FACULTAD DE CIENCIAS

PATRONES ESTACIONALES Y CIRCADIANOS DEL PAISAJE SONORO SUBMARINO Y RUIDO ANTROPOGENICO EN LA COSTA CHILENA, EFECTO SOBRE EL CONSUMO DE OXÍGENO EN UNA ESPECIE DE MISIDÁCEO

Por

Victor Alexander Molina Valdivia

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Director de Tesis: Iván Andrés Hinojosa Toledo

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Don **VICTOR ALEXANDER MOLINA VALDIVIA**

RUT 18537798-9

Alumno de la Carrera de **MAGISTER EN ECOLOGIA MARINA**

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DECANO

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TABLA DE CONTENIDOS

RESUMEN

El ruido submarino ha cobrado relevancia como una nueva fuente de contaminación, siendo incluido dentro en las estrategias de protección de varias agendas internacionales, las cuales han propuesto medidas de mitigación con el objetivo de atenuar los efectos negativos del ruido emitido por las actividades humanas. Dentro de los efectos negativos se han reportado cambios en el comportamiento, problemas temporales y permanentes de la audición, enmascaramiento de señales de comunicación y cambios en la fisiología de los organismos marinos. Chile, a pesar de ser uno de los primeros 5 países con mayor porcentaje de su zona económica exclusiva bajo alguna figura de protección, estas no consideran al ruido antropogénico submarino como una fuente de contaminación. Por lo tanto, este estudio se enfoca en evaluar la presencia del ruido proveniente del tránsito de embarcaciones en tres zonas ecológicamente relevantes de la costa de Chile: Isla Chañaral, Puñihuil y Golfo Corcovado. Isla Chañaral y Puñihuil mostraron marcados patrones estacionales y circadianos asociados a la presencia de embarcaciones, este patrón se refleja en los niveles de presión sonora, especialmente en las octavas 63Hz y 126Hz, que no superaron los 92 dB re1 µPa (mediana) a escala circadiana o estacional. En contraste, el golfo Corcovado no mostró patrones a ninguna escala temporal, pero la presencia de embarcaciones se mantuvo constante, con niveles de presión sonora elevados (>92 dB re 1 µPa - mediana) en las octavas 63Hz y 126Hz, a escala circadiana y estacional. Las implicancias biológicas de la presencia de este tipo de ruido sobre la fauna local aún no han sido del todo estudiadas y se necesita más información, sobre todo en grupos taxonómicos subrepresentados como los invertebrados. Hasta ahora, las posibles medidas de manejo que se podrían implementar en el actual escenario legislativo chileno solo contemplan medidas de mitigación durante el desarrollo de futuros proyectos que se ejecuten en el medio marino. Estas medidas son parte de una lista de criterios aun sin fuerza de ley, revelando que existe una necesidad apremiante de un marco normativo que considere al ruido antropogénico dentro de los ejes de protección y planes de manejo de áreas marinas protegidas. Posteriormente, se realizaron cultivos experimentales del misidáceo *Neomysis sp.* para respirometría, con el fin de estimar el punto de presión critica de oxígeno (P_{crit}). Los organismos llegaron al P_{crit} (42,611 mmHg - media) a los 170min. Esta información es esencial para futuros tiempos de exposición en experimentos para determinar el efecto del ruido de embarcaciones y otras antropofonías en el metabolismo de misidaceos. De manera conservativa, se determinó un tiempo de exposición de 90min para los próximos experimentos de exposición al ruido.

Palabras claves: Antropofonías, Áreas marinas protegidas, Metabolismo, Ruido submarino.

ABSTRACT

Anthropogenic noise in marine ecosystems has gained significance as a new source of contamination and has been incorporated into the protection strategies of various international agendas that have proposed mitigation measures to alleviate the negative effects of noise emitted by human activities. Among the reported adverse effects are changes in behavior, temporary and permanent hearing problems, masking of communication signals, and physiological disorders of marine organisms. Despite being among the top five countries with the highest percentage of exclusive economic zone under some form of protection, Chile does not consider underwater anthropogenic noise as a source of contamination.

This study evaluates the presence of noise from vessel traffic in three ecologically relevant zones along the coast of Chile: Isla Chañaral, Puñihuil, and Corcovado Gulf. Chañaral Island and Puñihuil exhibit marked seasonal and circadian patterns associated with the presence of vessels. This pattern was reflected in the sound pressure levels, especially in the 63 Hz and 126 Hz octaves, which did not exceed 92 dB re 1 µPa (median) on a circadian or seasonal scale. In contrast, the Corcovado Gulf showed no patterns at any temporal scale, but the presence of vessels remained constant, with elevated sound pressure levels (>92 dB re 1 µPa, median) in the 63 Hz and 126 Hz octaves on circadian and seasonal scale.

The biological implications of this type of noise on local fauna have not been fully studied, and more information is needed, especially for underrepresented taxonomic groups, such as invertebrates. Currently, potential management measures that could be implemented in the current Chilean legislative scenario only consider mitigation measures during the development of future projects in marine environments. These measures are part of a list of criteria that still lack legal force, revealing an urgent need for a regulatory framework that includes anthropogenic noise in the axes of protection and management plans for marine protected areas.

Subsequently, experimental cultures for respirometry were conducted using the mysid Neomysis sp. to estimate the critical oxygen pressure point (Pcrit). The organisms reached Pcrit (42,611 ± 9,070 mm Hg, mean ± standard deviation) at 170 min. This information is essential for future exposure times in experiments to determine the effect of vessel noise and other anthropophony on mysid metabolism. Conservatively, an exposure time of 90 minutes was determined for upcoming noise exposure experiments.

Key words: Anthrophonies, Marine protected areas, Metabolism, Underwater noise.

INTRODUCCIÓN GENERAL

El sonido está compuesto por la energía acústica emitida por la vibración de los objetos, generando perturbaciones mecánicas que se desplazan a través del agua de mar. Las onda sonoras generan movimientos de partículas y cambios en la presión hidrostática, conocidos como niveles de Presión Sonora (SPL, por sus siglas en inglés "Sound Pressure Level"), y son medidos en decibeles (dB) en 1 micro Pascal (μPa) (Bradley & Stern 2018). El sonido es una excelente señal en el medio marino debido a las ventajas que posee sobre otros tipos de señales ambientales como la luz o los olores, que se ven atenuados en el agua y dependen de la turbidez y corrientes. En cambio, el sonido es capaz de viajar 4,3 veces más rápido en el agua que en el aire, con mínima pérdida de energía en condiciones hidrográficas, batimétricas y oceanográficas específicas (NOAA, 2015).

En los ecosistemas marinos, los sonidos pueden provenir de tres tipos de fuentes: las abióticas o geofonías, como la lluvia, tormentas, oleaje, fuentes hidrotermales y terremotos, usualmente en frecuencias menores a 100Hz (Farmer & Xie 1989; Wilcock et al. 2014); las biofonías, generadas por organismos biológicos y que pueden ser interpretados como señales para otros organismos, como las vocalizaciones de mamíferos marinos y peces, y los chasquidos producidos por los crustáceos (Boon et al. 2009; Lillis & Mooney 2018; Filiciotto et al. 2019) y por último, las antropofonías, compuestas por los ruidos producidos por la actividad humana, como los sonares, las prospecciones sísmicas, el tráfico marítimo, los motores fuera de borda y el ruido asociado a la acuicultura (Andrew et al. 2011; Nieukirk et al. 2012; Kastelein 2014; Merchant et al. 2014; Guan et al. 2017; McCauley et al. 2017; Ruiz-Ruiz et al. 2019). Estas tres fuentes, componen el paisaje sonoro submarino en sus dimensiones espaciales y temporales (Pijanowski et al. 2011)

Los organismos marinos tienen órganos sensoriales que les permiten detectar estas fuentes sonoras, en términos de cambios de presión sonora y movimiento de partículas. Este último es especialmente relevante debido a que los tejidos tienen una densidad similar al agua de mar (Merchant et al. 2015). De esta forma, el sonido se vuelve fundamental en varios procesos del ciclo de vida, desde interacciones tan básicas como la detección de presas y depredadores en varios taxa (Wale et al. 2013a; Hughes et al. 2014; Simpson et al. 2015; Pieretti et al. 2017; Popper et al. 2020), en la comunicación y ecolocalización de cetáceos (Širović et al. 2007; David L. Bradley & Stern 2018), y en la orientación y asentamiento en peces (Tolimieri et al. 2000; Bolgan 2018) e invertebrados (Branscomb & Rittschof 1984; Hinojosa et al. 2016; Simpson et al. 2016; Anderson et al. 2021).

En este escenario, las antropofonías son una componente relativamente nueva de paisaje sonoro submarino, siendo el tráfico marítimo la principal actividad antrópica desarrollada internacionalmente, cuadruplicandose desde 1970 (Tournadre 2014), y actualmente sustenta el 80% del comercio global (United Nations Conference on Trade and Development 2020). Entonces, surge la pregunta sobre cuáles son los potenciales efectos negativos del ruido que emiten las embarcaciones sobre los organismos marinos. Hoy en día, los estudios sobre la ocurrencia y los efectos del ruido de embarcaciones en la fauna marina han cobrado relevancia, ya que se espera que el uso de embarcaciones siga creciendo en los próximos años.

En general los efectos del ruido sobre la fauna marina se agrupan en cuatro categorías principales: enmascaramiento de señales, cambios en el comportamiento, efectos anatómicos-fisiológicos y pérdida de audición temporales y permanentes. El enmascaramiento ocurre cuando el rango de frecuencias en las que se emite el ruido se superpone con las de una señal acústica de interés biológico, dificultando su detección y percepción por parte de los organismos (Thomsen et al. 2021). El comportamiento de los organismos marinos también se ve afectado por el ruido, manifestándose en respuestas como la evasión, cambios en los patrones de alimentación y buceo en mamíferos marinos, así como alteraciones en la natación y conductas anti-depredación en peces (Miksis-Olds & Wagner 2011; Voellmy et al. 2014; Popper & Hawkins 2019; Di Franco et al. 2020; de Vincenzi et al. 2021). La pérdida de audición es un efecto descrito en diversas especies tanto vertebradas como invertebradas cuando se exponen a niveles de presión sonora que causan daños en los órganos asociados a la percepción del sonido, siendo el nivel de pérdida auditiva dependiente de la sensibilidad del organismo y su exposición al ruido (McCauley et al. 2003; Nachtigall et al. 2004; Andrew et al. 2011; Peng et al. 2015; Southall et al. 2019a; Thomsen et al. 2021). Por lo tanto, dependiendo del daño, se pueden producir cambios temporales o permanentes en el umbral de detección del sonido (National Marine Fisheries Service 2018). Por último, los efectos anatómicos y fisiológicos del ruido incluyen barotraumas causados por la intensidad del sonido o cambios bruscos de presión, la secreción de hormonas de estrés, como el cortisol, y cambios en el consumo de oxígeno, reflejando alteraciones metabólicas producto de un mayor costo energético para suplir los efectos del ruido en los organismos marinos (Verslycke & Janssen 2002; Verslycke et al. 2003; Wysocki et al. 2006; Wale et al. 2013b; Ruiz-Ruiz et al. 2019; Chapina et al. 2020).

Hoy en día, el ruido antropogénico submarino ya es considerado una nueva fuente de contaminación por varias agendas internacionales (Lewandowski & Staaterman 2020; Duarte et al. 2021), y por lo tanto, el paisaje sonoro submarino debe ser considerado dentro de los ejes de protección de las áreas marinas protegidas y en futuros proyectos que involucren la emisión de ruidos al medio marino (Servicio de Evaluación Ambiental 2022). Sin embargo, a pesar de que Chile ocupa el primer lugar en el mundo en términos de áreas marinas protegidas dentro de su zona económica exclusiva, abarcando el 20% del área total (Fernández et al. 2021), este número de áreas marinas protegidas no se condice con planes de manejo efectivos que garanticen la protección real de estas áreas (Saavedra Gallo 2013; Petit et al. 2018; Andersen Cirera & Balbontín Gallo 2021; Fernández et al. 2021). A esto se suma los pocos estudios para evaluar el paisaje sonoro, la presencia del ruido antropogénico y sus efectos en los organismos marinos (Borie et al. 2017; Ruiz-Ruiz et al. 2019; Carrasco et al. 2021).

En este contexto, este estudio se enfoca en cuantificar la presencia y los niveles de ruido antropogénico en tres localidades de relevancia ecológica: Isla Chañaral, Puñihuil y Golfo Corcovado, con el objetivo de evidenciar determinar patrones en los niveles presión sonora en el paisaje sonoro submarino cercano a estas áreas.

La Isla Chañaral cuenta con la Reserva Marina Isla Chañaral, que abarca una milla náutica alrededor de la isla y se encuentra en la región de Atacama. Esta localidad es conocida por ser un área de alimentación para depredadores de alto nivel trófico, como mamíferos marinos y aves, en particular el pingüino de Humboldt (Pérez et al. 2006; Sepúlveda et al. 2009; Toro et al. 2016; Cárcamo et al. 2019). Las actividades antropogénicas en esta localidad se centran en la pesca artesanal y el turismo relacionado con la observación de cetáceos (Sepúlveda et al. 2018). Puñihuil se encuentra al norte de la isla de Chiloé y alberga tres islotes que son utilizados como zonas de nidificación para dos especies de pingüinos, el pingüino de Humboldt y el pingüino de Magallanes, así como área de tránsito de la ballena franca austral (Hiriart-Bertrand et al. 2010; Vernazzani et al. 2014). La pesca artesanal y el turismo son las principales actividades antropogénicas en esta zona (Gajardo Cortés & Ther Ríos 2011). El Golfo Corcovado se caracteriza como una zona de alimentación para mamíferos marinos, con una alta biomasa de zooplancton durante los meses de verano ((Hucke-Gaete et al. 2004, 2013a; Buchan & Quiñones 2016; Bedriñana-Romano et al. 2018; Buchan et al. 2021a, 2021b). Sin embargo, también se utiliza como ruta de navegación, lo que ha resultado en colisiones entre embarcaciones y cetáceos (Hucke-Gaete et al. 2004).

Así, el primer capítulo de esta tesis consiste en una aproximación temporal a escala anual y circadiana de los niveles de presión sonora en cada una de estas áreas. Teniendo en cuenta los diferentes niveles de actividades antrópicas en cada sitio, se espera que hayan marcadas variaciones entre estaciones y circadianas en Isla Chañaral y Puñihuil. En cambio, en Golfo Corcovado no se esperan variaciones a ninguna escala temporal, debido a que el tráfico marítimo en esta área responde a patrones de actividad pesquera artesanal, industrial y acuicultura que no dependen del turismo o de la estacionalidad.

Consecuentemente, además de determinar los niveles de ruido en el medio marino también es importante evaluar los potenciales efectos negativos que podría tener sobre los organismos marinos que habitan en zonas con alta presencia de antropofonías, especialmente en aquellos grupos subrepresentados como el zooplancton. La importancia de este grupo radica en que es un eslabón clave en la transferencia de carbono desde los productores primarios hasta los niveles tróficos superiores, por ejemplo, organismos como el Krill son la principal presa de peces y de grandes cetáceos como la ballena Fin *Balaenoptera physalus* y la ballena azul *Balaenoptera musculus* (Pérez et al. 2006; Berta & Lanzetti 2020; Buchan et al. 2021b), por lo tanto, es importante comprender los efectos del ruido antropogénico en este grupo de organismos. Hasta ahora, existen estudios sobre el ruido emitido durante prospecciones sísmicas o Air gun seismic survey. Esta actividad consiste en la emisión de sonidos de alta intensidad (156 dB re 1 µPa2 s−1 SEL) y baja frecuencia (20-500Hz) desde embarcaciones hacia el fondo marino donde penetra en el subsuelo y permite detectar yacimientos de combustibles fósiles en las capas inferiores del fondo marino. Se ha reportado que estas prospecciones son capaces de triplicar la mortalidad de larvas y adultos del zooplancton (McCauley et al. 2017). Sin embargo, Fields et al. (2019) bajo una aproximación en terreno a baja profundidad (6m), encontraron un aumento de la mortalidad solo a distancias inferiores 5m desde la fuente, y no encontraron efectos subletales en el copépodo *Calanus finmarchicus*. En este contexto, se hacen necesario más estudios para determinar los efectos del ruido sobre los distintos grupos de invertebrados y vertebrados que componen el zooplancton. Consecuentemente, el segundo capítulo de esta tesis se centrará en evaluar el efecto del ruido antropogénico sobre misidáceos. Estos organismos son crustáceos holoplanctónicos omnívoros que realizan migraciones verticales para alimentarse de productores y consumidores primarios, que a su vez, son presa de diversos depredadores tanto vertebrados como invertebrados, desempeñando un papel clave en las redes tróficas y flujo de carbono entre aguas superficiales y profundas (Hansson et al. 1997; Punchihewa & Krishnarajah 2013). Adicionalmente, son organismos que pueden resistir condiciones de laboratorio, lo que los convierte en buenas especies modelo para comprender los efectos del ruido antropogénico en el zooplancton. Específicamente, se avaluará el efecto del ruido de embarcaciones sobre el metabolismo de *Neomysis sp.* a través de los cambios en el consumo de oxígeno como indicador de un aumento en la demanda metabólica para sobrevivir a la presencia del ruido antropogénico.

CAPÍTULO 1

To submit in Marine Pollution Bulletin - ELSEVIER

Vessel Noise along Chilean coast, necessities for management and regulations

Victor Molina-Valdivia[1,4]*, Iván A. Hinojosa[2,3,4,6], Giuseppa Buscaino[5,] and Susannah Buchan[4]

[1]*Universidad Católica de la Santísima Concepción, Street, Concepción, 100190, Región del Biobío, Chile.

[2]Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Chile.

[3] Centro de Investigación en Biodiversidad y Ambientes Sustentables (CIBAS), Universidad Católica de la Santísima Concepción, Chile.

[4] Center for Ecology and Sustainable Management of Oceanic Islands (ESMOI), Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile (ESMOI).

[4]Centro de Investigación Oceanográfica COPAS Coastal, Universidad de Concepción, Concepción, Región del Bio Bio, 4070043, Chile.

[5]Institute for Coastal Marine Environment, National Research Council, Via del Mare, 6, Torretta Granitola, 91021, Campobello di Mazara, Italy.

Introduction

In marine ecosystems, sound is particularly relevant because of its advantages over other types of environmental signals, such as light and chemical signals, which are attenuated by water. In contrast, sound can travel at speeds of 1483 m/s in seawater (4.3 times the speed of sound in air), and with minimal energy loss under certain hydrographic, bathymetric, and oceanographic conditions (NOAA 2015). Due to these advantages, the sound constitutes an excellent signal to perceive the underwater soundscape which "encompasses all sounds in terms of spatial, temporal, along with their frequency attributes, and the types of sources contributing to the sound field" (Pijanowski et al. 2011).

The underwater soundscape is composed by three sources of sound: geophonies, which are composed of all sounds that come from meteorological or oceanographic processes such as rain, wind, waves, tidal currents, and earthquakes. Biophonies are sounds emitted by biological organisms such as vocalization, song and clicks from marine mammals, and grunt or croaks from fishes and snaps in invertebrates. Anthropophonies are composed of all noises emitted directly or indirectly by human activities carried out near or in the marine ecosystem, such as pile driving, ship noise, sonar, seismic surveys, and wind turbines (Nieukirk et al. 2004, 2012; Kastelein 2014; Guan et al. 2017; McCauley et al. 2017).

Underwater sound signals play a fundamental role in several biological processes such as communication, reproduction, prey detection, settlement, and orientation (Lindseth & Lobel 2018; Duarte et al. 2021), and the presence of anthropogenic noise may disrupt this perception process. One of the most common sources of noise is generated by vessel transit, as ships typically emit noise at low frequencies (<2kHz) through their engines and propellers, causing cavitation (André 2018; Haver et al. 2021). This falls within the audible range for various organisms, including marine mammals and fish, which also produce low-frequency sounds (Duarte et al. 2021). Therefore, it is plausible that the presence of ship and boat noise could disrupt the processes mentioned above. In fact, nowadays several studies have reported that this ship noise can impair orientation and survival of fish larvae (Holles et al. 2013; Nedelec et al. 2015; Simpson et al. 2016), and produce changes in behavior (Vieira et al. 2021a), anti-predatory responses (Spiga et al. 2017) and cooperative interactions (Nedelec et al. 2017) in adult fish. Marine mammals, can perceive and emit sound in a broad range of frequencies (Erbe et al. 2016), particularly mysticetes like blue whale (*Balaenoptera musculus*) and fin whale (*Balaenoptera physalus*) emit low-frequency (<200Hz) (Buchan et al. 2019; Redaelli et al. 2022) and high intensity vocalizations used mainly for communication over long distances (~50km) (Širović et al. 2007), then the potential effects for this group consider mainly masking (Erbe et al. 2016; Southall et al. 2019b) and changes in the vocalization behaviour (Mackenna 2011). In this way, ship noise constitutes a source of pollution and today the European Union´s Marine Strategy Framework Directive considers underwater noise a stressor and a new source of pollution in the oceans (Lewandowski & Staaterman 2020). In Chile, the underwater noise is consider in "National Oceanographic Program" (Comité Oceanográfico Nacional 2020) where one of the expected results by 2023 is the identification, reduction or elimination of the underwater anthropogenic noise. Additionally, the Ministry of the Environment has established in the 2020, the "Underwater Noise Operational Committee" with the purpose of serving as a technical advisory body for governmental entities. Furthermore, through the Environmental Impact Assessment Service (SEIA), the Evaluation Criteria for the prediction and assessment of impacts caused by underwater noise (Servicio de Evaluación Ambiental 2022) has been developed. This set of criteria, whose implementation is binding, establishes requirements and technical guidelines concerning underwater noise emissions. It specifically addresses the prediction and assessment of their impacts on marine fauna.

As anthropogenic noise emerges as a new form of marine pollution, it is essential to consider its inclusion in the management plans of marine protected areas to ensure effective protection. Among the measures proposed to mitigate the effect of vessel noise, restricting access, and reducing vessel speed near Marine Protected Areas (MPA) are the most feasible to implement. However, effective measures must be consistent with the biological and anthropogenic sound activity patterns. For example, the temporal scale of biological sound activity of organisms can vary at circadian and seasonal scales (Buscaino et al. 2016; Carrasco et al. 2021), and even by modifying the community structure (Carrasco et al. 2021). Anthropogenic activities such as boat traffic are also driven by environmental and economic conditions. For example, boats related to whale watching and tourism are most active during the summer months, when potential clients are more present, and the probability of sightings is higher. In contrast, heavily industrialized zones such as ports, where merchant vessels are constantly arriving and living the bays regardless of the season. Therefore, understanding biological and anthropogenic activity patterns is crucial not only for correlating their presence with sound pressure levels in the soundscape but also for comprehending patterns on short (hours to weeks) and long (months to years) timescales, facilitating the implementation of effective management measures, considering the periods of high biological activity during which anthropogenic noise is more likely to produce the afore mentioned effects.

Chile is ranked among the top five countries with a significant proportion of MPAs within its exclusive economic zone (20% under some form of protection) (Fernández et al. 2021). Nevertheless, there is a lack of corresponding management plans to ensure the protection of these areas, and the underwater soundscape is not adequately addressed or prioritized. In August 2022, the Ministry of the Environment published a list of "criteria" for evaluating present and future noise levels emitted by anthropogenic activities and projects in coastal areas. This information could be incorporated into reports submitted to the Environmental Impact Evaluation Service (SEIA) and considered by decision makers when approving or rejecting future coastal projects. Despite its relevance, scientific information regarding the presence of anthropogenic noise, sound pressure levels and occurrence patterns along Chilean coast is scare.

Therefore, the objective of this study is to describe the presence of vessel noise near three ecologically significant sites along the Chilean coast, with a specific focus on vessel noise, and to determine the extent of free-noise time.

Methods

Study Areas and data acquisition

At each site, a factory calibrated hydrophones were used to record the underwater soundscape, during at least ten months. The following three sites were chosen because of their ecological relevance and contrasting anthropogenic activities. These areas are renowned as nurseries, feeding grounds, and transit zones for large marine mammals and other cetaceans.

The northern site, Chañaral Island (**CI**), is in northern Chile (Fig. 1 – CI) and it has a reserve that covers one nautical mile around the island. The Chañaral Island MPA and surrounding waters are recognized as a feeding area for marine mammals (Sepúlveda *et al*. 2009; Pérez *et al*. 2006; Toro *et al*. 2016; Cárcamo *et al*. 2019), and birds, particularly the Humboldt penguin (Mattern *et al*. 2004; Dantas *et al*. 2019). In this locality, anthropogenic activities include artisanal fishing and tourism associated with whale watching. The acoustic data was collected by a Soundtrap ST300 hydrophone (http://www.oceaninstruments.co.nz/soundtrap-300/), setting a duty cycle of 10 minutes of recording (wav files) and 50 minutes of no recording with a sampling rate of 24kHz. This hydrophone was deployed very close to the MPA (29° 1.305'S – 71° 32.336'O) between November 01, 2017, and August 31, 2018, with a water column depth of 104 m and a hydrophone at a depth of 80 m.

The second site is located in the northern part of Isla Grande of Chiloé (Fig. 1- PN), the islets of Puñihuil (**PN**). This area does not have a MPA, but it does have the status of nature sanctuary with the aim of protecting the islets that constitute a nesting area for Humboldt and Magellanic penguins(Hiriart-

Bertrand *et al*. 2010) and as a transit zone for the southern right whale (*Eubalaena australis*) (Rojas-Cerda *et al*. 2021), and as feeding ground for Blue whale (Hucke-Gaete et al. 2004). In this locality, the artisanal fishing and tourism are the main anthropogenic activities carried out (Gajardo Cortés & Ther Ríos 2011). The acoustic data was collected by a Soundtrap ST300 hydrophone, that was installed at 70m depth from the surface in an 80m depth zone (41° 56'S - 74°07' W), with a continuous cycle 12h recording (wav files) with a 24kHz sampling rate, between July 01, 2018, to July 31, 2019.

The third site it's located further south of Chiloé Island is the Gulf of Corcovado (Fig 1. **CG**). This area has been described as a baleen whale feeding ground supported by high zooplankton biomass during the austral summer (Hucke-Gaete et al. 2004, 2013; Buchan & Quiñones 2016; Bedriñana-Romano et al. 2018; Buchan et al. 2021a, 2021b). However, it is also used for boat traffic, which results in collisions with cetaceans (Hucke-Gaete et al. 2004). For this reason, the Tic toc marine park has recently been declared, located on the continental margin (43°41'S - 73°18'W) since July 2020, covering 101.9 ha. Here, a SongMeter SM3M hydrophone was deployed (43°50'S – 73°30'O) 140m from the surface, in an area with a depth of 172m, and data were recorded continuously for 30min every 30min with a sampling rate of 4kHz, from 01 April 2018 to 28 February 2019.

Data analysis

Power spectral density analysis

To identify the presence of high-intensity sounds and explore long-term patterns, we used the PAMGuide Function in MATLAB to calculate the Power Spectral Density (PSD) and compute spectrograms for each season and site. The PSD is the standardized spectrum of sound levels across frequency expressed in dB re 1 $\mu Pa^2 Hz^{-1}$ (Merchant et al. 2015a). These monthly spectrograms were calculated with a time average of 10 min, Hann windows and 4096 samples windows size with 50% overlap.

Circadian and seasonal ship noise and octave-band pressure levels (OPL).

A binary ship detector was constructed using a MATLAB version of "Frequency Amplitude Variation" (FAV) analysis (Reis et al. 2019), where the amplitude differences between adjacent frequencies were extracted from 2 min time-average spectrograms matrix (Hann windows and 4096 samples windows size with 50% overlap) made with the PAMGuide function (Merchant et al. 2015a). If this difference exceeds a threshold of α=1.5 standard deviation, the number of peak differences is counted and considered indicative of ship presence. A confusion matrix was used to prove the efficiency of the detector, showed a

90% of effectiveness (Calculated wit a manually selected set of files with known presence and absence of vessel noise, n =100).

Thereafter, for the circular statistical analysis only the first 2 min segment of each hour from the FAV results were consider in the posterior analysis. The circadian patterns have circular distribution, then, the hour was transformed into radians by dividing 360° by 24h. A Rayleigh´s uniformity test was carried out in the Software PAST (4.11v) on each site for every season to determine the preferred time of the day in which the vessel noise was more recurrent.

Additionally, the results from 2 min segments results from FAV detector were used to calculate the percentage of time without vessel noise for each site for every season. Finally, using a custom Matlab script, the octave band pressure levels (OPL) (dB re 1 µ Pa, rms) were calculated for every 2-min file. The OPL correspond to the sound pressure level between a range of frequencies in a logarithmic scale, usually expressed as the Root Mean Square (RMS) of each band (Merchant et al. 2015a). Here we focus in low band pressure level following the suggestion of Merchant et al. (2014) and Vieira et al. (2021b) to assess the ship noise presence, therefore, only five octave band were consider in further analysis, 32, 63, 126, 251 and 501Hz. To find differences in bands pressure levels between hours and season, a Kruskal-Wallis test were carried out.

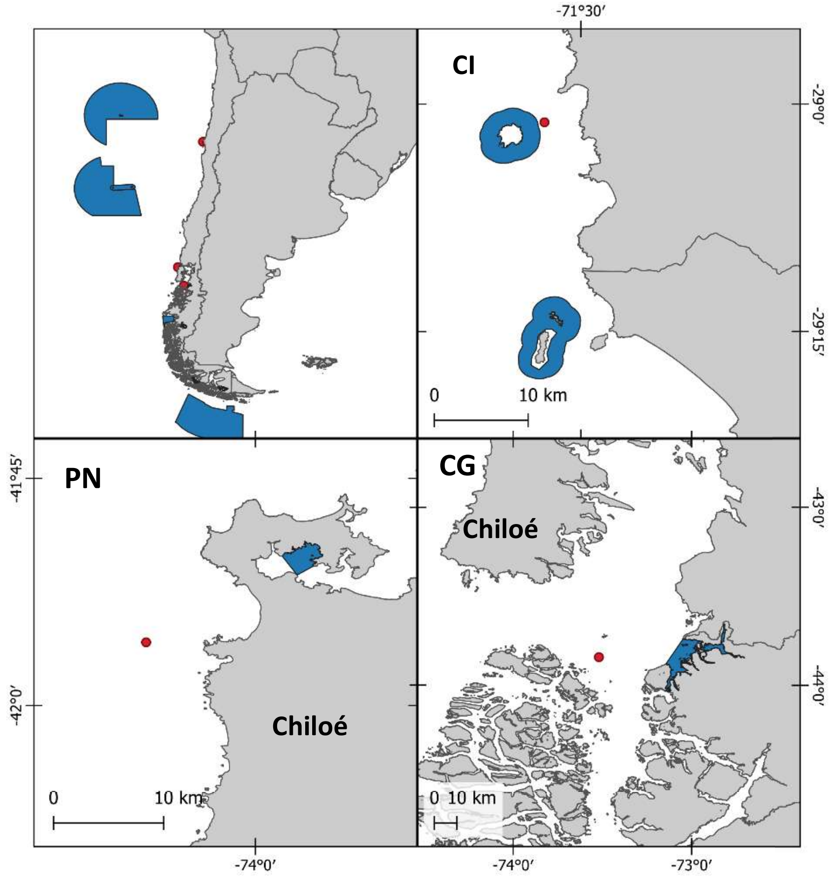

Figure 1. Location of recording sites along the Chilean coast. The first map (upper left) shows Chile, with the red dots representing the position where the hydrophones were deployed, and the blue areas corresponding to marine protected areas within the Economic Exclusive Zone (ZEE) in Chile. The next maps show a zoom at each site (CI: Chañaral Island; PN: Puñihuil; CG: Corcovado Gulf).

Results

Spectrograms / exploratory analysis

Clear differences among sites can be identified in the weekly spectrograms. In the long-term spectrogram from CI, a noise of higher intensity and between 100 and 200 Hz can be distinguished (Fig. 3 – Unknown noise). This noise is only present at night, between 22:00 and 03:00, and in the four seasons, but more intensively during spring and summer. In fact, at the 126 Hz octave, sound pressure levels reach 100 dB re 1µPa (RMS level). Additionally, higher intensity noise corresponding to outboard motors and merchant vessels are also observed between 12:00 and 18:00 throughout the year. In PN, noise from outboard motors and merchant vessel can be distinguished in the spectrograms. In CG, intense sounds covering the entire bandwidth can be observed during all seasons of the year, (Fig.2- Corcovado gulf). These sounds correspond to merchant vessels (Fig. 3 -Merchant vessel) that raise the average sound pressure levels up to 120 dB re 1µPa (RMS level), especially in the 126 Hz octave band.

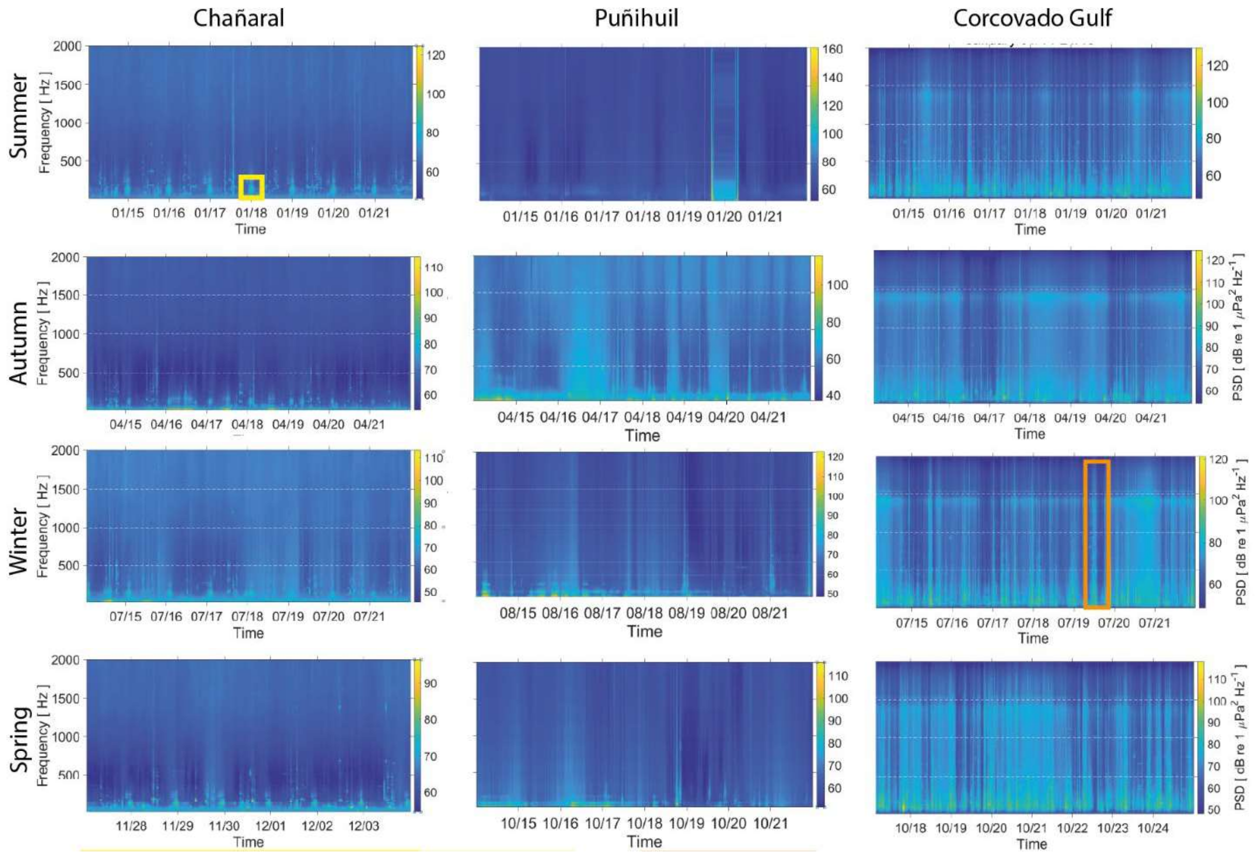

Figure 2. Long-term spectrogram per season from Chañaral island, Puñihuil and Corcovado gulf is representing. The coloured frames correspond to anthropophonies. Yellow frames correspond to an "unknown noise" consistently recorded during midnight (22:00 – 02:00) in all seasons. The orange frames correspond to a merchant vessel recorded, being visible in the entire spectrogram, elevating the noise levels mainly in low frequencies (<300Hz).

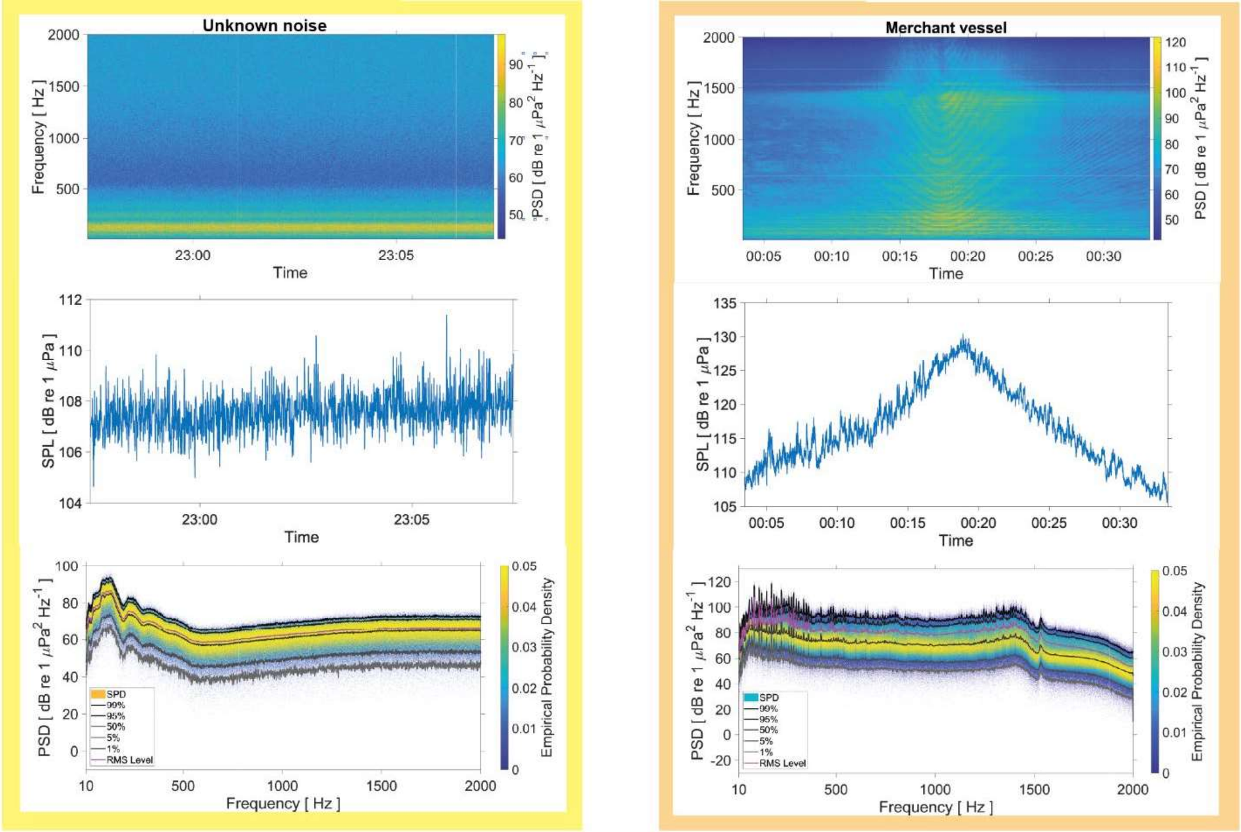

Figure 3. Acoustic metrics for anthropogenic noises founded Chañaral Island and Corcovado Gulf. The spectrograms of each noise are shown in the first row, the broadband sound pressure levels (SPL) in the second row, and the power spectral densities (PSD) with the empirical probability density in the color scale in the third row. In the yellow frame, is an "unknown noise" recorded on Chañaral Island, and the orange frames correspond to a merchant vessel recorded in the Corcovado Gulf.

Sound pressure levels and seasonal trends

In general, it can be found that all sites showed different levels of sound pressure between 10 and 2000 Hz. For example, in CI the sounds pressure levels were higher and more variable below 100 Hz, it was lower between 100-1000 Hz, and it was slightly higher levels above 1000Hz (Fig. 4). However, this was not the case for all seasons. In summer, sound pressure levels between 10-100Hz reach ~100 dB re 1µPa^2 Hz^{-1} (RMS level) and <90 dB re 1µPa^2 Hz^{-1} (RMS level) from 100 Hz to 2kHz. In autumn, the highest levels are reached between 10 and 40 Hz (~100 dB re 1µPa^2 Hz^{-1} RMS level), and after 40 Hz, the average sound pressure levels do not exceed 80 dB re 1µPa^2 Hz^{-1} (RMS level). In winter, sound pressure levels between 10 -400 Hz are highly variable and do not drop below ~100 dB re 1µPa^2 Hz^{-1}, and small peaks are observed between 200 and 1000 Hz. In spring, like winter, the average sound pressure levels between 10 and 300 Hz have high variability between ~100-120 dB re 1µPa^2 Hz^{-1} (RMS level).

The sound pressure levels in PN, showed a similar pattern to those found in CI in terms of higher sound pressure levels at low frequencies and lower levels at medium frequencies; however, the average sound pressure levels (RMS level) are generally higher and present peaks at different frequencies. In summer and autumn (Fig. 4), sound pressure levels below 100 Hz were between ~100-120 dB re 1µPa^2 Hz^{-1} (RMS level), with peaks at frequencies between 20-60 Hz and again between 80 and 100 Hz. In winter, the trend of high sound pressure levels between 100-120 dB re 1µPa^2 Hz^{-1} (RMS level) between 10-100 Hz was maintained; but above 100 Hz, the levels dropped to ~80 dB re 1µPa^2 Hz^{-1} (RMS level). In the spring, several sound pressure peaks can be distinguished above ~100 dB re 1µPa^2 Hz^{-1} (RMS level) between 80 and 200 Hz.

The sound pressure levels in CG showed a marked pattern of peaks that remained constant in all seasons. This pattern consisted of strong sound pressure peaks between 10-200 Hz, with the strongest peak observed between 10 and 20 Hz during summer, autumn, and winter. It is important to mention that, unlike the other locations that showed high variation in SPLs at low frequencies (<100 Hz), which also varied between seasons, GC did not show a great variation in sound pressure levels and remained constant throughout all seasons, except spring.

In terms of octave band pressure levels (OPL), in CI, minimal but significant differences were found among seasons in all octaves (Fig. 5 and Table 1), with the 32Hz octave showing higher levels than other bands, followed by the 63Hz band. In PN, significant differences were observed among seasons, with the 126Hz band consistently showing higher levels except in winter when the 63Hz band reached the highest levels. Consequently, winter was the season with the highest sound pressure levels (RMS). In GC, all octave bands showed similar seasonal trends, with autumn and winter having higher levels. However, despite these trends, the 32Hz octave band consistently showed significantly lower levels in all seasons compared to other bands.

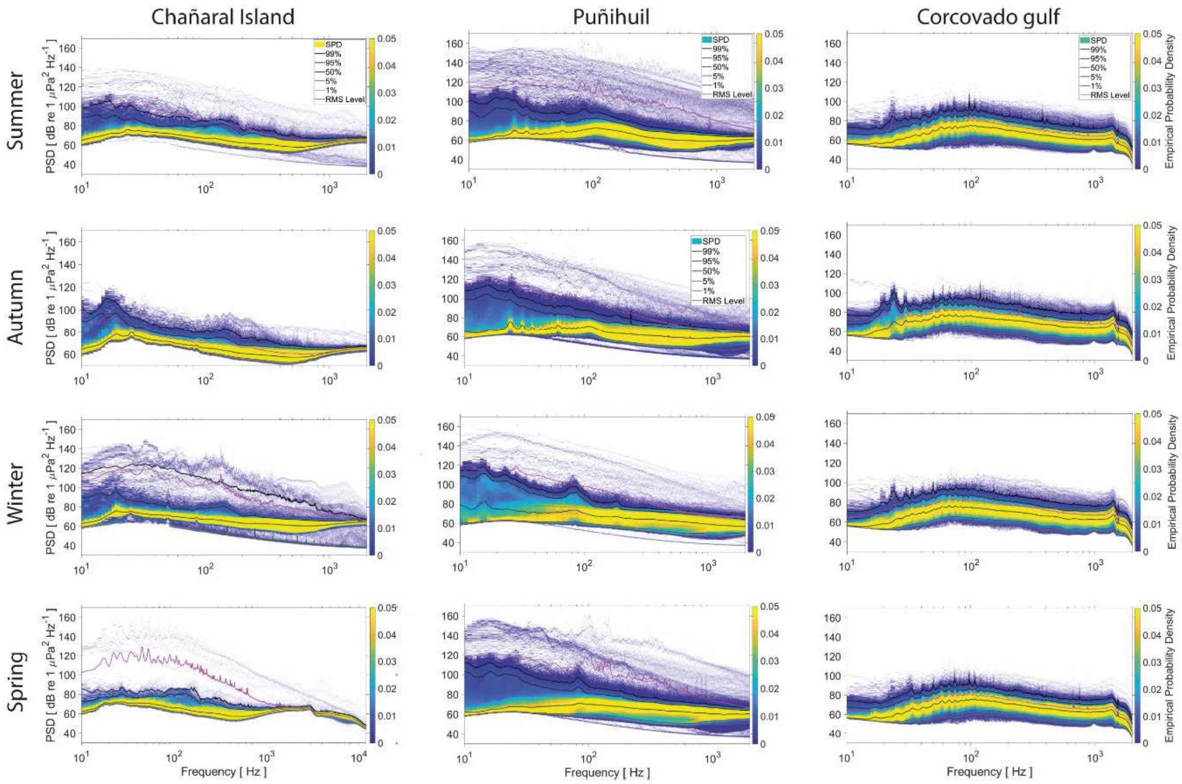

Figure 4. Noise levels represented in power spectral densities for the locations in each season. The violet line is the RMS level, and black lines are percentiles.

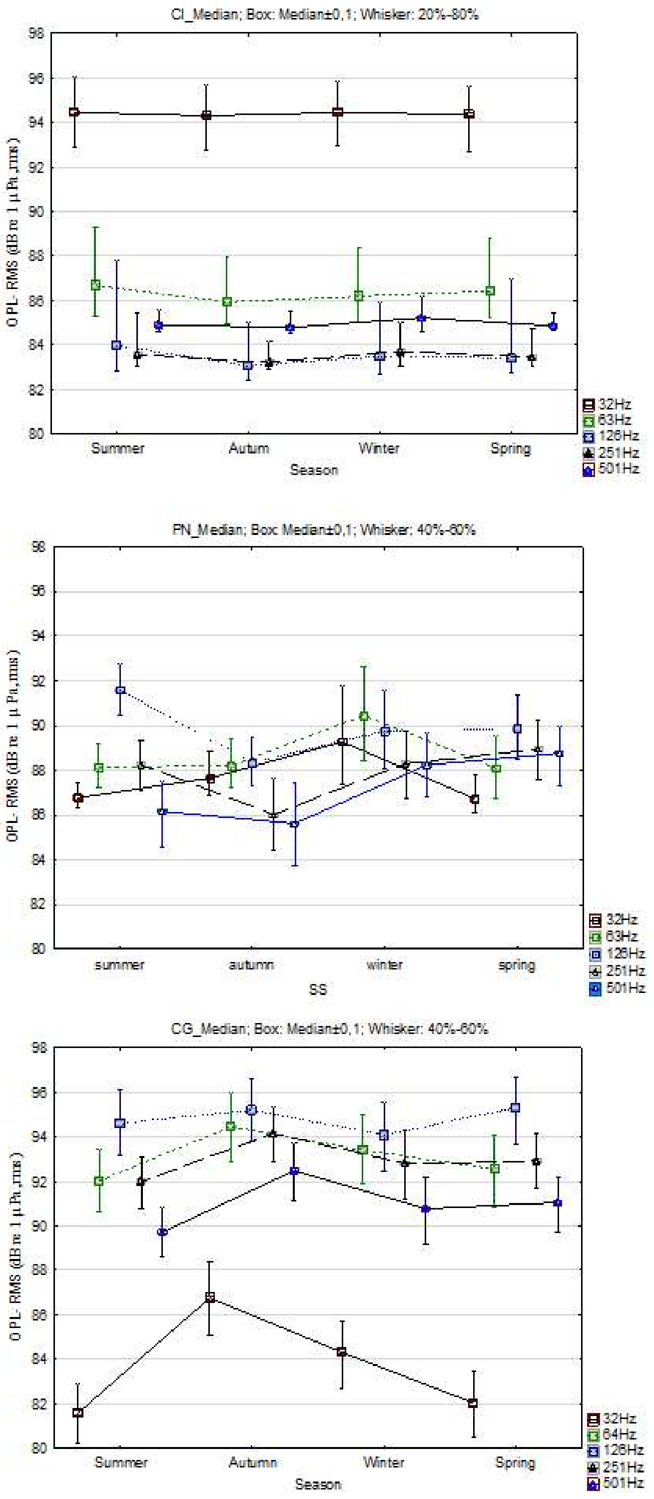

Figure 5. Seasonal variation of octave band pressure levels (32, 64, 126, 251 and 500Hz). the boxes represent the RMS level of the band, joined by a line to highlight the octave variations between stations. The whiskers represent the 20 - 80 percentile for Chañaral Island (CI), and 40 – 60 percentiles for Puñihuil (PN) and Corcovado gulf (CG). The Kruskal Wallis test parameters and *P* value are presented in table 1.

Table 1. Kruskal-Wallis test parameters for octave bands pressure levels among seasons for each site. Chañaral Island (CI) N=6448; Puñihuil (PN) N= 228829; Corcovado gulf (CG) N=11997.

Octave band	**CI**	*P*	**PN**	*P*	**CG**	*P*
32Hz	H = 12.76276	<0.05	H = 8060.965	<0.05	H = 1479.965	<0.05
63Hz	H = 134.4673	<0.05	H = 3347.632	<0.05	H = 414.3201	<0.05
126Hz	H = 279.2819	<0.05	H = 11250.72	<0.05	H = 57.00985	<0.05
251Hz	H = 248.2541	<0.05	H = 7764.579	<0.05	H = 260.2389	<0.05
501Hz	H = 164.8146	<0.05	H = 8520.477	<0.05	H = 407.8223	<0.05

Circadian patterns and free noise ratio

At each site, different patterns were found. In CI a clear pattern in the presence of boats was found, here boat noises were more abundant during the afternoon (12 to 18 h). This patter was consistent among seasons, but some changes on the circular mean were found, where the highest occurrence in summer was at 13:51, in autumn at 14:34, in winter was at 14.02, and at 11:46 in spring (Fig. 6 – Chañaral Island).

On the other hand, in PN a significant pattern was found only in summer and spring with a mean at 10:53 and 10:05 respectively, during autumn and winter the occurrence of boats did not show preferences for a particular time of day. CG was characterized by not showing a significant pattern in any season, with high occurrence during all hours of the day (Fig. 6 – Corcovado gulf). In terms of free-noise time, in CI a 72% was found, this percentage remained relatively constant throughout the year, being the site with highest free-noise time (Fig.8). A similar proportion was found in PN during summer, winter, and spring, with ~72% of free-noise time, while in autumn it decreased to 48%. On the other hand, in CG the percentages found did not exceed 15% throughout the year, this value was reached in winter, while in spring and summer it did not exceed 10%, therefore, Corcovado gulf was the site with highest presence of vessel noise among sites.

In terms of octave bands, in CI, the higher sound pressure levels (94.2 dB re 1 µPa, RMS) were found in the 32Hz with no significant differences, while the 63Hz, 126Hz, 251Hz, and 501Hz bands showed minimal but significant differences. Notably, in the 126Hz band was found a distinctive pattern, with higher sound pressure levels observed during midday and near midnight (Fig. 7). In Puñihuil, a strong pattern was observed across all octave bands, characterized by lower sound pressure levels during the night and higher levels starting from 12:00, reaching a maximum at 18:00, and subsequently decreasing towards midnight. in Corcovado gulf, in the 32Hz band can be observed the lowest sound pressure levels throughout all hours. Additionally, a pattern was evident in all octave bands, with higher sound pressure levels predominantly occurring during the night, while the lower pressure levels were observed from 12:00 until 18:00, followed by a rise in sound pressure levels across all octave bands.

Table 2. Kruskal-Wallis test parameters for octave band pressure levels among hours of the day for each location. Chañaral Island (CI) N=6448; Puñihuil (PN) N= 228829; Corcovado gulf (CG) N= 11997)

Octave band, Hz	IC	*P*	PN	*P*	CG	*P*
32	H = 30.53	0.134	H = 1035.01	<0.05	H =158.40	<0.05
63	H = 111.61	<0.05	H = 2078.21	<0.05	H = 215	<0.05
126	H = 619.47	<0.05	H = 4452.15	<0.05	H = 265.06	<0.05
251	H = 459.93	<0.05	H = 6866.36	<0.05	H = 178.50	<0.05
501	H = 372.35	<0.05	H = 4793.98	<0.05	H = 69.59	<0.05

Table 3. Circular stats parameters of Rayleigh test for circadian occurrence of vessel noise. Chañaral Island (CI); Puñihuil (PN); Corcovado gulf (CG); CM: Circular mean; ci: Confidence interval 95%; R: Rayleigh parameter.

	CI				**PN**				**CG**			
	CM	ci	R	*P*	CM	ci	R	*P*	CM	ci	R	*P*
Summer	13:51	13:25 - 14:16	0.51	<0.05	10:53	08:45 - 11.30	0.17	<0.05	1:50	19:50 - 07:50	0.003	0.96
Autumn	14:34	13:58 - 15:10	0.48	<0.05	4:58	22:34 - 10:34	0.03	0.2	2:36	20:36 - 08:36	0.01	0.67
Winter	14:02	12:56 - 15:09	0.34	<0.05	4:35	22:35 - 10:36	0.03	0.79	7:46	01:46 - 13:46	0.01	0.64
Spring	11:46	10:49 - 12:44	0.53	<0.05	10:05	08:00 - 13:46	0.07	<0.05	23:57	17:57 - 06:37	0	0.86

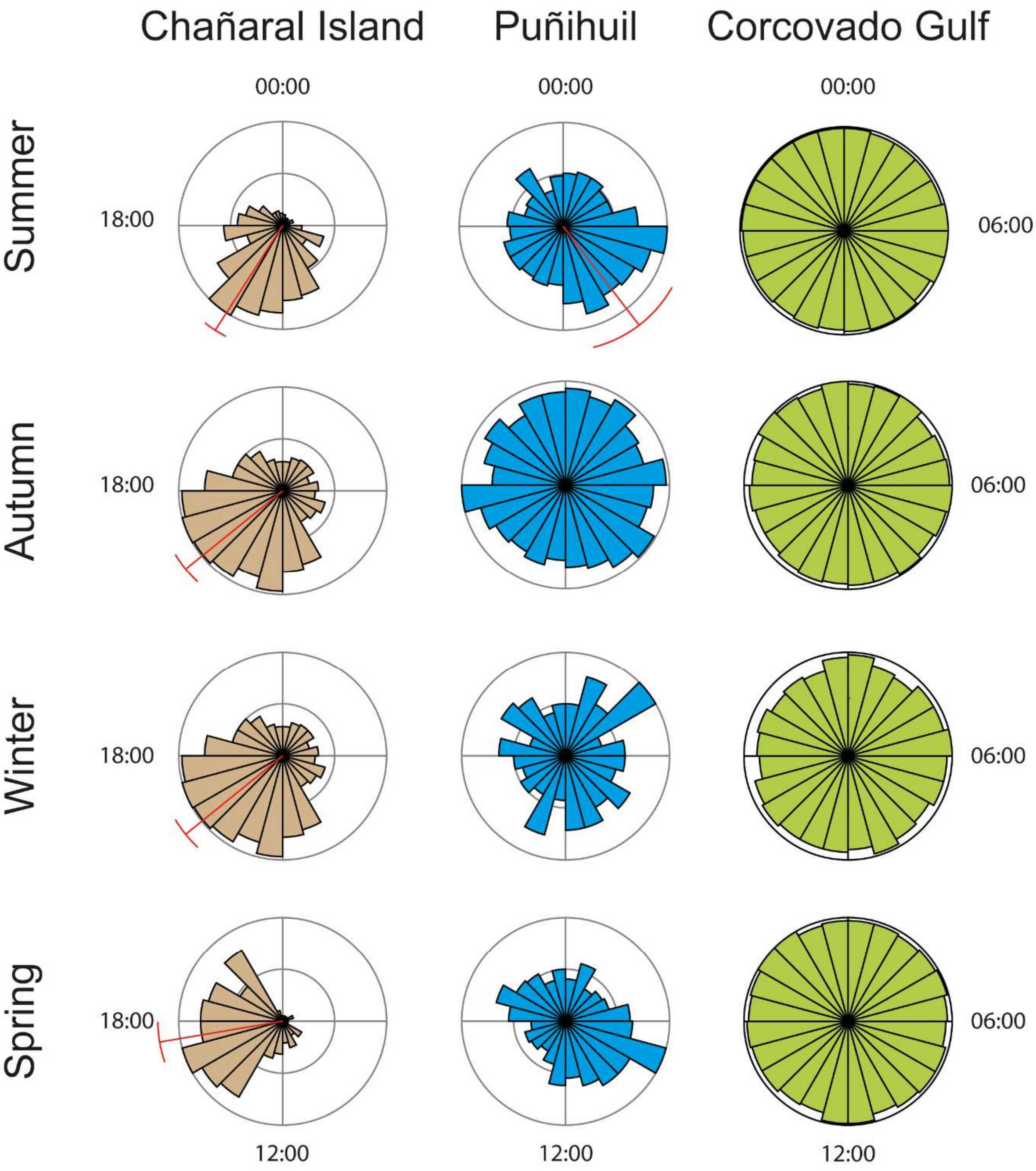

Figure 6. Circadian patterns of vessel occurrence on each location for each season. The red line shows significant pattern and the red arc correspond to the 95% confidence interval.

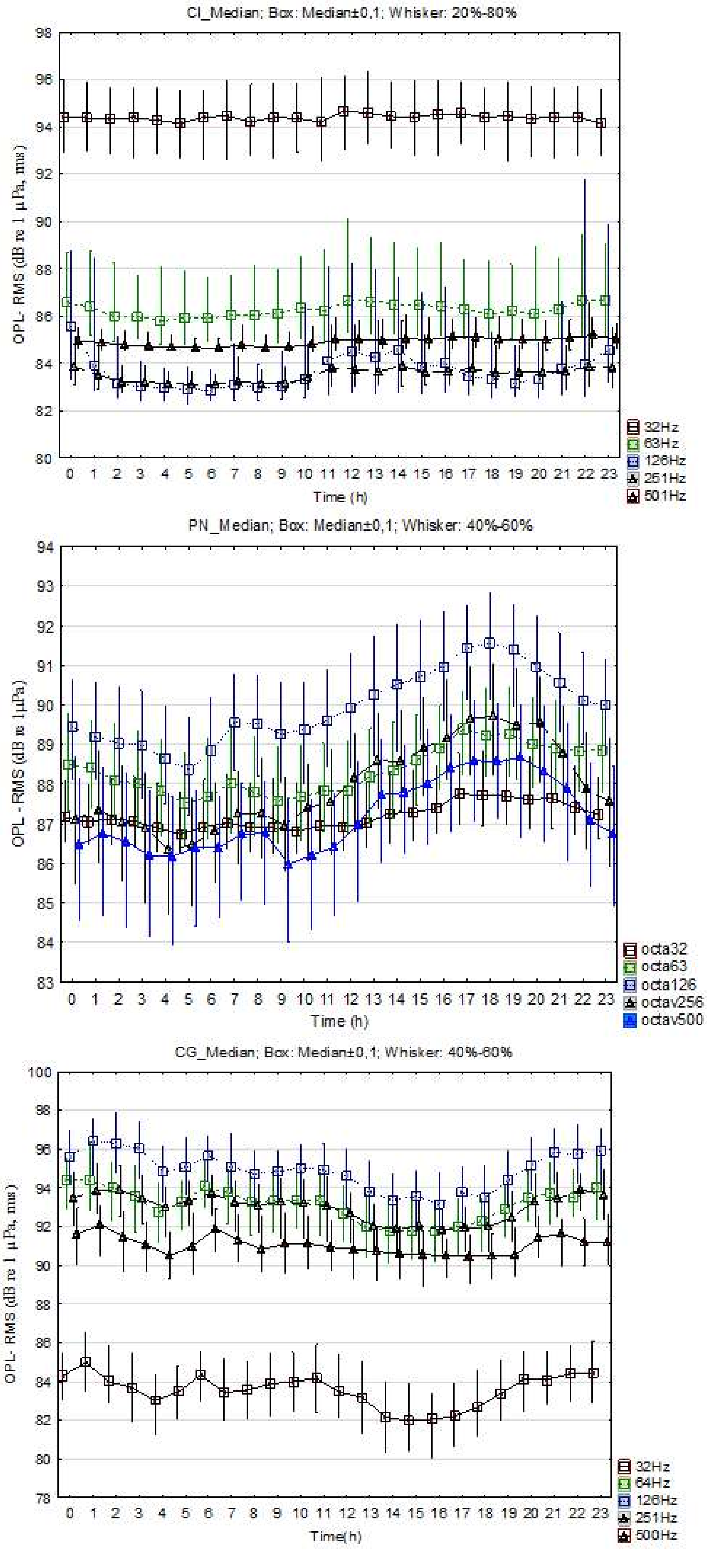

Figure 7. Circadian variation of octave bands pressure level for each location

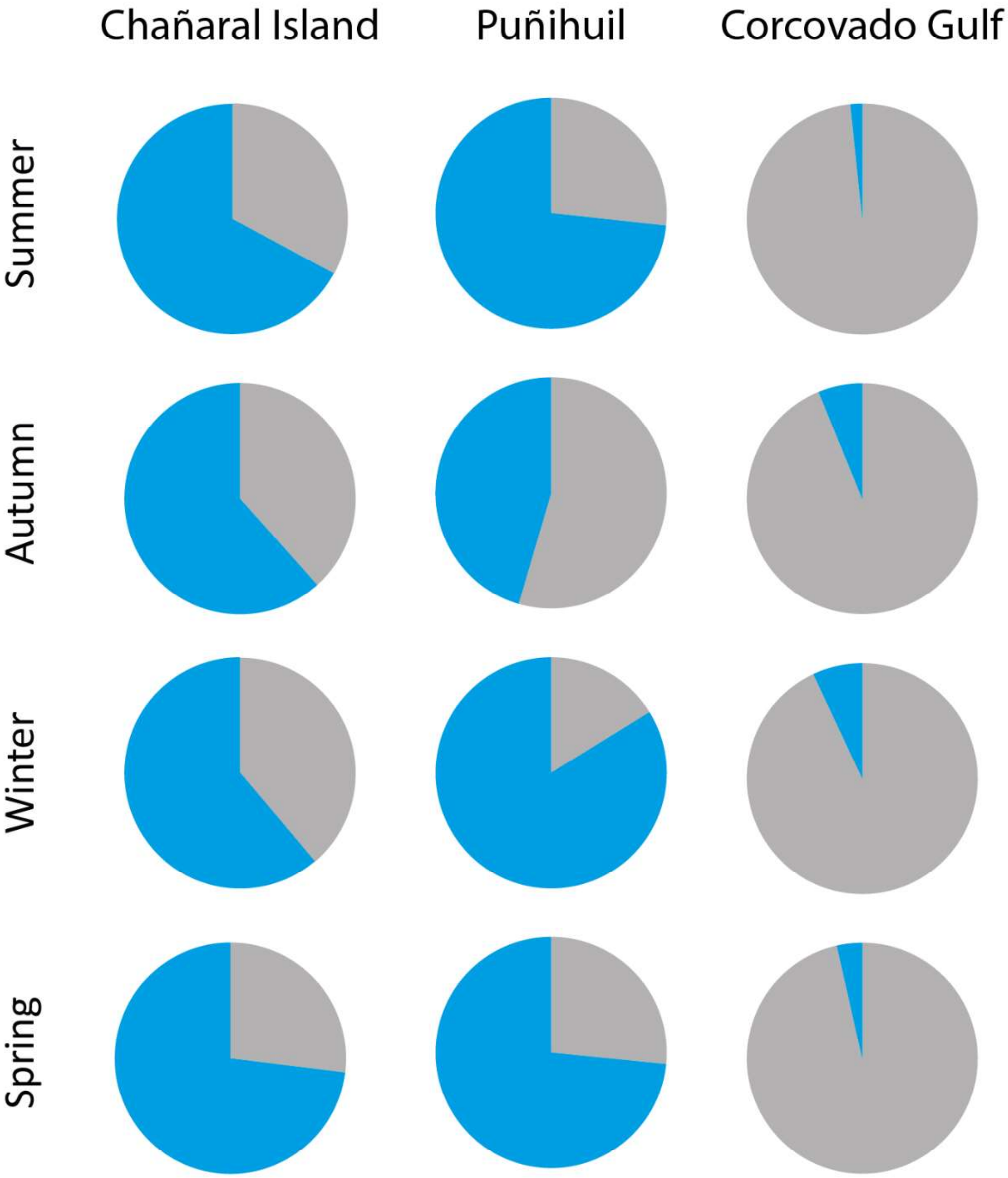

Figure 8. Percentages of noise-free time from vessel traffic calculated from 2-minute segments for each site and season. The cyan corresponds to noise-free time, and grey indicates periods when vessel noise is detected.

Discussion

This is one of the first studies to describe the noise levels emitted by vessels and their occurrence patterns on the Chilean coast. In general, vessel noise was present at all three sites throughout the year, but clear differences were observed among them. These differences are reflected in the contrasting seasonal and circadian patterns in vessel presence and octave band pressure levels, as well as in free-noise time. CI was the site with more free-noise time, and a clear circadian pattern in vessel presence was found in all seasons, between 12:00 and 18:00; this pattern was also observed in the 126 Hz octave band, where a higher OPL (>84 dB re 1 µPa, RMS) was found between 11:00 and 17:00. In PN, a circadian pattern in vessel presence was identified only during the summer, with a preference for hours between 06:00 and 12:00. OPL displayed a consistently strong pattern across all octave bands, especially in the 126 Hz octave. Higher OPL commenced at 12:00, peaking (>91 dB re 1 µPa, RMS) at 18:00, and gradually decreasing toward midnight. In CG, a uniform pattern was observed across all seasons in terms of vessel presence and OPL. Vessel presence remained almost constant at both the seasonal and circadian scales, and the free-noise time did not exceed 15% throughout the year. Autumn stood out as the season with the highest OPL in all bands, particularly the 126 Hz octave. Additionally, CG exhibited the highest levels among sites in all octave bands (>90 dB re 1 µPa, RMS), especially in the 126Hz octave (>94 dB re 1 µPa, RMS), except for the 32Hz octave, which did not surpass 86 dB re 1 µPa, RMS.

The level of industrialisation of each site seems to be responsible for the sound pressure levels in the soundscape, especially at 63 and 126Hz octave bands. The results of this study demonstrate how the presence of ships has a significant effect on sound pressure levels at circadian and seasonal scales, interacting with site-specific characteristics. Therefore, the potential effects on the organisms inhabiting these habitats should be assessed and management measures should be considered, especially in marine protected areas and sites of high ecosystem, social and economic value.

1. Vessel activity and noise patterns by site

On CI, the main vessel activity occurs between 12:00 and 18:00, presumably due to whale watching and artisanal fisheries activities (Sepúlveda et al. 2016, 2018). These partially support our first hypothesis due that the artisanal fishermen usually set sail early in the mornings between 6:00 and 8:00, but this was not reflected in the circadian patterns at any season. From the five octave bands, only in 126 Hz band can be identified a pattern. The sound pressure levels were higher in the two periods of the day, between 11:00 and 12:00, matching the vessel activity pattern (Fig. 6), and between 22:00 and 01:00, presumably due to noise from unknown sources (Fig.3). In Puñihuil, despite the absence of a pattern of vessel noise, except for summer, in autumn was found the higher presence of vessel noise, being constant in all hours of the day, consistently with the lowest free-noise time. Furthermore, a strong pattern in the octave band pressure levels was found, with higher levels from 09:00 to 23:00 and a peak at 18:00 in all octaves except at 32 Hz (Figure 6). In Corcovado Gulf was found the higher presence of vessel noise compared to the other sites. Interestingly, this noise was detected consistently throughout all seasons and at all hours of the day. On a circadian scale, the pressure levels were consistently lower between 12:00 and 18:00 across all octave bands. On a seasonal scale, pressure levels were higher during autumn but lower in both summer and spring. These differences could be related to the presence geophonies like waves and storms that are common during that season (Pérez-Santos et al. 2021). Furthermore, in this site was found the lowest free-noise time along all seasons (Fig.8).

2. Potential effects

The vessel generated noise levels described in this work (SPL and OPL) coincide with those described by other authors (Merchant et al. 2014; Haver et al. 2021; Vieira et al. 2021b) where the 63Hz and 126Hz were the most relevant. The noise and vessel patterns describe here, reveal that none of the sites are 100% free of human generated noise, even in those with MPA near the mooring hydrophone. Therefore, it is very likely that the fauna inhabiting the ecosystems of each location its being negatively affected, particularly those that mainly use the low-frequency range between 63hz and 126hz octaves. For example, according to the scale of impact of marine noise on marine mammals proposed by Southall et al. (2019), Baleen whales, such as Humpback, Blue, and Pacific Southern Right Whales, are particularly vulnerable. Their susceptibility arises from their auditory range and the frequencies at which they emit sounds, making them prone to masking and behavioral effects (Erbe 2002; Erbe et al. 2016). This is especially relevant in the Corcovado Gulf, where vessel noise is almost permanent (15% free-noise time) and constitutes a feeding and nursery ground for the Humpback (Hucke-Gaete et al. 2013) , Blue (Hucke-Gaete et al. 2004; Buchan & Quiñones 2016), Sei (Buchan et al. 2022) and Pacific Southern Right Whale (Seguel 2018; Rojas-Cerda et al. 2021). Puñihuil has been proposed as a non-migratory habitat area for the Southern Right Whale (*Eubalena australis*) (Rojas-Cerda et al. 2021) due to the near year around presence determined by acoustic monitoring of upsweep calls that are emitted between 50 – 120 Hz, therefore, masking effect also may be occurring, especially in autumn when the presence of vessel noise was higher. In the northern part of the Chilean coast, on CI, studies about the adverse effects of vessel presence are related to whale watching activities, for example, causing changes in behaviour in Fin whales (Santos-Carvallo et al. 2021) and bottlenose dolphins(Toro et al. 2021), but there is a lack of studies that assess noise.

Also, it is important to note, that these sites support large biomass of holoplanktonic invertebrates such as euphausiids (Buchan et al., 2021a; Pérez et al., 2006; Toro et al., 2016), which are capable of sustain a greater abundance of high trophic level predators such as cetaceans, especially during austral spring and summer. However, invertebrates have received much less attention despite the knowledge about the potential effect of anthropogenic underwater noise on this marine organism (Erbe et al., 2019; Popper et al., 2020; Wale et al., 2021). In fact, in Chile there is only one study on a benthic shrimp (*Rhynchocinetes typus*) that describes changes in reproductive behaviour in the presence of outboard vessel noise (Ruiz-Ruiz et al., 2019). Therefore, efforts must be made to assess and identify the effects of marine noise on marine mammals, invertebrates, and fishes that are key species in the trophic web of the coastal ecosystem of Chile.

3. Legal context of Chile and potential mitigations efforts or management solutions, evidence about the benefits of noise reductions efforts

As mentioned above, marine noise has been addressed as a new source of pollution by several international policy agendas such as the European Commission, IMO 2014, OSPAR 2017, and UN 2018, and mitigation and abatement measurements have been proposed to reduce or eliminate the noise emitted by vessels. In Chile, the need for the management and regulation of marine vessel traffic in MPAs has already been pointed out,with the aim of preventing behavioral effects in cetaceans exposed to whale watching (Santos-Carvallo et al., 2021; Toro et al., 2021), underwater noise (Colpaert et al., 2016), and collisions (Caruso et al., 2021). Until now, the government has published a list of criteria (Servicio de Evaluación Ambiental 2022) for the assessment of noise emitted during the construction and operation of future projects on the coast, indicating that the activities, type of noise to be emitted, area of influence, and potentially affected organisms must be identified, and mitigation and abatement measures must be considered when the project is carried out. Nevertheless, this applies only for future activities and not for current sources of noise, such as ports, salmon farms, marine traffic, etc., that are already installed near MPAs.

The implementation of regulations in the locations of CI, PN, and CG should be contingent upon several factors, including the current level of industrialization of maritime traffic, existing noise levels, and the potential socio-economic consequences for the local communities. To illustrate this, we consider the Chiloé ecoregion as a case study with a higher presence of vessel noise. Implementing restrictive measures in this region would pose significant challenges due to its geographical fragmentation, characterized by islands and the southernmost system of fjords and channels in the world. Maritime traffic plays a vital role in transportation, facilitating the movement of people, supporting artisanal and industrial fishing activities, and serving the aquaculture sector, which contributes the largest number of vessels in the region (Bedriñana-Romano et al., 2021). Therefore, spatiotemporal restrictions may not be welcomed by the community, especially in fisheries and aquaculture. As reviewed by (Merchant, 2019), internationally, there are ongoing efforts to promote the development of technologies aimed at designing new vessels with quieter propellers and hulls as well as innovations to mitigate the noise generated by engines and generators. In the local approach, implementing suitable noise reduction measures for existing vessels in Corcovado gulf may involve an initial step in monitoring the emitted noise levels at source and vessel speeds. It then recommends the adoption of operational measures, such as vessel speed reduction and incentive-based measures, to encourage noise reduction efforts by vessels that contribute the most to ambient noise levels. On the other hand, the CI exhibited the highest percentage of noise-free time. However, it is important to note that despite minimal noise levels, studies have already identified effects on marine mammal behavior attributed to whale-watching activities. In this scenario, it is more likely that spatiotemporal restrictions will be applied to ensure that the presence of ship noise does not increase.

Currently, there are studies in which such measures have been implemented to some extent, resulting in significant reduction in noise levels. For example, conducted a field experiment in which the vessel speed was restricted to 11 knots, resulting in a reduction of the mean broadband source levels for five categories of commercial vessels: containerships (11.5 dB), cruise vessels (10.5 dB), vehicle carriers (9.3 dB), tankers (6.1 dB), and bulkers (5.9 dB). Vakili et al. (2020) employed a trade-off approach to model the effects of adopting different speed limits on noise reduction, CO2 emissions, and fuel consumption. They found that such measures could reduce the noise emissions by up to 10 dB. In behavioural approach, Williams et al. (2021) found that reducing vessel speed not only reduces broadband and intensity of noise emitted, but also decreases the likelihood of disrupting orca foraging activities. Moreover, Nedelec et al. (2022) conducted field and laboratory studies and demonstrated that reducing noise through the adoption of mitigation measures can enhance the reproductive output of adults and, ultimately, the growth and survival of fish eggs and larvae.

Conclusions

The findings of this study highlight the imperative need to implement effective measures to sustainably manage marine traffic, particularly in areas where year-round noise pollution from vessels is prevalent, as observed in the Corcovado Gulf. Additionally, in areas where vessel noise currently has a lower prevalence, stricter measures should be adopted to protect these vulnerable ecosystems prior to projects that would increase vessel density near marine-protected areas, and stricter measures should be adopted to protect these vulnerable ecosystems. Further studies are needed to comprehensively assess the potential impacts of underwater noise on the diverse marine organisms inhabiting Chilean waters. These studies will contribute to a deeper understanding of the ecological consequences and to the formulation of evidence-based management strategies for mitigating the adverse effects of vessel noise in and around marine protected areas.

CAPÍTULO 2

To be submitted in marine pollution bulletin

Effect of vessel noise on metabolism of *Neomysis spp.*

Victor Molina-Valdivia[1,7]*, Iván Hinojosa[2,3,4], Mauricio Urbina[5,6] and Susannah Buchan[7]

[1]*Universidad Católica de la Santisíma Concepción, Street, Concepción, 100190, Región del Biobío, Chile.

[2]Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Chile.

[3] Centro de Investigación en Biodiversidad y Ambientes Sustentables (CIBAS), Universidad Católica de la Santísima Concepción, Chile.
[4] Center for Ecology and Sustainable Management of Oceanic Islands (ESMOI), Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile (ESMOI).
[5]Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de [6]Concepción, Concepción, Chile.

[6]Instituto Milenio de Oceanografía (IMO), Universidad de Concepción, Concepción, Chile.

[7]Centro de Investigación Oceanográfica COPAS Coastal, Universidad de Concepción, Concepción, Región del Bio Bio, City, 4070043, State, Chile.

Introduction

Marine ecosystems are subject to several anthropogenic stressors, with underwater noise pollution emerging as a significant concern in recent years, and it is considered a new source of pollution in several international agendas, such as the European Commission, IMO 2014, OSPAR 2017, and UN 2018. Among the sources of underwater noise, ships and outboard boats play a substantial role. Today, we know that 80% of world trade commerce its sustained by Vessels (United Nations Conference on Trade and Development, 2020) and the number of ships and capacity it's expected to increase in 87 – 102% between 2016 and 2030 (Kaplan & Solomon 2016). Consequently, the sound pressure levels emitted by these ships are also expected to increase, particularly in heavily trafficked coastal areas such as ports and straits. Vessel transit, emit low frequency sounds, rising the sound pressure levels (SPL) specially in 63, 126 and 251Hz octave bands (Vieira et al. 2021). In this range of frequencies, there are also several geological and oceanographic sounds (geophonies), such as earthquakes, waves, and biological sounds (biophonies), such as mysticetes songs (Širović et al. 2007; Buchan et al. 2014; Redaelli et al. 2022) and fish choruses (Popper & Hawkins 2019).The impact of noise pollution on marine organisms has gained attention because of its potential to disrupt crucial ecological processes and induce physiological stress in several taxa (Erbe et al. 2019; Murchy et al. 2019; Di Franco et al. 2020; Popper et al. 2020; Duarte et al. 2021). This evidence suggests that underwater noise can influence the behavior, physiology, and overall fitness of various marine organisms. Molina-Valdivia et al. (non-published data) recently described and quantified the noise emitted by vessels near three marine protected areas along the Chilean coast. Their findings revealed contrasting levels of noise, patterns, and free noise times between the areas. The site with a higher presence of vessel noise and free noise time did not exceed 15% in any season of the year, whereas the site with less presence reached 72% of the free noise time. These results highlight the need for management measures to address this new pollution source.

While previous research has predominantly focused on the effects of noise on large marine mammals and fish, invertebrates have been somewhat overlooked. However, studies evaluating the impact of vessel noise on invertebrates have revealed various negative effects. For instance, Wale et al. (2019) observed DNA damage, changes in filtration rates, and oxygen consumption in common mussel (*Mytilus edulis*). Charifi et al. (2018) found reduced valve opening and decreased filtered volume under chronic vessel noise conditions in pacific oyster (*Magallana gigas*). In crustaceans, Wale et al. (2013a) reported disrupted feeding and slower escape responses in the presence of their predator in the European green crab (*Carcinus maenas*). Tidau & Briffa (2019b, 2019a) found changes in shell size selection times and aggregation behaviors in the hermit crab (*Pagurus bernhardus)*. Physiologically, Wale et al. (2013b) observed increased oxygen consumption in *C. maenas* when exposed to vessel noise, and Ruiz-Ruiz et al. (2019) documented alterations in reproductive behavior and heightened oxygen consumption in the rocky shrimp *Rhynchocinnetes typus*. While studies have increasingly highlighted the impact of underwater noise pollution on marine organisms, they have primarily focused on the adult life stages of organisms. Smaller planktonic stages and holoplanktonic invertebrates have been notably underrepresented in the research on the effects of underwater noise. These invertebrates play a key role in coastal ecosystems by acting as link in the carbon flux between trophic levels. To comprehensively understand the broader ecological consequences within coastal ecosystems, it is essential to include these organisms in the assessment of the potential effects of vessel noise (Gill et al. 2015; Williams et al. 2015; McCauley et al. 2017; Wale et al. 2021).

With the aim to contribute to fill this knowledge gap, this study examinate the effects of vessel noise in *Neomysis sp*. a Mysidacean species that inhabits the coastal waters of Chile (Cornejo 2000) in a controlled laboratory setting. Mysidaceans are holoplanktonic invertebrates that migrate from deeper layers during the day to the upper layers at night and are commonly found in nearshore environments. Mysidaceans represent an essential component of the food web and act as both prey and predators (Punchihewa & Krishnarajah 2013). The physiological effects of vessel noise are assessed by quantifying the metabolic impact of noise exposure through changes in oxygen consumption rates, allowing us to gain insight into the potential energetic costs imposed by underwater noise. By studying the effects of underwater noise pollution on key species, we can contribute to a more comprehensive understanding of the ecological consequences of anthropogenic noise. This knowledge can aid in the development of mitigation strategies and formulation of sound management policies aimed at minimizing the impacts of underwater noise pollution on vulnerable marine organisms.

Methods

Acquisition and Acclimation

Mysidaceans (*Neomysis spp*.) used in this study were collected near a *Macrocystis pyrifera* forest in Talcahuano Bay (36°41’23” S – 72°58’29” O). The organisms were capture using snorkeling with a hand net and were subsequently transported to the Estación de Biología Marina Abate Juan Ignacio Molina of the Universidad Católica de la Santísima Concepción in Lenga (36°45’39” S – 73°10’28” O), which is located in the bay of San Vicente, adjacent to Talcahuano. During transportation, the mysidaceans were kept in a thermal container (approximately 13°C) with constant aeration. Once in the laboratory, the organisms were acclimated for one week at a temperature of 13°C, the same temperature as their natural environment. During acclimation, constant aeration was maintained, and mysidaceans were fed with *Artemia salina* nauplii aged less than 24 hours after hatching. On the eighth day of acclimation, the mysidaceans were randomly selected, divided into groups of 8 male and female individuals without eggs, and subjected to a 24-hour pre-treatment period with no food provided. This action was taken to prevent digestion-related metabolism influencing oxygen consumption during subsequent treatments. It important to note that during the 7-day acclimation period and the 24-hour pre-treatment, the individuals were kept in silence, isolated from ambient laboratory noise.

Critical Oxygen Pressure Point ($\mathbf{P_{crit}}$)

Prior to conducting the experiments, it was necessary to determine the exposure times for each treatment, considering the well-being of the organisms and preventing hypoxic conditions from inducing changes in metabolic rates. To achieve this, the critical oxygen pressure point was determined, this is defined as the oxygen pressure at which an organism is no longer capable of sustaining its basal metabolism (Wood 2018). Then, six specimens were randomly selected, excluding the ovigerous females. The specimens were kept without food for 24 hours prior to measurements and then individually placed in 0.025 L glass respirometry chambers with enough volume to allow movement during the experiments. All respirometry chambers were submerged in a controlled temperature bath at 14°C. The respirometry chambers were filled with filtered seawater (0.2 µm) and equipped with a Pyro science Fiber optic oxygen sensor (oxrob10). Oxygen sensors were connected to a console Fire Sting O_2 that measured the partial pressure of oxygen every 2 minutes. Once the experiment started, the mysidaceans were left undisturbed to acclimate for at least 30 minutes and then allowed to deplete the oxygen inside the respirometry chamber until there was no movement in the thoracic appendages and pleopods. Subsequently, the specimens were wet weighted to determine the metabolic rate.

Noise treatment and Experimental design

Once the exposure time was determined (90 min), mysids that successfully passed the acclimatization and 24-hour fasting were randomly selected and individually placed in 4 hermetic chambers, under the same respirometry setup described above. A nested ANOVA design was applied, consisting of four treatments and a control group. Each treatment involved the playback of a 90-minute recording. During the mysids culture viability trials, the specimens responded with accelerated and erratic swimming after manipulation. Therefore, the initial 30 min consisted in ambient sound, followed by the actual treatment for the subsequent 60 min. This approach aimed to mitigate any potential behavioral effects caused by handling the individuals that could influence metabolism and therefore oxygen consumption.

To design the playbacks for each treatment, 16 sounds were selected from underwater soundscapes in Concepción Bay, including 8 sounds from outboard motor noise and 8 sounds from large vessel activities, recorded with a hydrophone (Soundtrap ST600, sampling rate: 98kHz, flat sensitivity: -176). The playbacks were created using Audacity 3.2.3. Under a nested ANOVA design, four types of outboard motorboat noises were tested for each treatment, and the same design was used for the merchant vessel noise. Four individuals were exposed to each type of noise, resulting in a total of 16 individuals per treatment, in addition to 16 individuals in the control group (N = 80). This design aimed to avoid bias associated with pseudo-replication that could occur when using only one type of engine noise or a mix of them (Kroodsma et al., 2001).

The outboard motorboat noise factor consists of two levels of noise found by Molina-Valdivia et al. (unpublished data) in Chañaral Island, Puñihuil, and Corcovado Gulf. The first level had 16 minutes of noise evenly distributed within the 60-minute treatment (72% free-noise time), while the second level had 31 minutes of noise evenly distributed (48% free-noise time). For the merchant vessel noise factor, a similar approach was carried out, with the first level having 16 minutes of noise evenly distributed within the 60-minute treatment (72% free-noise time), and the second level with 51 minutes of noise (15% free-noise time). The respective treatments' sounds were played back through a submersible speaker (University Sound UW30, Nominal frequency response: 100 Hz - 10 kHz; Maximum output level: 153 dB at 150 Hz; http://www.lubell.com/UW30.html), positioned at least 1 meter away from the respirometric chambers, also the chambers were placed 50 cm away from the tank's edges (Fig. 1). These distances were chosen to prevent resonance of the noise on the tank walls, which could exaggerate sound intensity and bias individual responses (Wale et al., 2021). To ensure that the sound pressure levels emitted by the speakers were representative of those recorded in the marine environment, a calibrated hydrophone (Soundtrap ST600 HF, 90 min continued recording cycle with a sampling rate of 48kHz), was installed within the tank. The hydrophone was placed at the same distance and depth as the respirometry chambers.

Statistical Analysis

To determine the P_{crit} segmented linear models were fitted to the relationship between metabolic rate and oxygen pressure. Following the experimental design, nested ANOVA was performed to test the differences between noise treatments. Statistical analyses were performed in R version 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org).

Results

Respirometry and P_{crit}

During the experiments, no mortalities were recorded among any of the six individuals; however, abrupt movements and agitated swimming were observed during the first 15 min after the specimens were placed in respirometry chambers. Subsequently, the behaviour of mysidaceans shifted to a resting state with constant movement of their appendages and occasional vertical swimming. This pattern persisted until approximately 160 min, when vertical swimming became more frequent. At 180 min, the mysidaceans positioned themselves at the bottom of the respirometry chambers, and their appendages exhibited progressively slower movements until no movement was recorded, and then the experiment stopped.

According to the segmented linear models (Table 1), a P_{crit} of 42.611 ± 9.070 mmHg (mean ± SD) (Fig. 1) was estimated between 170 and 250 min from the start of the experiments (Fig.2). Therefore, for the noise exposure experiments, a prudent exposure time of 60 min plus 30 min of acclimation to the respirometry chambers (totalling 90 min) was determined. This approach was adopted to avoid potential effects due to manipulation and hypoxia within the chambers.

Table 1. Segmented linear model parameters and P_{crit} estimated for each individual. WW: wet weigh; SE: Standard Error.

N	Sex	WW (g)	P_{crit} (Hgmm)	SE	*P*
1	F	0.013	39.861	2.334	<0.05
2	M	0.011	27.414	1.181	<0.05
3	M	0.009	42.223	6.441	<0.05
4	F	0.010	52.355	6.831	<0.05
5	F	0.0144	51.558	6.909	<0.05
6	M	0.0128	42.260	7.094	<0.05

A

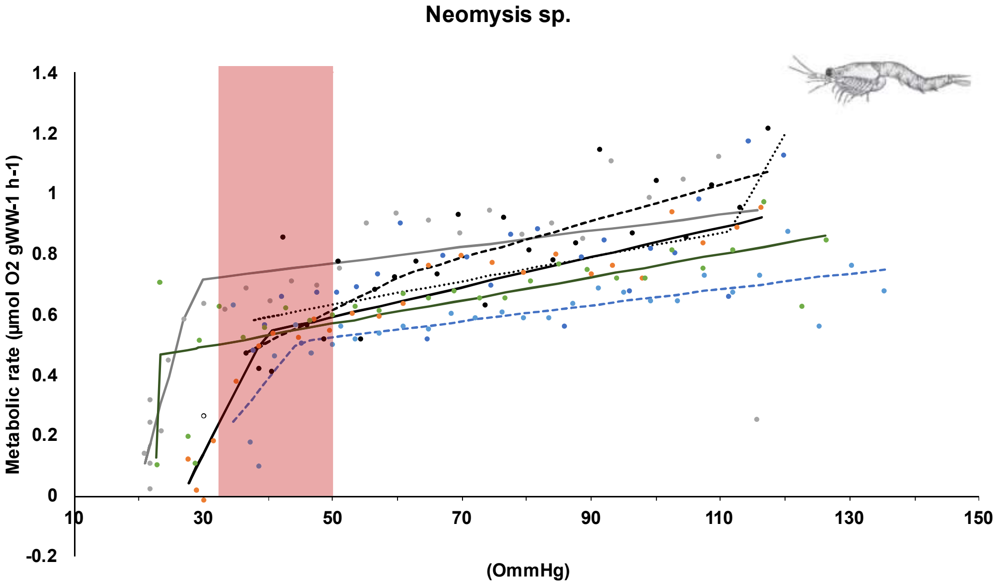

Figure 1. Relation between partial pressure of oxygen and the metabolic rate for each organism. The red area represent the P_{crit} zone estimated with the segmented linear models.

B

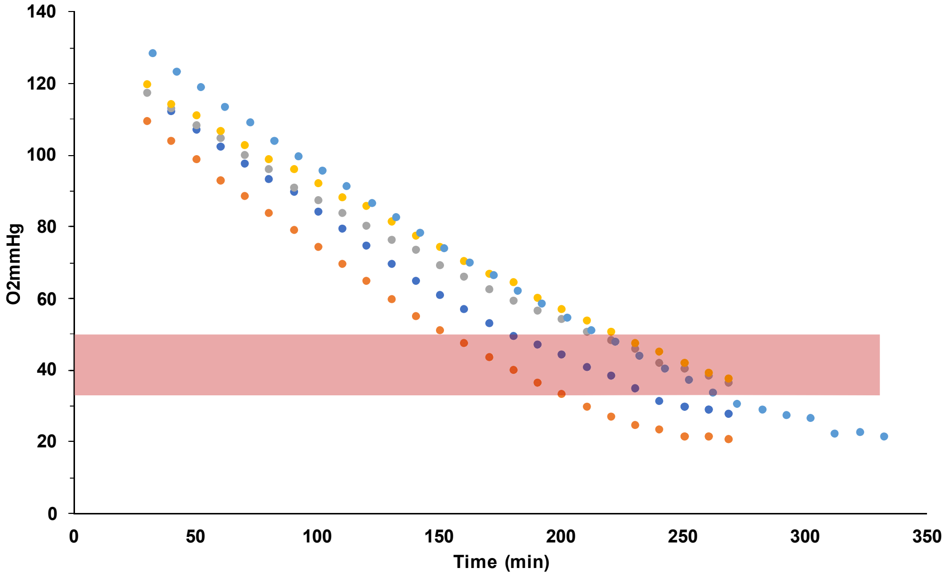

Figure 2. Relation between time and partial pressure of oxygen. The red area represents the P_{crit} zone.

Noise exposure treatments

Unfortunately, the speakers and amplifier used in the experiments experienced a malfunction and did not reproduce the complete soundscapes, specifically failing to emit sounds between 100 – 1400Hz (Fig. 3). This frequency range encompasses the peak frequencies of the two types of vessel noise that were intended to be tested. Furthermore, above 1400Hz the sound pressure levels rise but were quite different compared to those measured in field. Then no experiment could be carried out.

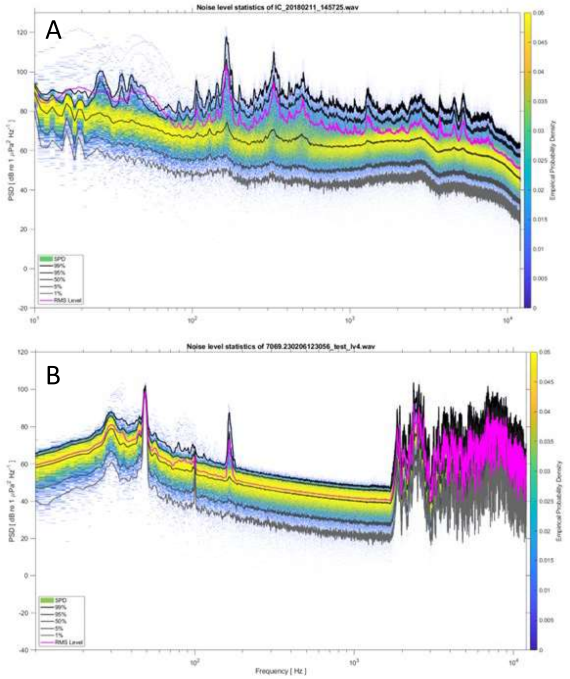

Figure 3. Comparison between of sound pressure levels (SPL) emitted by a motorboat in Chañaral Island (A), and (B) the same motorboat but emitted in the experimental tank (3m diameter).

Discussion

Despite the inability to conduct the experiments, the P_{crit} of these organisms could be determined, which is particularly relevant considering that this species inhabits bays where upwelling events may reduce oxygen levels, leading to mysidaceans strandings in beach. In fact, Hernández-Miranda et al. (2017) described one of this events following the influx of hypoxic waters, hypothesizing that Neomysis sp.

possesses a hypoxia resistance threshold of 1 mL O_2 *L^{-1}, a value slightly lower than the one obtained in the respirometry (2.12 mL O_2 *L^{-1}).

CONCLUSIONES GENERALES

En este estudio, se ha evidenciado cómo la presencia de embarcaciones y los niveles de presión sonora varían entre estaciones y localidades. Por ejemplo, áreas con bajos niveles de ruido como Isla Chañaral, donde el 72% del tiempo se encuentra libre de ruido y presentó un marcado patrón circadiano en la presencia de embarcaciones y los niveles de presión sonora en las octavas de 63Hz y 126Hz. Por otro lado, el Golfo Corcovado con presencia casi permanente de embarcaciones (15% libre de tiempo libre de ruido), y variaciones estacionales de los niveles de presión sonora. Los potenciales efectos de los constantes niveles de presión sonora generados por el tránsito de embarcaciones son especialmente relevantes para aquellos organismos sensibles a los sonidos de baja frecuencia en las octavas de 63 y 126 Hz. Por ejemplo, misticetos o ballenas con barbas y peces, que emiten y perciben sonidos que son importantes para la comunicación, orientación y detección de presas en este rango de frecuencias. Por lo tanto, el enmascaramiento de estas señales podría tener consecuencias en el comportamiento y alimentación de estos organismos. En este escenario es importante considerar el marco legislativo actual con el objetivo de analizar medidas manejo que se podrían implementar en los presentes y futuros proyectos que se lleven a cabo en el medio marino. En este sentido, hoy solo se han planteados criterios sin fuerza de ley, para futuros proyectos, y no para los actuales. Por lo tanto, se necesitan un marco normativo que permita implementar medidas de manejo efectivas en la reducción de los niveles de presión sonora desde fuentes antropogénicas, en este caso embarcaciones, sobre todo, en cercanías a áreas marinas costeras protegidas con una alta relevancia ecológica como las mencionadas en este estudio. Si bien, ya existen estudios en otras partes del mundo que muestran los efectos positivos de la implementación de medidas como la reducción de velocidad y la restricción de acceso a las embarcaciones, debemos reconocer que implementar estas medidas en zonas geográficas altamente fragmentadas como Golfo Corcovado y la zona de fiordos y canales, es especialmente desafiante. Ya que las embarcaciones son imprescindibles en el transporte y abastecimiento de la comunidad local y en el desarrollo de la acuicultura.

Es importante mencionar también, que se necesitan más información científica sobre los efectos del ruido antropogénico en las especies chilenas. Actualmente, estos son escasos y no representan la gran variedad de los organismos que habitan esta costa.

En este sentido, los resultados de los experimentos de respirometría en misidaceos proporcionan información valiosa, a pesar de las dificultades técnicas encontradas en los tratamientos de exposición al ruido. No se produjeron muertes durante los experimentos y los misidáceos mostraron cambios de comportamiento, con un $\mathbf{P_{crit}}$ de oxígeno de 42,610 ± 9,070 mmHg.

El tiempo de exposición establecido de 90 minutos pretendía mitigar los impactos potenciales de la manipulación y la hipoxia dentro de las cámaras de respirometría. Sin embargo, una avería en el equipo impidió la ejecución de los experimentos de exposición al ruido, lo que afectó especialmente al examen previsto de los efectos del ruido en los rangos entre 100 y 1400 Hz.

A pesar de este contratiempo, el $\mathbf{P_{crit}}$ determinado tiene importancia, especialmente para los misidáceos que habitan bahías donde ocurren eventos de surgencia. Estos resultados concuerdan con registros previos de varamientos de cardúmenes de misidaceos en presencia de aguas hipóxicas con 1 mL O2 $*L^{-1}$, levemente menores a los 2,120 mL O2$*L^{-1}$ determinados en este estudio. Finalmente, a pesar de las limitaciones del experimento, el estudio aporta información valiosa para comprender las respuestas fisiológicas de los misidáceos a los factores de estrés ambiental, lo que subraya la importancia de seguir investigando en este ámbito considerando al ruido submarino como una nueva fuente de estrés.

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Universidad Católica de la Santísima Concepción

ACTA DE EXAMEN DE GRADO

En Concepción de Chile, a 29 de DICIEMBRE de 2023 , vista y revisados los requisitos de Título/Grado presentados por:

Don **VICTOR ALEXANDER MOLINA VALDIVIA**

RUT 18537798-9

Alumno de la Carrera de **MAGISTER EN ECOLOGIA MARINA**

Sede **CONCEPCIÓN** Jornada **DIURNO**

De la Universidad Católica de la Santísima Concepción, la Comisión Examinadora ha otorgado las siguientes calificaciones:

"PATRONES ESTACIONALES Y CIRCADIANOS DEL PAISAJE SONORO SUBMARINO Y RUIDO ANTROPOGENICO EN LA COSTA CHILENA, EFECTO SOBRE EL CONSUMO DE OXÍGENO EN UNA ESPECIE DE MISIDÁCEO"

Nombre	**Calificación**
ÁNGEL URZÚA OSORIO **MIEMBRO INTERNO COMISIÓN DE TESIS**	**6,70 (SEIS , SETENTA)**
MAURICIO LANDAETA DIAZ **MIEMBRO EXTERNO COMISIÓN DE TESIS**	**6,70 (SEIS , SETENTA)**
IVAN HINOJOSA TOLEDO **DIRECTOR DE TESIS**	
SUSANNAH BUCHAN . **CO-DIRECTOR DE TESIS**	
JORGE LEON MUÑOZ **MINISTRO DE FE**	
CALIFICACION FINAL DE EXAMEN	**6,70 (SEIS , SETENTA)**

SECRETARIO ACADÉMICO **DECANO**