 101 to 300; 4 = 301 to 500; 5 = 500). To obtain the interval with the highest vocalization of the species, the day was divided into six periods according to the solar calendar of the city of Rio Grande, RS, Brazil: dawn, morning twilight, morning, afternoon, afternoon twilight and night (Tab. 1).

	Dawn	Morning twilight	Morning	Afternoon	Evening twilight	Night
Spring	0 – 3 a.m.	3 – 5 a.m.	5 – 12 a.m.	12 – 19 p.m.	19 – 20 p.m.	20 p.m. – 0 a.m.
Summer	0 – 4 a.m.	4 – 6 a.m.	6 – 12 a.m.	12 – 19 p.m.	19 – 20 p.m.	20 p.m. – 0 a.m.
Autumn	0 – 5 a.m.	5 – 7 a.m.	7 – 12 a.m.	12 – 17 p.m.	17 – 18 p.m.	18 p.m. – 0 a.m.
Winter	0 – 5 a.m.	5 – 7 a.m.	7 – 12 a.m.	12 – 18 p.m.	18 – 19 p.m.	19 p.m. – 0 a.m.

Table 1. Periods of the day defined according to the solar calendar in Rio Grande, RS, Brazil.

The non-parametric Kruskal–Wallis test was used for the comparative analysis of the sound emission rate of the southern black drum throughout the day in the PLE. When there was a significant difference, the post hoc Nemenyi test was applied to verify which groups were different from each other. The non-parametric Mann–Whitney test was used to compare the acoustic parameters of southern black drum (duration and dominant frequency) in the wild and in captivity. All statistical analyzes were performed in R software.

#### **3. RESULTS**

The detection of the southern black drum reproductive chorus in the PLE occurred only in the spring and was more intense at night (Kruskal–Wallis p < 0.05).

However, the interval with the highest intensity of sound emission occurred between 6 pm and 8 pm (late afternoon and evening twilight) (Fig. 3). The temperature and salinity conditions in the PLE at the time of peak vocalization are shown in Table 2.



Figure 3. Distribution of vocalization detections of the southern black drum (*Pogonias courbina*) during spring quantified by the average of the categories of audio analyzed per hour (0: no detection, 1: 1 to 40, 2: 41 to 100, 3: 101 to 300, 4: 301 to 500 and 5: > 500) over the days sampled in the PLE. The data represented in gray were not analyzed due to masking caused by anthropogenic noise.

Table 2. Temperature and salinity conditions at the moments of highest intensity of the southern black drum (*Pogonias courbina*) vocalization in the PLE (mean ± standard deviation).

	Time (BRT)	Temperature (°C)	Salinity
06 Nov 2020	7 – 8 pm	$20,4 \pm 0,02$	3,2 ± 0,15
09 Nov 2020	6 – 8 pm	$20,8 \pm 0,15$	$5,39 \pm 0,77$

A total of 140 pulses of the species were analyzed, 70 from captivity and 70 from the PLE (Tab. 3), and we found no significant difference between them (Mann–Whitney p > 0.05). This similarity between the pulses indicates that the sound emissions found in the PLE were emitted by the same species.

Table 3. Mean  $\pm$  standard deviation (minimum-maximum) for two acoustic parameters of the southern black drum *Pogonias courbina* in the wild (PLE) and in captivity (EMA).

	PLE n=70	EMA n=70
Duration (ms)	208 ± 31,9 (144 – 274)	238,5 ± 37,4 (124 – 274)
Dominant frequency (Hz)	116 ± 46,6 (46,9 – 281,2)	149 42,1 (93,8 – 281,2)

#### 4. DISCUSSION

The pulses emitted by the southern black drum during the spawning period are considered long duration pulses, and to date, there are no records of other sciaenids that present a reproductive chorus with these characteristics (Amorim 2006, Ladich and Fine 2006, Tellechea et al., 2011a). However, very little is known about these pulses in the Southern Hemisphere, especially regarding their daily and seasonal temporal patterns. Table 4 summarizes the main studies with southern black drum around the world. It is noteworthy that the species of the genus *Pogonias* found in the Northern and Southern Hemispheres were considered the same until 2019, when they were differentiated as *Pogonias cromis* and *Pogonias courbina*, respectively (Azpelicueta et al., 2019). Yet, differences in sound emissions had been previously detected between the specimens from the two regions (Tellechea et al., 2011a).

Species	Habitat	Location	Methodology	Dominant Frequency (Hz)	Duration (ms)	Daily pattern	Season of the year	Salinity (psu)	Temperature (°C)	Reference
				Nort	hern Hemisp	here				
P. chromis	Bay and coastal areas	Gulf of Mexico	Vessel	-	_	Late afternoon – early evening	January - April	10 – 27	15 – 24	Saucier and Baltz 1993
P. chromis	Estuary	Savannah Harbor	MAP	-	-	Late afternoon – early evening	March - June	16 - 32	14.3 – 9.1	Collins et al. 2001
P. chromis	Estuary	Florida	MAP	94	$600 \pm 22$	Late afternoon – early evening	January - March	-	18 - 22	Locascio and Mann 2011
P. chromis	Estuary	Cape Coral	MAP	-	-	Night	January - April	21.7 - 22.8	18.5 – 25.7	Locascio et al. 2012
P. chromis	Estuary	South Carolina	MAP	-	-	Late afternoon – early evening	March – May	-	13.1 – 24.3	Monczak et al. 2017
				Sout	hern Hemisp	here				
P. courbine	Estuary	La Plata River	MAP	$128 \pm 5.08$	184 ± 6.5	Late afternoon – night	December – January	20 - 27	18 - 24	Tellechea et al. 2011a
P. courbine	Estuary	Rio Grande	МАР	116 ± 46.6	149 ± 42.1	Late afternoon – early evening	September	3.1 - 6.8	20.5 - 20.7	The present study

1 Table 4. Comparative table of the available information on the reproductive chorus of *Pogonia cromis* and *Pogonia courbina*, including the present study.

Tellechea et al. (2011) performed the only description of the reproductive chorus of *P. coubina* in the Southern Hemisphere to date. The authors determined values of the dominant frequency and duration of pulses, as well as their temporal variation. In the present study, the values of both parameters were similar to those found by Tellechea et al. (2011), but with a higher standard deviation both in captivity and in the wild. This variation is likely mainly due to the difference between the total length of the individuals who were vocalizing, as there is a direct relationship between the size of the individual and the values of acoustic parameters (Tellechea et al., 2011), increasing for duration and decreasing for dominant frequency. Therefore, as our recording in captivity was simultaneous for 14 individuals and there were at least 4 males (responsible for emission of the reproductive chorus), a high standard deviation was expected, as in the wild.

Although no significant differences were detected between the values of the acoustic parameters between the two environments, the dissimilarity of the sound pulses seen in the spectrogram (Fig. 2) is noteworthy. This difference may be linked to the difference between the sound fields, especially in relation to sound pressure (Duncan et al. 2016, Gray et al. 2016, Rogers et al. 2016), as the walls of the tank generate very different particle movements than are found in nature (Rogers et al. 2016) and can cause changes in the acoustic signal. Additionally, the greater intensity of the sound pulse of the individuals in captivity may be related to their distance to the SoundTrap, since the tank represents a much smaller area when compared to the natural habitat.

The daily and seasonal pattern of the chorus produced by the southern black drum in the PLE, in turn, agrees with previous studies of fish that emit chorus associated with reproduction (Locascio et al., 2012, Breder, 1968; Mok and Gilmore, 1983; Luczkovich et al., 1999; Aalbers, 2008). Both in the Northern and Southern Hemispheres, fish of the genus *Pogonias* emit chorus in the late afternoon and early evening during spring and summer (Tab. 4). These results partially corroborate the results of our study, as no pulses associated with reproduction of the species were detected during the summer. In this context, it is important to increase the sampling period in the region to characterize the daily and seasonal patterns of the species. In addition, there is a contrast in relation to the salinity values found in the PLE at the time of chorus, where the region stands out for the lowest value.

Although it has been recorded in other regions (Vieira et al., 1998, Machi et al., 2003), the southern black drum is an estuarine-dependent species (Fischer, 2011), unlikely to use the estuarine zone during the spawning season under low salinity conditions. However, despite the low salinity at the time of chorus emission, it is important to note that the *La Niña* period was beginning in Brazil during sampling. In this period, due to the predominance of drought periods, the entry of salt water into the estuary occurs more intensely and favors the entry of marine species into the region, especially those considered estuarine-dependent (Garcia et al., 2001). Furthermore, Santos et al. (2016) demonstrated, based on bycatch data, that the species has formed reproductive aggregations in the PLE. These aggregations occur at depths less than 10 meters (Machi et al., 2003), therefore, it is suggested that the individuals were close to the sampling equipment during the sound emissions.

Studies with passive acoustic monitoring (PAM) have been used to collaborate with fish management and monitoring actions, as this methodology is able to define the spatiotemporal patterns of different species in a habitat, as well as their preferred reproductive conditions (e.g., water temperature and salinity) (Picciulin et al., 2020). However, to ensure the accuracy of the spatial resolution of the spawning area, complementary studies are needed to investigate the sound transmission loss model (Biggs and Erisman 2021).

Considering the potential anthropogenic impacts that the PLE region is facing, including illegal fishing activities (Costa et al. 2018), PAM can be a tool to assist in the protection of fish species that depend on the region to carry out vital activities. In particular, PAM can help identify seasonal and daily patterns of distribution and key areas for reproduction of endangered species.

#### **5. CONCLUSION**

Passive acoustic monitoring in the region of the Naval Station of Rio Grande in the Patos Lagoon estuary allowed the detection of the reproductive chorus of the southern black drum (*Pogonias courbina*) through manual analysis of the spectrograms. This result coincided with previous studies conducted in the Southern Hemisphere, demonstrating the strong seasonal and daily influence of these sound emissions in the Patos Lagoon estuary. In this context, this methodology proved to be an applicable tool for future studies on distribution and identification of fish species. Considering that this is the first record of the reproductive chorus of the species in the region, a greater sampling effort in the PLE region is urged for the complete characterization of the daily and seasonal pattern of the southern black drum sound emissions. This information may favor protective measures for this species, which is threatened with extinction and uses the region in the spawning period.

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## **APÊNDICE 3**

### MATERIAL SUPLEMENTAR

Catálogo Acústico das Embarcações (Estuário da Lagoa dos Patos)

Tipo de Embarcação	Distância do SoundTrap (m)	Página
Lancha de passeio	118	93
Navio cargueiro	215	94
Navio cargueiro	300	95
Pesca artesanal	42	96
Pesca artesanal	217	97
Pesca artesanal	473	98
Pesca industrial	60	99
Pesca semi industrial	68	100
Praticagem	210	101



Tipo de embarcação: Lancha de passeio	<b>Data</b> : 08/10/2021
Material: Fibra de vidro	Distancia do SoundTrap (m): 118
Comprimento total (m): 3 a 6	Sentido de navegação: Entrando no ELP
<b>Motor (HP)</b> : Popa (90 a150)	Hora da gravação: 14:12





Tipo de embarcação: Navio cargueiroData: 08/10/2021Material: FerroDistancia do SoundTrap (m): 215Comprimento total (m): 218,45Sentido de navegação: Saindo do ELPMotor (HP): Centro (desconhecido)Hora da gravação: 16:30





Tipo de embarcação: Navio cargueiro	<b>Data</b> : 08/10/2021
Material: Ferro	Distancia do SoundTrap (m): 300
Comprimento total (m): 186,4	Sentido de navegação: Entrando no ELP
Motor (HP): Centro (desconhecido)	Hora da gravação: 14:36







98

## Informações Gerais

Tipo de embarcação: Pesca artesanal	<b>Data</b> : 08/10/2021
Material: Madeira	Distancia do SoundTrap (m): 42
Comprimento total (m): 12 a 14	Sentido de navegação: Saindo do ELP
<b>Motor (HP)</b> : Centro (62 a 115)	Hora da gravação: 15:51









Informações Gerais		
Tipo de embarcação: Pesca artesanal	<b>Data</b> : 10/12/2021	
Material: Madeira	Distancia do SoundTrap (m): 217	
<b>Comprimento total (m):</b> 12 a 14	Sentido de navegação: Saindo do ELP	
<b>Motor (HP)</b> : Centro (62 a 115)	Hora da gravação: 15:51	





Informações Gerais			
Tipo de embarcação: Pesca artesanal	Data: 28/10/2021		
Material: Madeira	Distancia do SoundTrap (m): 473		
Comprimento total (m): 12 a 14	Sentido de navegação: Saindo do ELP		
<b>Motor</b> ( <b>HP</b> ): Centro (62 a 115)	Hora da gravação: 16:22		







101

## Informações Gerais

Tipo de embarcação: Pesca industrial	<b>Data</b> : 28/10/2021
Material: Ferro	Distancia do SoundTrap (m): 60
<b>Comprimento total (m):</b> 19 a 24	Sentido de navegação: Saindo do ELP
Motor (HP): Centro (325 a 360)	Hora da gravação: 16:33







Tipo de embarcação: Pesca semi industrial	<b>Data</b> : 10/12/2021
Material: Madeira	Distancia do SoundTrap (m): 68
<b>Comprimento total (m):</b> 12 < 20 AB	Sentido de navegação: Entrando no ELP
Motor (HP): Centro (200 a 260)	Hora da gravação: 10:53





Tipo de embarcação: Praticagem	<b>Data</b> : 08/10/2021
Material: Ferro	Distancia do SoundTrap (m): 210
Comprimento total (m): 14	Sentido de navegação: Saindo do ELP
Motor (HP): Centro (desconhecido)	Hora da gravação: 16:37

de

