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Temperate Mesophotic Soundscape Diversity and Pollution

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ABSTRACT

Temperate Mesophotic Ecosystems have both economic and ecological importance, however, they are currently understudied and scarcely protected, leaving them vulnerable to anthropogenic and environmental disturbances. In recent years, novel methodologies have facilitated the study of these ecosystems. One of these methods is acoustic monitoring, which can measure the diversity and health of ecosystems and can reach depths and species that are not easily accessible using other methods. Several acoustic indices have been developed to measure diversity using the soundscape, however these have mostly been used in terrestrial ecosystems. In this study, we analysed the soundscape of two mesophotic ecosystems in the central coast of Chile to assess the reliability of acoustic indices in marine ecosystems by comparing them with diversity measures based on environmental DNA (eDNA). In addition, the effects of the emergence of Oxygen Minimum Zones (OMZ) on the soundscape and acoustic indices was explored.

To this end, first fish sounds from the soundscape were characterised and counted. Next, from the eDNA data, species richness was calculated and based on the acoustic data, eight indices and a soundscape metric, Sound-Pressure Level (SPL), were measured for two frequency bands. From this, a principal component analysis was performed. The first principal component was subsequently used for further analysis. Both qualitative and quantitative analyses were conducted to compare the indices and soundscape to the eDNA data and study the effect of hypoxic conditions on the acoustic indices and SPL.

It was found that there was substantial difference between indices, showing that there is a lot of variability in what they measure. Furthermore, there were similarities in the diversity based on the eDNA and some acoustic indices, and in particular a combination of indices reflected the species richness well. Both sites showed significant variability, with a higher diversity observed near a Marine Protected Area (MPA). Moreover, similar results were found in terms of number of sound-producing species and number of signals detected in the soundscape, both being higher in Algarrobo. Finally, the results showed that there was no significant difference in the results of the indices between oxygen categories.

PREFACE

My journey to this thesis started a couple of years ago, when I decided to study how music and sound are important in an environmental and ecological context. The focus was originally on how music can drive social change to tackle environmental and sustainability issues. Along the road, which had many twists and turns, I found myself diving into the role of sound in ecosystems. I was in awe that we knew so little about it, despite sound being ubiquitous in the living world. How could it not, then, be present in most processes of living things, especially underwater where it travels so much farther than on land? Thus, when the possibility to write my thesis about the soundscape of a poorly studied ecosystem was presented to me, it seemed the perfect way to bring my master's to an end.

This research was conducted as part of my master's thesis, to complete the programme Sustainable Development at Utrecht University. I am incredibly grateful to the many people that supported me along this journey and enabled me to dive into a topic I am so passionate about. First and foremost, I would like to thank my UU supervisor, Dr. Angeles Garcia-Mayor, who very quickly brought me into contact with the researchers with whom I could conduct my research, and who provided valuable guidance and support throughout the entire process. I also need to thank Dr. Alex Genin, who was the link between UU and the marine research centre (ECIM) of the Pontificia Catholic University of Chile, for always being willing to help and offering important feedback on my work. I am also extremely grateful to my supervisor there, Dr. Ricardo Beldade, who took me on board the project and gave me so much of his time and guidance in the exploration of this new field.

This research was also a contribution to the NUTME project based at ECIM. I am grateful for the entire team of this project for their support and readiness to help me through the problems I encountered, as well as conducting the technical aspects of the data collection and processing. I also had the opportunity to visit the lab at the Austral University, where I was guided through the processing of eDNA data. For this, I would like to thank Paula Ramirez Moenne-Loccoz who took me under her wing and was always ready to answer the many questions I had. Further, I want to thank Mauro Zucconi Ramirez for the countless hours spent assisting me in R. Finally, I am grateful to all the members of the lab who made the challenges I encountered along the way so much lighter.

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LIST OF ABBREVIATIONS

ACI	Acoustic Complexity Index
AEI	Acoustic Evenness Index
ASV	Amplicon Sequency Variance
BI	Bioacoustic Index
dB	Decibel
DOC	Dissolved Oxygen Content
eDNA	Environmental DNA
GLM	General Linear Model
H	Acoustic Entropy
Hz	Hertz
M	Median of the amplitude envelope
MPA	Marine Protected Area
NDSI	Normalised Difference Soundscape Index
OMZ	Oxygen Minimum Zone
PAM	Passive Acosutic Monitoring
PC	Principal Component
PCA	Principal Component Analysis
SE	Spectral Entropy
SPL	Sound-Pressure Level
TE	Temporal Entropy
TME	Temperate Mesphotic Ecosystem

1. INTRODUCTION

Whereas shallow reefs have been abundantly studied, their mesophotic counterparts, especially in temperate regions, have been, to a large extent, left in the dark. In recent years, novel technologies, and methods, such as acoustic monitoring and eDNA, have broadened the scope of research capabilities, making the study of mesophotic ecosystems more accessible. This research investigates the diversity and susceptibility of a Temperate Mesophotic Ecosystem (TME) to changing conditions, and as such contributes to the 14th sustainable development goal, life below water, which aims to "conserve and sustainably use the oceans, seas and marine resources for sustainable development" (UN, 2021, p.54)

1.1. TEMPERATE MESOPHOTIC ECOSYSTEMS

TMEs occur at depths of 30 to 150m (Slattery & Lesser, 2012), with diversity generally being highest at 30m where disturbances occur most frequently (Williams et al., 2019). Despite limited knowledge, TMEs are undeniably intrinsically and economically important, containing rare species such as red and black corals, as well as fisheries-targeted species providing essential fishery resources (Soares et al., 2020; Williams et al., 2019) TMEs also have the potential to be significant sources of biotechnological products and function as vital carbon sinks (Soares et al., 2020). An increase in knowledge in their diversity, composition and vulnerabilities can shine a light on their ecological and economic value. This would allow us to better predict how the system will react to changing conditions and anthropogenic activities which would ultimately facilitate efficient protection and conservation strategies. This study took place at two sites off the coast of central Chile, a region which is dominated by the Humboldt Current System, resulting in it being one of the most productive coastal areas in the world, supporting a high diversity of benthic, demersal and pelagic species (Hernández-Miranda et al., 2012).

1.2. EMERGING TECHNOLOGIES TO SAMPLE MESOPHOTIC REEFS

TMEs are not well studied and are difficult to access but new technologies are able to better characterise these ecosystems. Over the last decade, passive acoustic monitoring has shown to be a valuable asset in the investigation of soundscapes in marine ecosystems (Nedelec et al., 2015; Lin et al., 2021; Kaplan et al., 2015; Lamont et al., 2022). Soundscapes are composed of biological, environmental, and anthropogenic information through sound production and propagation within the ecosystem (Lin & Tsao, 2020). There are around 800

species of fish that are known to produce sounds, out of an estimate of 33,000 fish species on earth (Popper & Hawkins, 2019). Reefs in particular are inhabited by many soniferous organisms, including fish and invertebrates (Lammers et al., 2008). The sound they produce can come from a variety of biological processes, including mating (courting and spawning), feeding, competition, social cohesion and recruitment (Mooney et al., 2020; Radford et al., 2008; Lillis et al., 2013; Piercy et al., 2014; Montgomery et al., 2006).

Soundscape analysis can be used to determine abundance, diversity, density of species and individuals, and ecosystem health (Kikuchi et al., 2015; Nedelec et al., 2015; Lin et al., 2021; Kaplan et al., 2015; Lamont et al., 2022; Piercy et al., 2014), and has advantages over other methods that evaluate the same parameters. For instance, cryptic species, such as the snapping shrimp, are difficult to detect visually, but can easily be picked up with hydrophones (Mooney et al., 2020). The soundscape can also detect disturbances, biological responses to disturbances, as well as changes in environmental conditions that influence sound production and propagation (Bertucci et al., 2017). Moreover, hydrophones can be deployed for extended periods while recording continuously or intermittently, providing long term data which is needed to adequately quantify processes and disturbances such as noise pollution. Certain hydrophones also provide access and data from ecosystems that are beyond reach of more traditional methods, which is the case for mesophotic reefs (Aguzzi et al., 2019). However, soundscape analysis is not without its limitations. For instance, it cannot cover all the diversity present, as it can only give an account of sound-producing species. Such limitations are being addressed by combining different relatively novel techniques to characterise local communities.

Environmental DNA (eDNA) refers to the DNA traces sampled from the environment that through metagenomics offer information regarding community composition (Taberlet et al., 2018). This method offers the possibility to monitor species diversity, at a lower cost than is possible with traditional methods (Rees et al., 2014) especially in mesophotic reefs. So far it has been used to detect abundance of species (Lacoursière-Roussel et al., 2016) for species identification and monitoring (including rare and invasive species) (Sepulveda et al., 2019; Jerde et al., 2011), diversity of assemblages (Di Capua et al., 2021), and species or taxa composition (Closek et al., 2019; Stat et al., 2019). Because eDNA ultimately gets broken down, it is assumed to provide a good indication of the presence of a species at a particular point in time, alive or recently deceased (Taberlet et al., 2018). However, since it can also enter a particular environment by travelling with currents (Watts & Miksis-Olds, 2018), or by other sources including sewage and through faeces from other animals (Rees et al., 2014), it is not

always an unbiased representation of an ecosystem's composition. Since eDNA has only been developed and applied recently, many studies have combined it with other (traditional) methodologies to compare its accuracy. In deep marine systems, these include baited remote underwater video (BRUV) (Stat et al., 2019; Jeunen et al., 2020), bottom trawling (Closek et al., 2019; Stoeckle et al., 2021), tow-nets (Kelly et al., 2017), and visual fish survey (Port et al., 2016). Although eDNA is very efficient in detecting organisms, significantly reducing the required personal effort and time (93 to 0.174 days person effort for positive detection) (Rees et al., 2014; Jerde et al., 2011), eDNA faces "blind spots" (Jeunen et al., 2020). These consist of elements including variability in PCR efficacy, sensitivity of the assay, amplicon length, and primer degeneracy, which lead to failure to detect certain species (Jeunen et al., 2020). Thus, using a combination of methods can mitigate these errors. So far, studies have used eDNA primarily to assess diversity and assemblages of fish and other vertebrate organisms (Closek et al., 2019; Thomsen et al., 2012; Miya et al., 2020), with few studies assessing invertebrate diversity, such as the one conducted by Di Capua et al. (2021) on metazoan diversity and this master thesis.

1.3. THREATS TO TEMPERATE MESOPHOTIC ECOSYSTEMS

TMEs are scarcely protected and face many threats brought forth by anthropogenic activity (Rocha et al., 2018), including fishing-associate habitat destruction by trawlers and pollution (plastic and discarded fishing gear) (Smith et al., 2019). Other threats comprise increased sedimentation rate and turbidity, mining, and other previously unseen anthropogenic pollutants such as noise originating from human activity (Smith et al., 2019; Rodríguez et al., 2021; Lin et al., 2021). In addition, the expansion in area and potentially frequency of natural events, such as the emergence of Oxygen Minimum Zones (OMZs) related to climate change are also likely to impact these ecosystems residing at greater depths (Smith et al., 2019; Stramma & Schmidtko, 2019).

The study site in the central coast of Chile is especially vulnerable to the latter, as the Humboldt Current System contains a sub-surface water mass characterised by high nutrient and low oxygen concentrations, which leads to an extensive OMZ. This study consists of two sites, one of which is more prone to upwellings than the other (Aiken et al., 2008; Ferreira et al., 2018). This has an influence in the community composition of the ecosystem, for instance comprising species more tolerant to low oxygen levels (Gallo & Levin, 2016). During the spring and summer, coastal upwelling events happen frequently, in which this sub-surface

water is brought to the surface (Thiel et al., 2007; Hernández-Miranda et al., 2012; Pizarro-Koch et al., 2019), leading to severe hypoxic conditions and mass mortality of the benthic and pelagic fauna (Hernández-Miranda et al., 2012). Upwelling events are caused by wind-stress that push the warm water away from the coast, replacing it by cold water that emerges from the deep ocean (Wright et al., 2012). This results in decreased temperatures and oxygen levels and increased nutrient concentrations which have damaging effects on the ecosystems (Smith et al., 2019). When an ecosystem experiences hypoxia for a long period of time, the annual secondary production, biomass growth of heterotrophic organisms, becomes low, and the benthic fauna disappears (Diaz & Rosenberg, 1995). According to Altieri et al. (2017), coral reefs can experience mass mortality due to low oxygen concentrations, which is observed to be worse at greater depths, making TMEs more vulnerable to such events. Furthermore, hypoxia can make reefs more susceptible to coral white plague disease, which is also more prevalent in mesophotic reefs (Chaves-Fonnegra et al., 2021). Research in temperate mesophotic ecosystems has shown that recovery from hypoxic conditions can take decades (Hughes et al., 2020).

1.4. TMES, OMZS AND eDNA

The soundscape can be a powerful tool in monitoring biodiversity changes related to specific environmental conditions, such as temperature, pH, and oxygen. This allows passive acoustic monitoring to be used to monitor health of ecosystems and environmental change that arises because of climate change and habitat degradation. Passive acoustic monitoring (PAM) is a semi-automated method that allows continuous or semi-continuous data collection over large periods of time. Post-sampling data treatment is equally becoming automated with examples mostly from terrestrial systems holding much promise (Thomas & Davison, 2020). eDNA is rapidly growing in popularity as a biodiversity assessment tool, however sampling is not yet automated and metagenomic processing is expensive and requires bioinformatic expertise. Sampling of infrequent events such as OMZs can benefit from continuous or semi-continuous sampling afforded by passive acoustic monitoring. However, there have been very few studies looking into the effect of environmental conditions on the soundscape. Most have focused on temperature or wind speed (Bruce Martin & Cott, 2016; Putland et al., 2017; Monczak et al., 2019; Ceraulo et al., 2020). At the time of publishing, there were no publications on the effect that OMZs have on the soundscape known to the author. This is an important knowledge gap that is ecologically

relevant as upwelling events and OMZs are likely to have a strong influence on sound production and propagation.

It is known that factors such as water density, temperature and pressure, have an influence on the attenuation of sound (Larsen & Radford, 2018), and these are often paired with upwelling events and OMZs. Moreover, low oxygen levels have severe negative consequences on aerobic organisms. According to Vaquer-Sunyer & Duarte (2011) hypoxia lowers the survival rate of benthic organisms to 74%, thus likely reducing the overall sound level and source diversity of the soundscape. OMZs are also expected to alter community composition and diversity, with mobile organisms avoiding hypoxic zones, instead finding refuge in shallower areas. The species left behind would be those that have a higher tolerance to oxygen-poor conditions (Galic et al., 2019). In addition, it influences interactions between fish, such as increased aggression and dominance, predator-prey interactions, changes in parental care, behaviour of fish schools, and reduced reproductive activity (Chapman & Mckenzie, 2009; Galic et al., 2019). Studies have documented both an increase and decrease in activity under oxygen stress for both fish and invertebrates. In general, under hypoxia suppressed metabolic activity leads to decreased activity level for both fish and invertebrates. However, under severe hypoxia the activity increases as an acute escape response (Galic et al., 2019; Champan & McKenzie, 2009; Diaz & Rosenbert, 1995). Chapman & McKenzie (2009) observed that demersal bentho/pelagic fish species decreased their activity under oxygen stress, in contrast to pelagic schooling fishes which became very agitated.

1.5. RESEARCH AIMS, QUESTIONS AND HYPOTHESES

The goals of this research are (1) to compare the diversity of fish and invertebrates in two sites within a temperate mesophotic ecosystems with different historical upwelling regimes (Aiken et al., 2008; Ferreira et al., 2018) in the central coast of Chile using acoustic biodiversity indices and community diversity assessed by eDNA and (2) to assess how the diversity detected by acoustic indices is affected by seawater oxygen levels, thus determining the relevance of the soundscape as a methodology to study changes in mesophotic ecosystems as a function of OMZ emergence. In addition, a first description of the soundscape of mesophotic ecosystems is provided, including the spatial variability thereof in terms of biological and anthropogenic noise. Following this, the first research question is stated as follows:

RQ1: "*How diverse are TMEs according to their soundscape and genetic organismal traces identified with eDNA?*"

The second research question is:

RQ2: "*How do oxygen levels influence the soundscape of a temperate mesophotic ecosystem?*".

The hypotheses per research questions are:

RQ1:

H1: There is a high variability in species richness and acoustic diversity between the two sites, with a higher diversity (both acoustic and eDNA) in Las Cruces.

H2: Several acoustic indices are strongly related to species richness.

RQ2:

H3: There is a significant correlation between oxygen and the acoustic indices.

H4: The acoustic indices differ between contrasting oxygen levels.

2. METHODS

This research consisted of two methodologies: eDNA and PAM. The latter was conducted semi-continuously for a period of 75 days, whereas sampling for eDNA occurred twice, once before and once during the time that the acoustic monitoring was taking place (figure 1). The data collection for both methods was conducted in the same location, at the same depth. Oxygen data was collected alongside the acoustic data, providing continuous data for the entire period.

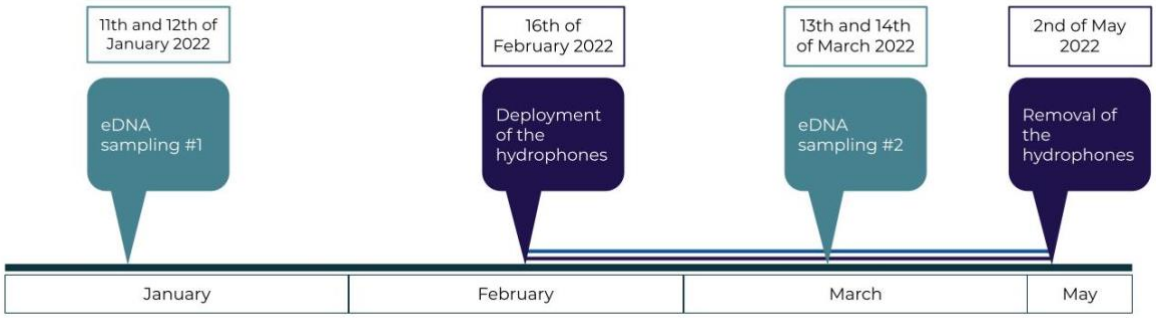

Figure 1. Timeline of the data collection.

The eDNA data and acoustic data (recordings) were further processed into diversity measures for two groups: fish and invertebrates, which corresponded with low and high frequency bands. A number of analyses, both quantitative and qualitative were performed on these indices, as well as on the raw data. Figure 2 gives an overview of the research design.

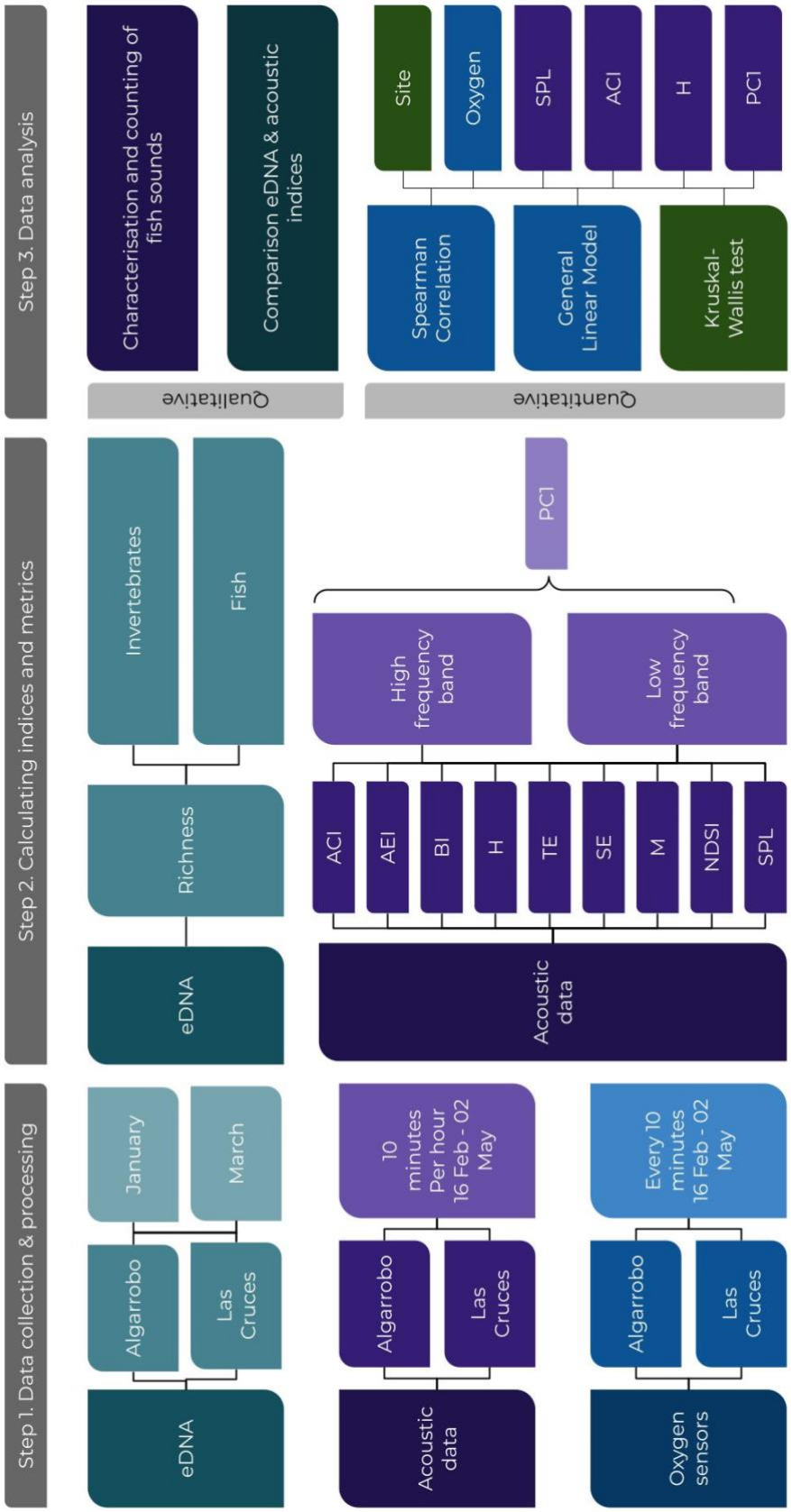

Figure 2. Research design.

2.1. STUDY AREA

The focus of the study was on temperate mesophotic reefs in the central region of Chile at two study sites located roughly 2km from the coastline, one near the Algarrobo and the other off the coast of Las Cruces (figure 3). Data collection spanned between January and April 2022. The study sites were chosen based on the prevalence of upwellings and their similarity in oceanographic processes. More specifically, at one site upwellings occurred more frequently (Algarrobo), whilst at the other they were less common (Las Cruces) (Aiken et al., 2008; Ferreira et al., 2018). eDNA and PAM were used at each site over rocky substrate at 30m depth.

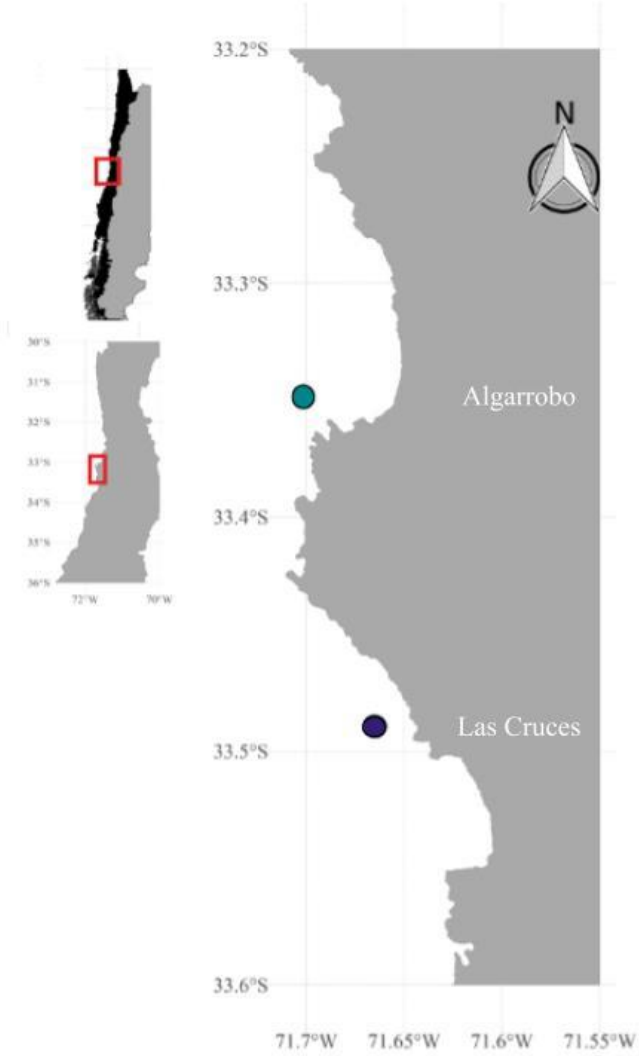

Figure 3. Map of sampling sites. The green dot represents the site near Algarrobo, where upwelling is more frequent. The purple dot represents the site near Las Cruces, where upwelling is less frequent, and which is close to a marine protected area.

2.2. SAMPLE COLLECTION AND PROCESSING

2.2.1. eDNA

2.2.1.1. SAMPLING

eDNA was sampled in the proximity of the hydrophones in the months of January and March. For more details on the eDNA sampling, see appendix A. The water samples were collected by boat using 1l Niksin bottles that sampled water at a depth of 30m. At each site three replicates were taken. Upon collection, the samples were filtered with manual vacuum pumps and 0.22 µm pore size filters. In addition to the samples, a control sample was included, for which molecular grade water was filtered. The samples were subsequently stored in the laboratory at 4°C in plastic tubes with 1ml of lysis buffer. eDNA was extracted from the filters a maximum of 2 months after they were collected.

2.2.1.2. LABORATORY ENVIRONMENT

The processing of the eDNA samples were conducted at the *Genética y Ecología Molecular laboratory* of the Universidad Austral de Chile. Prior to use, all surfaces were cleaned with bleach solution and ethanol before the samples were processed. Furthermore, all eDNA samples were handled in a laminar flux chamber fitted with a Hepa filter, exclusively used for eDNA samples, which was cleaned with DNA*Zap*™ and subsequently irradiated with UV light for 10 minutes. DNA*Zap*™ was also used to clean any equipment with which the samples were handled.

2.2.1.3. EXTRACTIONS

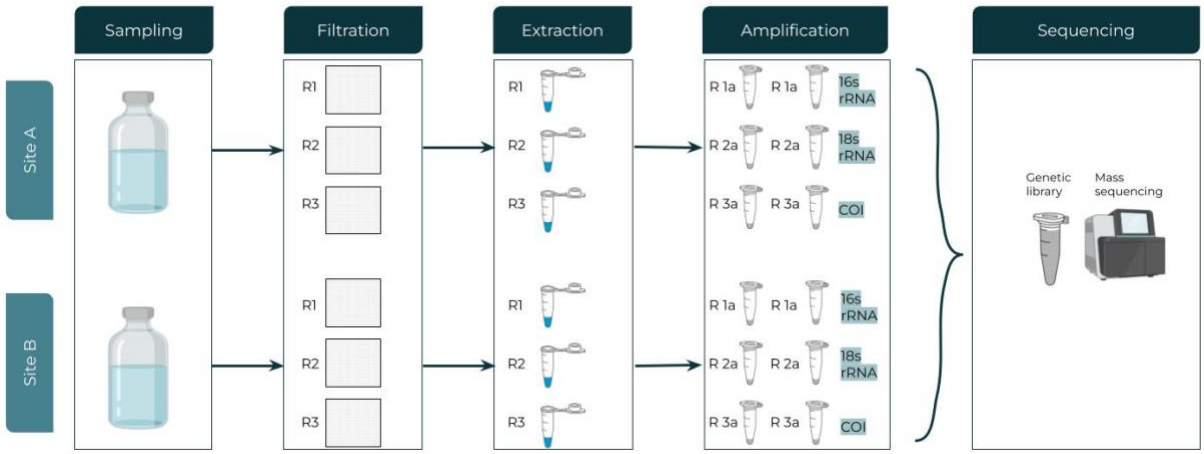

Figure 4. Schematic representation of the procedure for the sampling and processing of the eDNA.

To extract the eDNA, each tube was placed in a Mini-Beadbeater-16 at 2.5 x 1,000 stroke/min for 1 minute, after which they were opened in a laminar flux chamber and 500ml of the supernatant was transferred to a microcentrifuge tube of 1.5ml. The DNA was subsequently extracted with a GeneJET Genomic DNA Purification Kit as indicated by the manufacturer's protocol (ThermoFisher, 2016). From each 2ml tube which contained the filter, 500 µL of the lysis solution was removed and transferred to a 1.5ml tube. Next, 50 µL of the Proteinase K solution was added and mixed with vortex. The samples were then incubated for 3 hours. The DNA was extracted from the samples using 60 µl of elution buffer. For each batch of DNA samples, a control sample was included. Once the DNA had been extracted, the purified samples were stored at -20°C.

2.2.1.4. PCR AMPLIFICATION

eDNA was amplified with a two-step PCR method. The samples were first amplified with three primer sets that target specific sections of the 16s rRNA (Deagle et al., 2007; Berry et al., 2017), COI (Leray et al., 2013) and 18s rRNA genes (Fonseca et al., 2010). This combination of primers was used to maximize the discovery and identification of vertebrate and invertebrate taxa in the samples. The primers consisted of spacers, internal barcodes and sequencing primers. Four different barcodes were synthesized for each splitter, consisting both of forward and reverse primers. Each sample was assigned a unique combination, allowing their subsequent differentiation through bioinformatics. Each sample, including the controls, were

amplified in duplicate. The samples were grouped together by sampling point, after which they were cleaned using AMPure XP® (Beckman Coulter, Brea, CA, USA) to remove an excess of non-useful products (splitters and dimers). DNA sizing and quantifying using a fragment analyser (Agilent Technologies, Santa Clara, USA) and Qubit® (Life Technologies, Carlsbad, USA). Finally, all samples were joined together at equimolar final concentrations before sequencing in a Miseq illumina sequencer.

2.2.1.5. SEQUENCE DATA ANALYSIS

The sequences from the Illumina primer were first demultiplexed allowing for only one mismatch in the spacer or barcode. The information from the barcode was then processed with the Anacapa tool kit (Curd et al., 2019). Using the NCBI and EMBL databases, a CRUX reference library for each of the three primer sets were created. Following this, the first Ancapa pipeline was run to filter the sequences by quality and to generate Amplicon Sequence Variants (ASV). First, adapters and primers were trimmed for each raw demultiplexed file, then low-quality reads where Q was less than 30 were removed, and finally the reads were sorted by primer sequence. Next, the sequences went through a custom script, which sorted them as forward, reverse or unmerged read files. They were then denoised, merged, tested for chimeric sequences, and grouped into ASVs using the DADA2 program (Callahan et al., 2016). However, they were only left as an ASV if they appeared in the dataset a minimum of four times. The ASV.fasta files were subsequently introduced in the second Ancapa pipeline so that the taxonomy could be assigned for each ASV. All ASVs were aligned to the CRUX reference catalogues. Only the 100 best hits were kept. Finally, the ASVs were put into BCLA, and bootstrap confidence scores were assigned to taxonomic assignments.

2.2.1.6. CONTAMINATION DENOISING AND DATA CONSOLIDATION

The data produced by Ancapa was imported to R v 4.1.3 with the phyloseq package (McMurdie & Holmes, 2013) after having been converted into phyoseq class objects. Contaminant ASVs that appeared in the negative controls were then removed, as well as ASVs that were present in an equal or lesser proportion as the negative controls, for which the threshold was 0.5. Lastly, an assignment at the genus and species level with a bootstrap confidence higher or equal to 80% were accepted. Duplicate species were removed, and further filtration was conducted for the results of the COI and 18s primers, so that only invertebrates were included. The 16s primers did not need further filtration, since it only included fish species. Finally, only reads in

which at least the genus had been identified was included in the results. However, for the calculation of diversity, also reads which had not been identified at genus or species level were included.

2.2.2. PASSIVE ACOUSTIC MONITORING

PAM was conducted using two hydrophones (Sound traps ST-300 from Ocean Instruments, NZ) deployed on February 16, 2022, by SCUBA divers on rocky bottoms (figure 5). Site A (-33.348236°, -71.701469°) was located near Algarrobo. Site B (-33.489661°, -71.665048°) was located near Las Cruces. The hydrophones were factory calibrated following the Ocean Instruments manual (OceanInstruments, 2021). For both locations the depth of the hydrophones was 33m. At each site, the instruments were cable tied to a rope and held vertically between a buoy and a weight. The hydrophones were configured for semi-continuous registration (10 min per hour) at a maximum sampling frequency of 40 kHz. The deployment lasted for 75 days after which the hydrophones were removed on the 2nd of May 2022 by SCUBA divers. From the hydrophones, a sound file (.wav) was acquired with the manufacturer's software (OceanInstruments, 2021). This was analysed with Audacity® Cross-Platform Sound Editor, and with paPAMv2 (Nedelec et al., 2016).

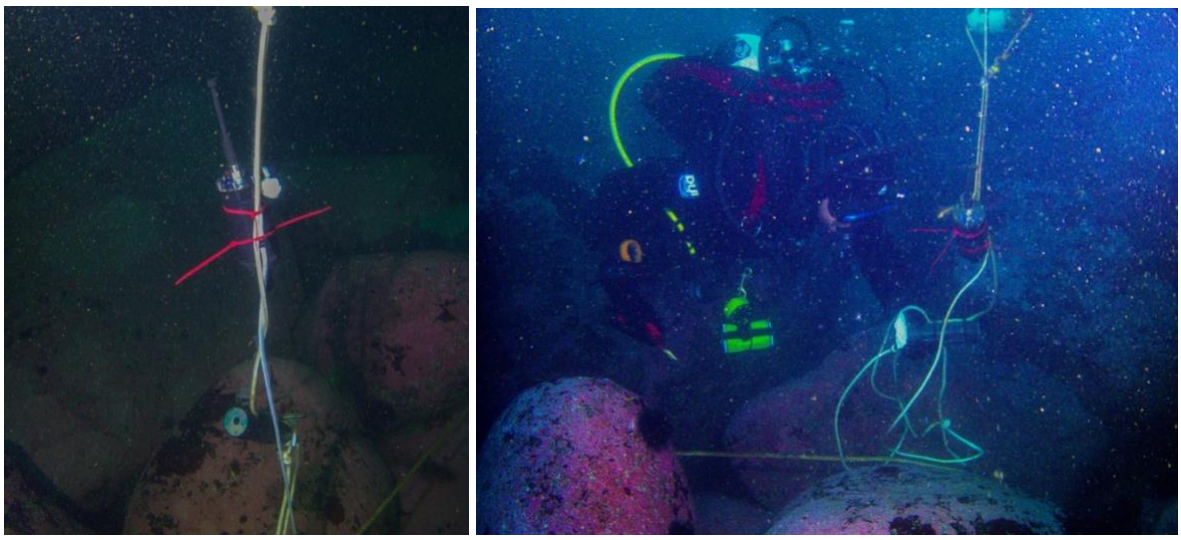

Figure 5. Placement of the hydrophones in the study site by SCUBA divers.

Temperature, salinity, and density all have an influence on sound propagation. CTD data was taken together with the sampling of eDNA. These are provided in table 1.

Table 1. Salinity, temperature, density and depth data taken together with the eDNA samples.

	LC 11/01/2022	ALG 12/01/2022	LC 13/03/2022	ALG 14/03/2022
Absolute Salinity (g kg^{-1})	34.64	34.708	34.693	34.604
Conservative temperature (°C)	11.429	11.114	11.621	12.021
Density (kg m^{-3})	26.538	26.546	26.401	26.27
Depth (m)	30	32	23	26

2.2.3. OXYGEN SENSORS AND TEMPERATURE

Oxygen sensors (PME miniDOT) were deployed together with the hydrophones (at a depth of around 30m) and continuously measured temperature and dissolved oxygen levels throughout the sampling period. The miniDOTs were last calibrated in October 2021. The oxygen data was processed on R (ver 4.1.3). Temperature data was measured along with the recordings performed by the hydrophones. A measure was taken at 1-minute intervals at the same time that the hydrophones recorded the acoustic data. This temperature data was extracted using R (ver 4.1.3), only the first value for every recording was used.

2.3. DATA ANALYSIS

2.3.1. SUBSAMPLING

A large part of the analysis was conducted using a subsample, to equalise the number of (limited) recordings within and outside of the oxygen minimum zones. In selecting subsamples, we first tested whether there was an effect of day and night on the different indices and metrics. Whether the recordings were taken during the day or night was calculated based on the R function “getSunlightTimes” from the suncalc package (Thieurmel & Elmarhraoui, 2019), which indicated the sunrise and sunset times for each day. The recordings taken between the sunrise and sunset times were categorised as day and the others as night. To estimate whether there was a difference in the index values of SPL, ACI and H, during the day or night, we used a Kruskal-Wallis test. These showed that most indices had a

significant difference in mean for recordings taken during the day and at night for both frequency bands (table 2).

Table 2. Results of the Kruskal-Wallis test on differences in day and night for SPL, ACI, PC1 and H for each frequency range. The significative correlations are given in red ($p < 0.05$).

Acoustic index and frequency range	**H**
SPL low	0.48536
SPL high	122.34
PC low	14.303
PC high	129.26
ACI low	0.18492
ACI high	24.896
H low	17.638
H high	362.58

Recordings were selected for three categories: normal, hypoxia and severe hypoxia. For the latter, the threshold level was 0.7 mg/L of dissolved oxygen content, as this is the point where metabolic activity is severely affected by the lack of oxygen and mass mortality potentially occurs (Hernández-Miranda et al., 2012; Hernández-Miranda et al., 2010; Diaz & Rosenberg, 1995; Gallo & Levin, 2016). The second threshold, for hypoxia, was put at 2.8 mg/L, which is where some effects including migration can already be observed (Diaz & Rosenberg, 1995). To create the subsamples, the recordings were filtered to the ones taking place when oxygen levels were below or equal to 0.7 mg/l. Since there were significant differences between day and night, it was decided to only choose samples taken at night. This was also expected to decrease the anthropogenic noise within the recordings. Following this, the number of recordings remaining was 11 for Las Cruces and 16 for Algarrobo. For the other oxygen ranges (hypoxia and normal) an equal number of recordings taken at night were selected randomly.

2.3.2. COUNTING AND CHARACTERISATION OF FISH SOUNDS

Frist, a manual counting and characterisation of fish signals was conducted. This was performed in Audacity®, by listening to the recordings and looking at the spectrograms. Since it was not possible to listen to every recording, the recordings from the categories

“severe hypoxia” and “normal” from the subsample described above were used for the analysis. This means that 22 recordings from Las Cruces (11 within and 11 outside of an OMZ) and 32 recordings from Algarrobo (16 within and 16 outside of an OMZ) were analysed. The signatures were described based on frequency range, duration, and number of pulses. Since the number of recordings differed between sites, the percentage of recordings the signatures appeared in was calculated. A conservative approach was taken to the characterisation of fish signatures, which entailed that if there was any doubt that two signals were the same, they were counted as a separate signal. Thus, it is possible that there were more signatures than the ones that were described and counted. In addition, the number of recordings in which there was anthropogenic noise was counted.

An example spectrogram in figure 6, a fragment of the soundscape (33 seconds), shows distinct sound signatures The two frequency ranges are clearly visible, with the high range constituting of clicks produced by invertebrates, and the low range comprising a variety of sounds produced by fishes (Kennedy et al., 2010).

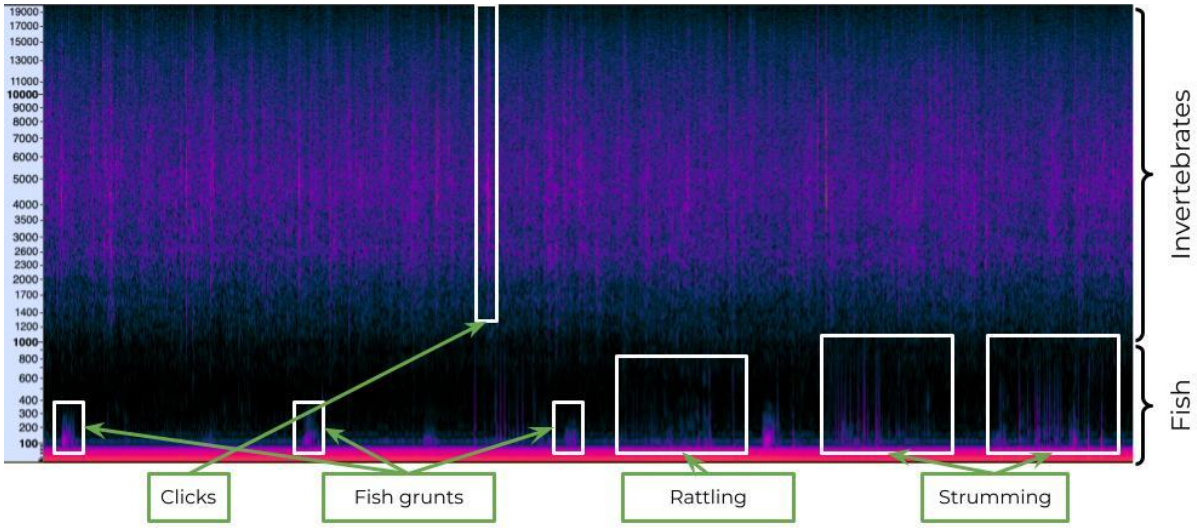

Figure 6. Spectrogram of a segment of 33 seconds of a recording taken in Algarrobo on the 16th of March at 03:30. The intensity is visualised by the colours, with a brighter colour (pink) indicating a higher sound intensity (in dB).

2.3.3. CALCULATION OF INDICES

All calculations were performed in R (ver 4.1.3), using the packages *seewave* (ver 2.2.0., Sueur, Aubin, & Simonis, 2008), *soundecology* (ver 1.3.3, Villanueva-Rivera & Pijanowski, 2018) and *tuneR* (ver 1.4.0, Ligges et al., 2018). For each 10-minute recording, the following indices were calculated: Acoustic complexity index (ACI), Acoustic Entropy (H), Bioacoustic index

(BI), Temporal Entropy (TE), Spectral Entropy (SE), Median of the amplitude envelope (M), Normalised Difference Soundscape Index (NDSI). A description of each index is given in table 3. On top of the acoustic diversity indices, Sound-Pressure Level (SPL) was used as a descriptive metric of the soundscape. SPL measures the average variation in pressure of acoustic energy of the medium (in this case seawater) that is caused by sound (Haxel et al., 2013). With the exception of NDSI, all indices and SPL were calculated for full bandwidth (0-20kHz), low frequencies (0-1kHz, 0.01-1kHz for SPL) and high frequencies (1-20kHz). These frequency ranges were chosen to make a distinction between fish sound production (low frequencies) and invertebrates or benthic sound production (high frequencies). The respective ranges have been shown to be linked to fish and benthic diversity (Kennedy et al., 2010). NDSI was only calculated for full bandwidth since the index calculated the ratio between low and high frequencies. From the results of the eDNA sampling, the species richness was calculated per site.

Table 3. Overview of acoustic indices calculated for each recording.

Biodiversity index	**Definition**	**Sound-analysis domain**
Acoustic Complexity Index (ACI) (Pieretti et al., 2011)	The ACI measures the absolute difference (d_k) between two adjacent values of intensity (l_k and $l_{(k+1)}$) in a single frequency bin (Δ_{fi}), all of which are subsequently totalled.	Frequency-time-amplitude
Acoustic entropy (H) (Sueur et al., 2008)	H is a normalised index calculated from the Shannon diversity index which measures the number of frequency bands and amplitude modulations, increasing as the amount of random noise increases.	Frequency-time-amplitude
Bioacoustic index (BI) (Boelman et al., 2007)	BI calculates the area between a threshold value and the mean spectrum curve.	Frequency-amplitude
Temporal entropy (TE) (Sueur et al., 2008)	TE calculates the evenness of the amplitude envelope in relation to time.	Time-amplitude
Spectral entropy (SE) (Sueur et al., 2008)	TE calculates the evenness of the amplitude envelope in relation to frequency.	Frequency-amplitude
Median of the amplitude envelope (M) (Depraetere et al., 2012)	M measures the overall amplitude of acoustic signals.	Amplitude
Acoustic evenness index (AEI) (Villanueva-Rivera et al., 2011)	AEI measures the evenness across frequency bands.	Frequency
Normalised difference soundscape index (NDSI) (Kasten et al., 2012)	NDSI is a normalised index which provides a measure of the relationship between the biophony and anthrophony.	Frequency

After visualising the results of the indices and SPL, it was decided to cut out data of the first month of recording (16th of February – 14th of March). This choice was made because the

data in these recordings included a significant number of outliers due to unusual anthropogenic activity, especially around Las Cruces, which was confirmed by listening to the .wav files. Given the overlap of the anthropogenic sounds and the bioacoustics signals, the above-mentioned period was removed from the analysis and the remaining 3605 recordings were analysed.

2.3.4. STATISTICAL ANALYSES

To maximize the information provided by the combined acoustic diversity indexes in characterizing the soundscape we used multivariate analysis. Because different indices have a high variability in what they measure (table 3), all indices were used to define sound diversity for each 10-minute recording. A principal component analysis (PCA) was subsequently used to look for differences among samples based on the diversity indices calculated for each of them. This approach could allow us to visualise differences among samples. Therefore, two PCAs were performed on the results of the acoustic biodiversity indices, one for the high frequency range and one for the low frequency range. The contribution of each index to the first and second principal components, as well as the spatial relationship among data points, was visualised with a two-dimensional scatter plot. The values for the principal component were used in further analysis, as a combination of all the acoustic diversity indices that contributed to it.

Further analyses were conducted on SPL and PC1, as well as ACI and H. These two indices were chosen as they are the most used and reliable indices for describing diversity in marine ecosystems (Pieretti & Danovaro, 2020; Harris & Radford, 2014; Ross et al., 2021). In marine environments, ACI has been shown to be useful since it is not strongly affected by continuous noise, such as the passage of ships (Ross et al., 2021). Furthermore, it has been proven to be effective to measure the biological diversity in (temperate) reef fish communities, to be correlated with important ecosystem functions, as well as other index's such as Pielou's evenness and the Shannon index (Pieretti & Danovaro, 2020; Harris & Radford, 2014). Acoustic entropy (H) is also a commonly used index for marine soundscapes. It has been shown to be associated with richness irrespective of conditions (Ross et al., 2021).

To test the differences between site for acoustic indices, a Kruskal-Wallis test was conducted on SPL, ACI, H and PC1. For studying the effect of oxygen on the soundscape, first, a Spearman correlation was performed between oxygen and SPL, ACI, H and PC1. This was followed by a general linear model (GLM) per index and frequency band, resulting in eight

models. For every model the following independent variables were selected: Dissolved Oxygen Content, temperature, and site. The effect of Dissolved Oxygen Content and site together was also included in the model. The GLM was complemented by a Kruskal-Wallis test which also tested the difference in SPL, ACI, H and PC1 for the different oxygen categories.

3. RESULTS

3.1. THE SOUNDSCAPE OF THE MESOPHOTIC REEF

The soundscape of both study sites was characterised by 13 signals (table 4), several which appeared in almost every recording (figure 7), namely stridulations, grunts, growls, tapping sounds and croaks. Stridulations were one of the longest signals, lasting between 0.5 to 5 seconds. They also had quite a large frequency range, spanning between 0 up to 1300 Hz and consisting of a few pulses sometimes far apart and other times almost blending together. The two other sounds ubiquitous in the soundscape were fish grunts and growls. These were more limited in both frequency range (both going up to 350) and duration (lasting a fraction of a second) and consisted of only one pulse. The croak was one of the shorter signals, occurring only within higher frequencies (still within the low frequency range). Finally, the tapping was often found to consist of several pulses, although it sometimes also appeared as one pulse. The frequency range was similar to that of the grunts and growls.

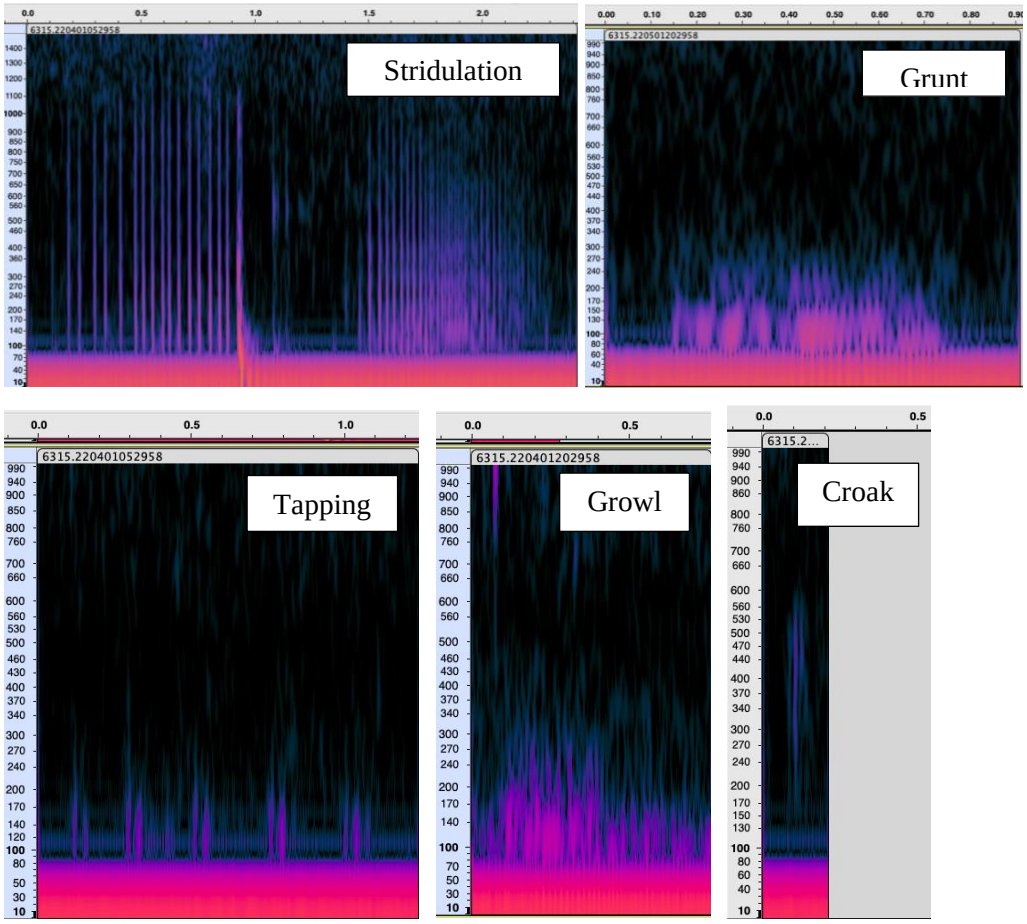

Figure 7. A selection of fish signatures found in the recordings. The x-axis represents frequency (in Hz), the y-axis indicates time (in seconds), and the colour visualises the sound intensity (in dB).

Table 4. Overview and description of fish signatures (sound labels by author).

Name	Frequency band (Hz)	Duration (s)	Number of pulses
Stridulation	0-1300	0.5-5	>10
Grunt	0-350	0.7	1
Croak	200-650	0.1	1
Tapping	80-200	1	10
Rattling	70-800	2	>10
Trilling	70-800	0.2	4
High Tap	200-600	0.05	1
Clac	100-500	0.05	1
Growl	60-350	0.5	1
Laugh	0-800	2	10
Cackling	1000-1500	0.6	7
Wheezing	130-700	0.3	1
Woosh	400-750	0.3	1

Another aspect of the soundscape which was found in many recordings was anthropogenic noise, usually in the form of ships passing nearby. This could last an entire recording, or just a couple of seconds. The percentage of recordings comprising anthropogenic noise for each site and category can be found in table 5.

Table 5. Percentage of recordings in which anthropogenic noise can be heard, both within and outside of OMZs for both sites.

	Algarrobo		**Las Cruces**	
	OMZ	***No OMZ***	***OMZ***	***No OMZ***
Anthropogenic noise	12.5	31.25	81.8	45.5

The characterisation of fish sounds resulted in the description of 13 signatures, 13 observed in Algarrobo and 11 in Las Cruces (figure 8 and 9). Notable differences between both sites were the occurrence of the laugh and wheezing sound in Algarrobo which were absent in Las Cruces. Furthermore, the recordings in Las Cruces tended to be quieter than in Algarrobo, with signatures appearing less frequently within the recordings. The characterisation of fish

sounds was performed for recordings under severe hypoxia and normal oxygen conditions. We observed that there was no difference in the abundance of signals detected between both conditions, in terms of percentage recordings in which the signatures were detected. However, in Las Cruces many of the signals occurred more often outside of the OMZ in comparison to under severe hypoxia (figure 8 and 9). This difference is most notable for the croak and rumbling sound. For Algarrobo, the opposite pattern was visible, where many signatures occurred more frequently within the OMZ.

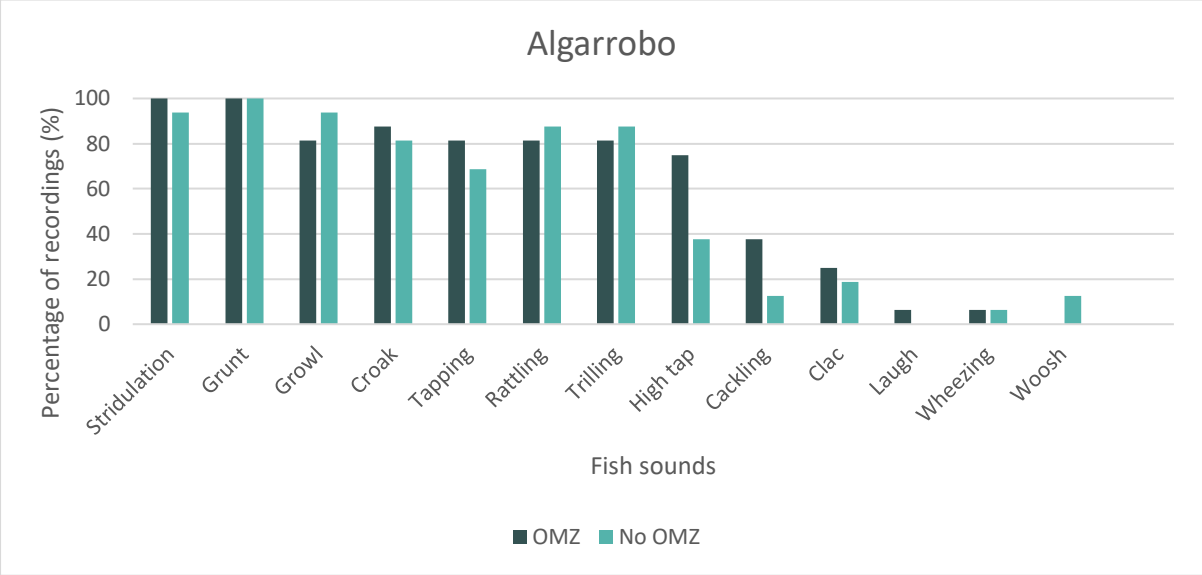

Figure 8. Comparison of the percentage of recordings in which each signature appeared for the categories of severe hypoxia (OMZ) and normal (no OMZ) in Algarrobo.

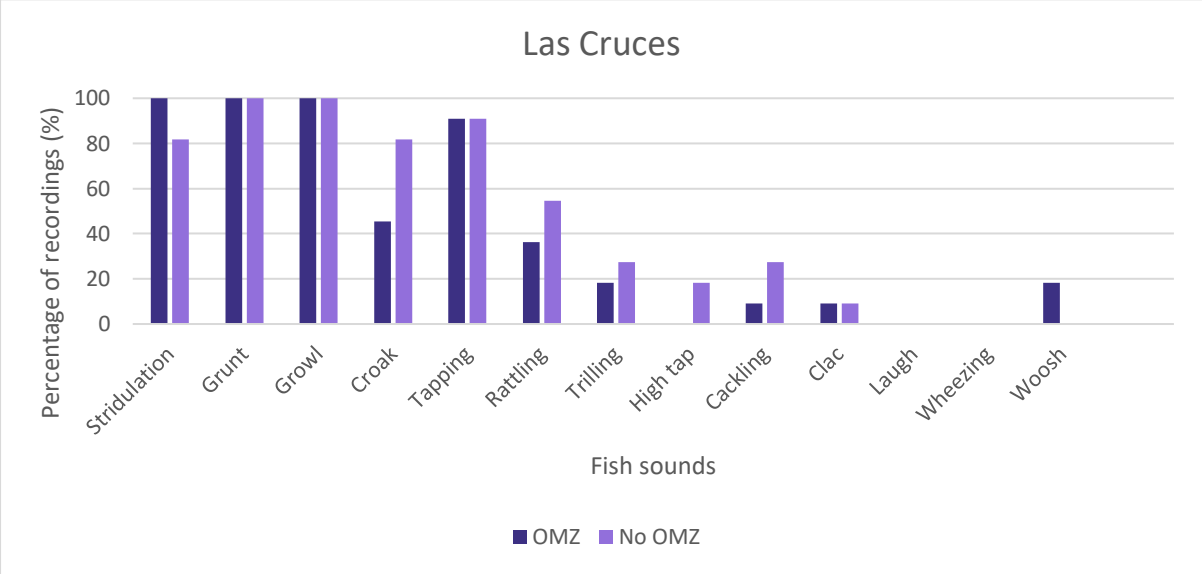

Figure 9. Comparison of the percentage of recordings in which each signature appeared for the categories of severe hypoxia (OMZ) and normal (no OMZ) in Las Cruces.

3.2. DIVERSITY OF THE TME

3.2.1. eDNA

A list of the taxa identified using eDNA which are potential contributors to the soundscape can be found in table 6. A full list of taxa resulting from the eDNA sampling is in appendix B. The majority of identified taxa of invertebrates were sampled in Algarrobo, whereas most fish identified were sampled at both sites (table 7). The arthropods identified could be placed in four groups: copepods (18 out of 32), insects, including beetles, flies, and bugs (8 out of 32), and barnacles (3 out of 32). There were also two species belonging to the class malacostraca. The results of the summer samples after all the decontamination and filtering for invertebrates consisted of 7 fish and 33 invertebrates species in Algarrobo and 8 fish and 25 invertebrate species in Las Cruces. The samples taken in autumn resulted in 1 fish and 41 invertebrates in Algarrobo and 6 fish and 54 invertebrate species in Las Cruces.

Table 6. List of (potential) sound-producing fish and invertebrate species identified with eDNA per season and site.

Genus	**Species**	**Phylum**	**Site**	**Season**
		Fish		
Chromis	*Chromis enchrysura*	Chordata	Both	Both
Engraulis	*Engraulis ringens*	Chordata	Both	Both
Genypterus		Chordata	ALG	Summer
Merluccius		Chordata	Both	Summer
Merluccius	*Merluccius productus*	Chordata	ALG	Summer
Sebastes	*Sebastes oculatus*	Chordata	ALG	Summer
		Invertebrates		
Calyptraeotheres	*Calyptraeotheres politus*	Arthropoda	ALG	Autumn

Table 7. Percentage of species identified per site out of total species identified for fish and invertebrates.

	Fish	***Invertebrates***
Both	45.5	33.9
Las Cruces	18.2	30.6
Algarrobo	36.4	35.5

3.2.2. ACOUSTIC DIVERSITY OF THE TME

The acoustic diversity indices had varying results, especially between different frequency ranges (figure 10). The differences between sites were more pronounced for high frequencies than low frequencies, with Las Cruces having higher ACI values, but lower H values. For low frequencies, ACI, H and SPL are all slightly higher in Las Cruces. For ACI the values for low frequencies were higher than for high frequencies, in contrast to H and SPL where the values for the low frequencies were considerably lower than for high frequencies. For all three indices there was a significant difference in values for Algarrobo and Las Cruces, for both frequency ranges. The results of the Kruskal-Wallis test can be found in appendix D.

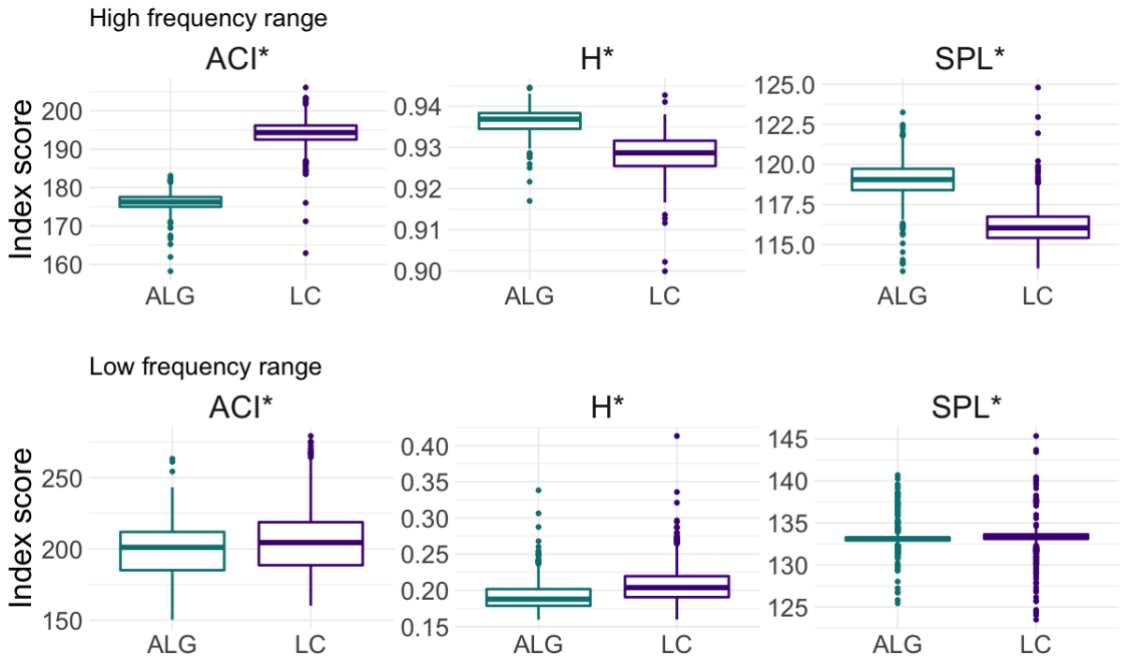

Figure 10. Boxplot of the respective score values for ACI, H, PC1 and SPL, divided between site (green for Algarrobo and purple for Las Cruces) and frequency range. The stars indicate significant differences ($p<0.05$) between sites based on Kruskal-Wallis tests.

3.2.3. PRINCIPAL COMPONENT ANALYSIS

Two PCAs were conducted, one per frequency range. The first principal component of the high frequency range explained 65.6% of the variability, whereas the first principal component of the low frequency range explained 43.6% of the variability (table 8). In the former, the indices contributing most strongly (>0.38) to PC1 were H (-), TE, SE (-), and NDSI. In the low frequency range this was ACI, AEI, H (-), TE (-) and M (-).

Table 8. Overview of first principal component and eigen-value contribution rate per frequency range for each acoustic index.

Index	High	Low
ACI	-0.0474805	0.38700843
AEI	0.31243614	0.38947289
BI	0.20133264	0.21359017
H	-0.3866852	-0.4044126
TE	0.44445351	-0.4115181
SE	-0.4194353	-0.1152941
M	0.36293613	-0.4210714
NDSI	0.45263827	-0.3597555
Explained variance (%)	**65.6**	**43.6**

These results were visualised in a scatter plot of the scores of the first and second principal components for each recording, with data points categorised according to Site (figure 11). The plots showed clear clustering of the data points, with the recordings taken in Las Cruces correlating more with ACI and AEI for high frequencies and ACI, SE and H for low frequencies. In Algarrobo, the recordings were affiliated with NDSI, H, M and TE for high frequencies, and AEI, NDSI, TE and ME for low frequencies.

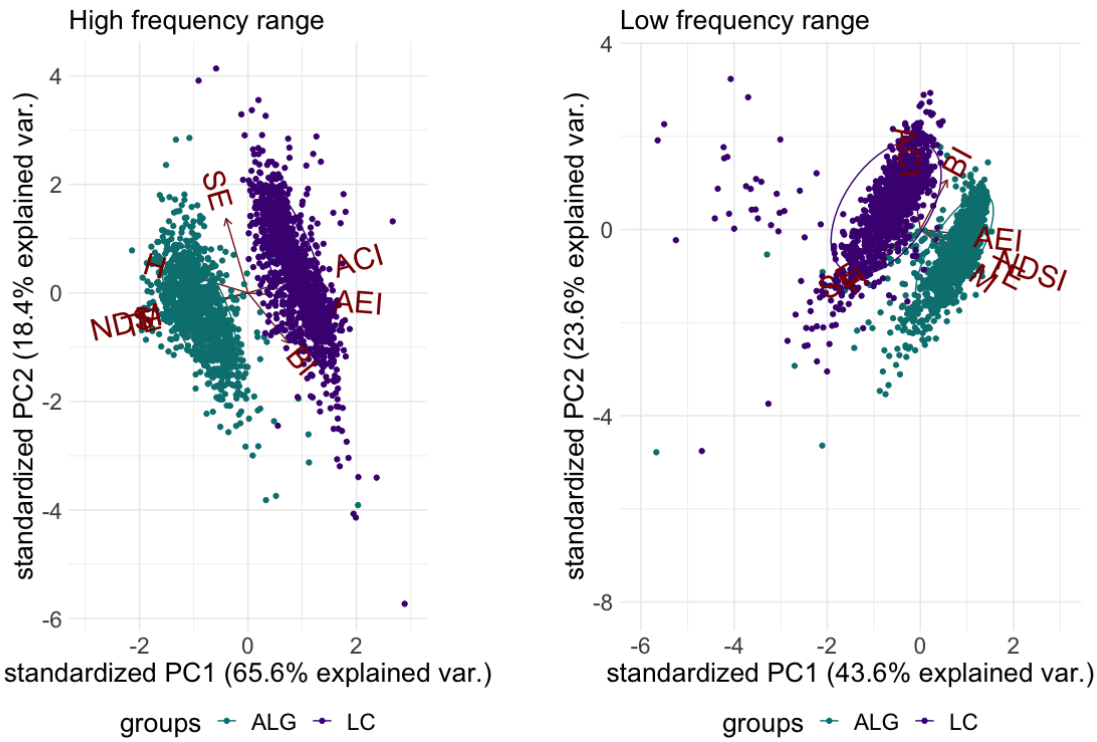

Figure 11. Two PCA scatter plots of the acoustic indices for full range, with data points categorised between sites.

3.2.4. COMPARISON OF eDNA AND ACOUSTIC DIVERSITY MEASURES

The higher species richness identified with eDNA in Las Cruces was best reflected in BI, ACI and AEI. BI also had the same pattern in the difference in species richness between invertebrates and fish (table 9). For H, although the change in species richness between sites did not reflect the results of species richness, the difference between invertebrates and fish, and the high and low frequency bands, was similar. M had completely opposite results to species richness, with higher values for the low frequency bands and the recordings taken in Algarrobo. NDSI, indicated higher values in Algarrobo, meaning that the ratio of biological to anthropogenic sound was higher there compared to Las Cruces.

Table 9. Mean results of the diversity indices, with species richness based on eDNA, and the acoustic indices based on the soundscape. The results are given for both frequency ranges and sites.

	Algarrobo		**Las Cruces**	
	Invertebrates	***Fish***	***Invertebrates***	***Fish***
Species richness	37	4.0	39.5	7.0
	High frequency range	***Low frequency range***	***High frequency range***	***Low frequency range***
SPL	119.057036	133.128095	116.125609	133.261971
ACI	175.630647	199.85092	194.239807	205.518509
AEI	0.52307703	0.89027489	0.576674	0.87939474
BI	24.1977341	1.61232984	25.3074732	1.63374789
H	0.93690581	0.19160478	0.92849111	0.20704316
TE	0.98488904	0.97186232	0.97603263	0.95347888
SE	0.95128011	0.19718556	0.9512896	0.21715203
M	1.0405E-06	1.5275E-06	4.8341E-07	9.7281E-07
NDSI	0.685921582		0.516437944	

3.3. EFFECT OF OXYGEN ON THE SOUNDSCAPE

Two analyses were performed to look at the effect of oxygen on the acoustic indices. First, a Spearman correlation was conducted on the entire dataset of both oxygen and SPL for both sites and frequency ranges. There was a significant Spearman correlation between oxygen and almost every index, with the exception of the low frequency band in Las Cruces for SPL and the high frequency band in Las Cruces for ACI. The high frequency range of SPL had the strongest correlation with oxygen. A time series of both oxygen and SPL are given in figure 12. A more complete time series plot can be found in appendix E.

Figure 12. Time series of oxygen together with the high frequency range of SPL((re 1 µPa2)/dB) for Algarrobo and Las Cruces.

Next, a general linear model was conducted using the subsample, comparing recordings within the three categories of oxygen levels: severe hypoxia, hypoxia and normal. The covariables included in the model were temperature and site. Here, we observed a significant difference in SPL for the high frequency range only between the two sites ($p<0.001$): M = 119.3, SD = -0.1 for Algarrobo and M = 116.3, SD = -0.2) for Las Cruces (figure 13). There was no significant effect of oxygen or temperature on SPL for either frequency ranges. Similarly, the results of the GLM only indicated a significant difference between sites for the high frequency range ($p<0.001$): M = *175*.3, SD = 0.3 for ACI in Algarrobo, M = 194.2, SD = 0.4 for ACI in Las Cruces (figure 14), M = 0.938, SD = 0.0003 for H in Algarrobo and M = 0.931, SD = 0.0004 for H Las Cruces (figure 15). For PC1, the GLM conducted on the high frequency range showed that there was a significant difference between sites ($p<0.001$): M = -2.52, SD = 0.118 in Algarrobo and M = 1.42, SD = 0.142 in Las Cruces. The GLM for PC1 conducted on the low frequency range also indicated a significant difference between sites ($p<0.001$): M = 1.34, SD = 0.143 in Algarrobo and M =-1.02, SD = 0.172 in Las Cruces (figure 16). The results of the Kruskal-Wallis test indicated that there was no significant difference between oxygen categories for the indices. The results thereof can be found in appendix D.

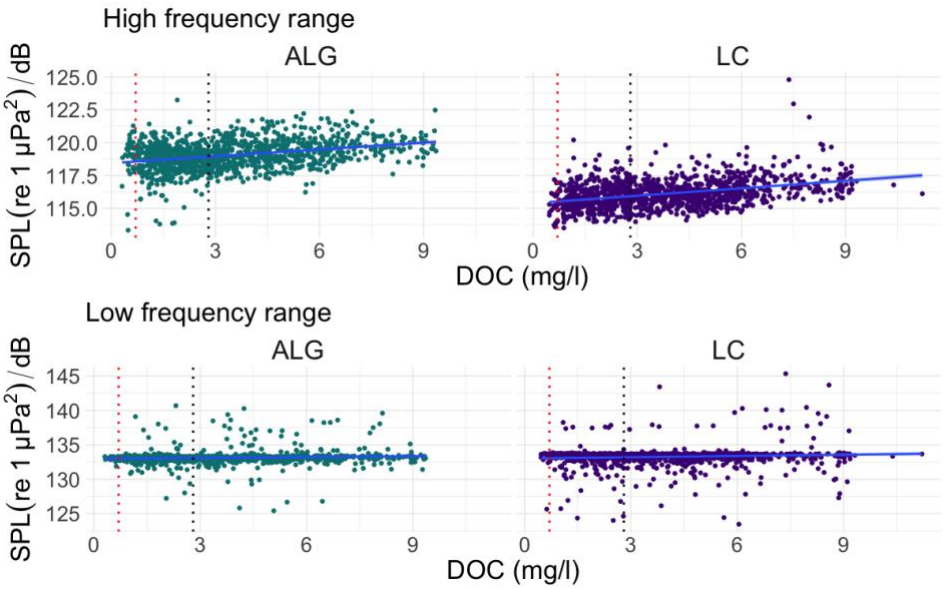

Figure 13. The relationship between SPL and oxygen for both Algarrobo and Las Cruces, divided between high and low frequencies. The red and black dotted lines indicate the thresholds for severe hypoxia and hypoxia respectively. There was a significant correlation for the frequency range in Algarrobo (rho = 0.308, p<0.001) and Las Cruces (rho = 0.361, p<0.001), as well as for the low frequency range in Algarrobo (rho = 0.101, p<0.001) but not for Las Cruces (rho = 0.03, p = 0.3026)

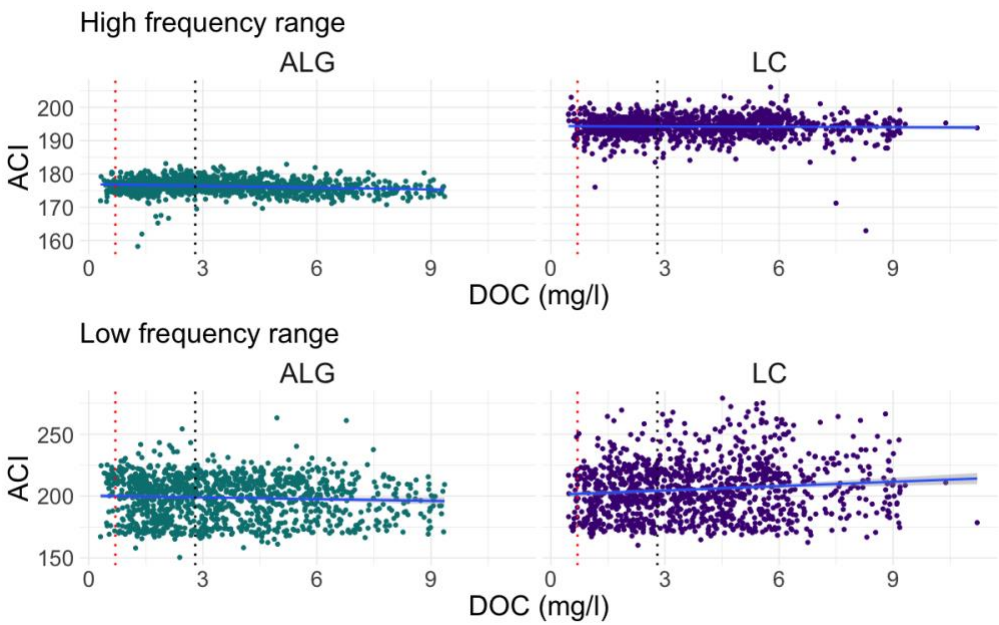

Figure 14. The relationship between ACI and oxygen for both Algarrobo and Las Cruces, divided between high and low frequencies. The red and black dotted lines indicate the thresholds for severe hypoxia and hypoxia respectively. There was a significant correlation for the high frequency range in Algarrobo (rho = -0.185, p<0.001) but not Las Cruces (rho = 0.015, p = 0.605), and for the low frequency range in Algarrobo (rho = -0.06, p<0.05) and Las Cruces (rho = 0.11, p<0.001)

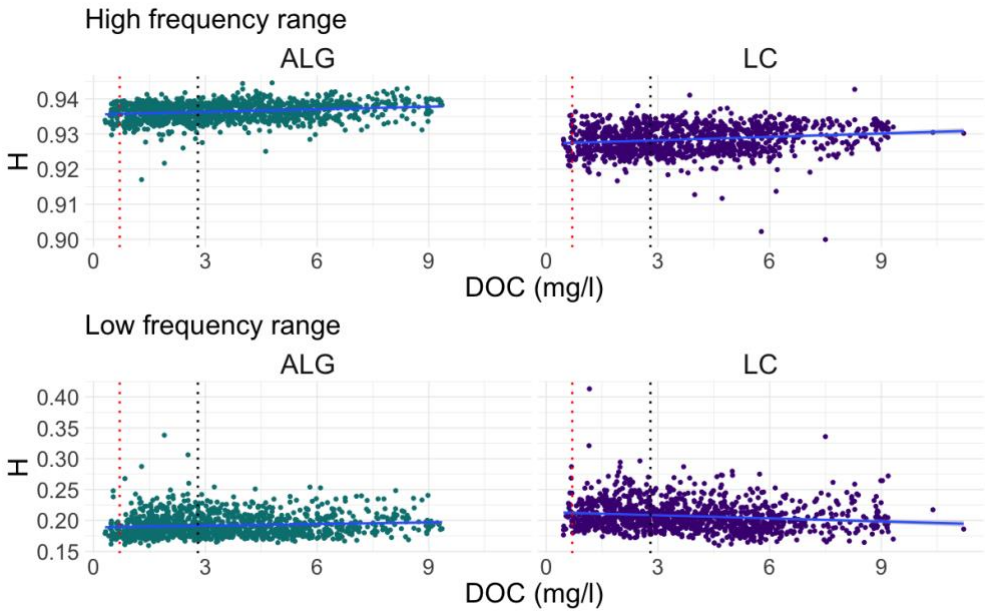

Figure 15. The relationship between H and oxygen for both Algarrobo and Las Cruces, divided between high and low frequencies. The red and black dotted lines indicate the thresholds for severe hypoxia and hypoxia respectively. There was a significant correlation for the high frequency range in Algarrobo (rho = 0.174, p<0.001) and Las Cruces (rho = 0.14, p<0.001), and for the low frequency range in Algarrobo (rho = -0.06, p<0.05) and Las Cruces (rho = -0.179, p<0.001).

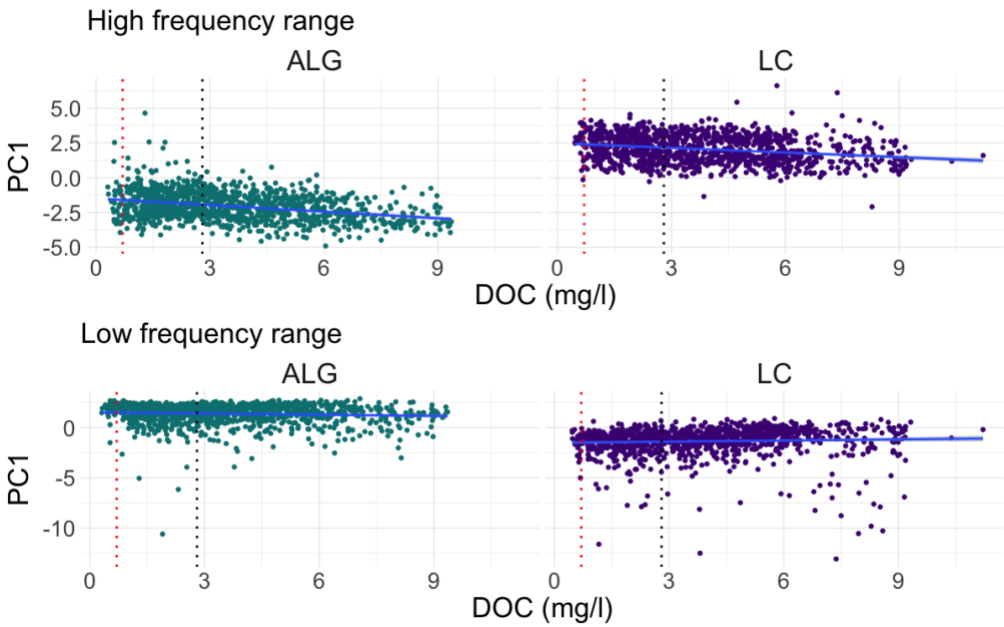

Figure 16. The relationship between PC1 and oxygen for both Algarrobo and Las Cruces, divided between high and low frequencies. The red and black dotted lines indicate the thresholds for severe hypoxia and hypoxia respectively. There was a significant correlation for the high frequency range in Algarrobo (rho = -0.285, p<0.001) and Las Cruces (rho = -0.243, p<0.001), and for the low frequency range in Algarrobo (rho = -0.087, p<0.05) and Las Cruces (rho = 0.227, p<0.001).

4. DISCUSSION

This research is novel in multiple aspects. It is the first study which combines and compares acoustic monitoring with eDNA. By doing so, it allows for further exploration of the reliability of acoustic indices for the investigation of marine ecosystems and in particular of the mesophotic reefs which are challenging to access. This is important, since most of the research so far using acoustic indices has taken place in terrestrial ecosystems (Minello et al., 2021). In addition, this is the first research to investigate the effect of OMZs on the soundscape and acoustic indices. As such, this research is an important step in contrasting methodologies (eDNA and acoustic monitoring) to study the diversity, health and response to disturbances of marine ecosystems.

4.1. DIVERSITY OF THE TME

In this study the diversity of marine animals, specifically fish and invertebrates, was compared using two methodologies: eDNA and acoustic monitoring at two sites in central Chile. The PCA showed clear clustering of sites, confirming the variability in diversity, likely resulting from the differences in upwelling regimes and presence of the marine protected area. As expected, Las Cruces, characterised by a less dynamic upwelling system (Aiken et al., 2008) and where a marine protected area is in place revealed a higher diversity. Furthermore, the results of this study point towards a similarity in the diversity measured from acoustic monitoring and eDNA. Since there was much variability within the acoustic indices, only a select number of indices corresponded to the measure of species richness calculated from the results of eDNA. Although Ross et al. (2021) found that H related most closely to species richness, this study indicated that other indices, namely ACI, AEI and BI, better followed the pattern between sites and frequency bands.

eDNA collected at the two sites showed spatial differences: in the number of species (and genera) present but also temporally: variation at different sampling times. eDNA revealed a higher diversity in Las Cruces than in Algarrobo. The fact that species richness was higher in Las Cruces for both invertebrates and fish could be attributed to the marine protected area, that hosts higher abundances and biomass than surrounding areas (Navarrete et al., 2010). The consecutive eDNA sampling seasons at each site returned different diversity values highlighting potential local changes in the fish and invertebrate community but also potentially may have been due to methodological issues (poor amplification of 16S and consequent poorer performance in detecting fish). Sampling of other coastal communities

with eDNA have shown the same general trends: high variability in diversity across space and time (Handley et al., 2019; O'Donnell et al., 2017).

The taxa identified with eDNA encompassed species and genera that are known to produce sound, such as *Chromis* (De Amorim, *1996), Merluccius* (Groison et al., 2011), *Sebastes oculatus* (Kasumyan, 2008; Nichols, 2005) and *Genypterus* (Parmentier et al., 2018). In total, 14 sounds were identified in the soundscape of the two TMEs, some of which have been described in other papers. One of these is the growl, which is similar to one documented by Parmentier et al. (2018) of the species *Genypterus chilensis*. Similarly, the grunt sound identified in this research is somewhat similar to the sound produced by *Genypterus maculatus*. Another fish genus found in the eDNA sample whose sound production has been documented is *Chromis*. De Amorim (1996) described the signals from *Chromis viridis* as click-like sounds. This could for instance be linked to the trilling sound identified in the recordings. Lamont et al. (2022) also described a number of fish sounds, including a croak, grunt and a laughing sound, and Tricas & Boyle (2014) described the growl and grunt sound, associated with the Holocentridae family. It is important to note that fish can make a number of different signals whilst some signatures that may appear very similar might in fact be produced by different species (Lamont et al., 2022). The invertebrates identified with eDNA mainly contained species that are not likely to produce sounds and therefore contribute to the soundscape, such as cnidarians, annelids, and bryozoans. Most literature on sound production of invertebrates has focused on crustaceans (arthropods) (Hazlett & Winn, 1962; Kikuchi et al., 2015; Popper et al., 2001), but there have also been studies on the sound production of bivalves and sea urchins (Radford et al., 2008; Di Iorio et al., 2012; Júnior et al., 2019).

Fish and invertebrate community diversity was also assessed via acoustic diversity indices, that were combined via multivariable approaches and compared across sites and within or outside of oxygen minimum zones. We found high variability in what was measured by the different acoustic indices, and the extent to which they correlated between each other fluctuated with site and frequency range. In both PCAs H and ACI were at opposite sides of the graph, showing that they had the largest amount of variability in what they measured, consequently varying in the results for sites and frequency bands.

Since H is a measure of the randomness of sound production in the soundscape, increasing with the number of vocalising species and evenness of the acoustic environment (Andrews & Dick, 2021), it was logically higher for high frequencies which was dominated by clicks of random frequencies and intensities. On the other hand, ACI calculates the

variability over time which was stronger in the low frequency band (Pieretti et al., 2011). Thus, using both indices is useful, because only a very diverse soundscape would have high values in both, as it would consist of a large variety of sound sources strongly fluctuating over time. Therefore, the most diverse site according to the combination of both indices, would be Las Cruces, specifically in the high frequency band, which also exhibited the highest species richness. The only other acoustic index which had the same result, was the Bioacoustic Index. Although this index has been used very little, Elise et al. (2019) found that it was also useful as a proxy for ecosystem functions.

As mentioned previously, a number of acoustic indices have not yet been tested in marine ecosystems and thus their interpretation in this context needs to be cautious. NDSI is one of these indices, which was created to measure the ratio of anthropogenic and biological noise in terrestrial ecosystems, more specifically looking at bird calls (Kasten et al., 2012). So far, the results of this index comparing Las Cruces and Algarrobo seem to correspond with the prevalence of anthropogenic noise in both sites, with more pollution in Las Cruces.

Both SPL and H had a lower value in Las Cruces than in Algarrobo for the high frequency range. For H this could be explained once more by the anthropogenic noise in Las Cruces, since noise of ships passing through tend to be constant and at one frequency, thus lowering H values. However, anthropogenic noise also increases SPL, which was not observed. In addition, the presence of the MPA would be expected to increase SPL levels in Las Cruces (Borie et al., 2021). A possible explanation for these results could be lower geophonic noise in Las Cruces. Since Las Cruces is more sheltered from upwelling conditions taking place in the rest of the coast, meaning there is reduced wind speed and wave activity (Aiken et al., 2008), the geophony would be substantially lower in comparison with Algarrobo.

An interesting result, which contradicts with the findings of diversity is the number of sound-producing fish species identified with eDNA in both sites, with Algarrobo having a higher number of species known to produce sound. This result is similar to what we found when counting fish sounds in the spectrogram, where a higher number of signatures were observed in Algarrobo. Yet, the acoustic diversity indices indicated a higher acoustic diversity in Las Cruces. This could be due to non-biological noise impacting the results of the indices. Bolgan et al. (2018) tested ACI on a vocal fish community and found that under some settings the index highlighted anthropogenic noise more than that of vocalising fish, and that it was not good at making a distinction between the abundance of and diversity of sound. Since more anthropogenic noise was identified in Las Cruces than Algarrobo, it is

possible that the indices were strongly influenced by this. It is also important to consider that there was some room for mis-assignment in the interpretation of fish signature, since a lot of signatures overlap in some way. For future research, possible errors could be avoided by having multiple judges analysing the soundscape.

An essential distinction between the eDNA and acoustic monitoring is the scale at which they operate. Whereas the soundscape monitors species that are relatively close to the hydrophones, and thus are locally present, organismal traces picked up by eDNA can travel some distances thus not necessarily providing a picture of the local community (Taberlet et al., 2018). Furthermore, the reach of either methodology is not clear as it is related to many factors, such as currents, wind speed and other organisms for eDNA (Taberlet et al., 2018), and sound propagation for the soundscape. It is important to note that whilst this research studied a mesophotic reef, the communities described here might not have been limited to the mesophotic reef.

4.2. EFFECT OF OXYGEN ON THE SOUNDSCAPE

The second objective of this study was to investigate whether the soundscape can be used to determine changes in diversity resulting from the emergence of OMZs. Although this has not been widely researched yet, there have been some studies looking into the effect of disturbances on the soundscape. Simmons et al. (2021), for instance, wrote about changes in the soundscape caused by a hurricane. In that study, no significant change in SPL for the low frequency-band was observed, nor was there a difference in snapping shrimp activity. Similar results were found in this study, as there was no significant difference found in SPL, ACI, H or PC1 within and outside of OMZs, for either frequency bands.

On the other hand, significant correlations were found between acoustic diversity and oxygen. Yet most of these correlations were weak and may not truly indicate a relationship between oxygen and the indices. The only regressions which were notable were the ones for the high frequency range of SPL. However, this might not be a result of oxygen alone. There are other conditions which arise with OMZs which likely played a role. For instance, temperature has an influence on sound propagation (Richards, 1998). The effect of temperature on the soundscape has been studied previously, in which it was observed that during upwelling events, colder temperatures generally led to a decrease in noise levels (Louza et al., 2019). This is supported by Calazan et al. (2019), who investigated the influence of upwellings on acoustic signals. They observed that in the presence of the

upwelling stream, the transmission loss increased by about 10 dB. Therefore, it is probable that the influence of oxygen levels on SPL were linked to increased sound attenuation resulting from lower temperatures. This effect of temperature would not be as noticeable in indices such as ACI and H since attenuation is directly linked to SPL but not to variability and randomness of the soundscape.

The analysis with oxygen was strongly limited by the fact that oxygen levels dropped below 0.7 mg/l very few times, and when it did it was only for several hours. Although fishes and crustaceans are affected relatively quickly by hypoxia, meaning that the effect should be visible within a short time frame (hours-days) (Hernández-Miranda et al., 2012), it would be useful to repeat the analyses with data in which a longer lasting OMZ event is included. The limited occurrence of OMZs also restricted the number of recordings which could be used for the analysis and meant that they were spread out over quite a large amount of time. This could have a significant influence, as studies have shown a strong seasonal and monthly effect on the soundscape (Radford et al., 2008). It would therefore be useful to redo the analyses with larger subsamples, less spread out over time and in longer-lasting OMZs.

4.3. IMPLICATIONS OF THE FINDING AND FURTHER RESEARCH

This study contributes to the growing research on the soundscapes of ecosystems around the world. As there have been few studies on this focusing on mesophotic reef, and especially those in temperate areas, the description of the soundscape gives a better understanding into these systems. For instance, it is clear that the protection thereof is still lacking, as MPAs seem to be critically polluted by anthropogenic noise. The fact that there was no distinguishable difference in acoustic diversity and SPL between the different oxygen conditions could point towards the fact that monitoring the soundscape is not a reliable indicator of responses to disturbances, since this was also found by Simmons et al. (2021). However, it is possible that the results were strongly influenced by the limitations regarding the sample size and the anthropogenic noise. Thus, the research would have to be repeated without those limitations to draw more definite conclusions.

This study is also a first step in the documentation of fish sounds in mesophotic reefs. This is crucial in the development of passive acoustic monitoring as a method for investigating ecosystems, which is limited by the few and incomplete databases. Parsons et al. (2022) discusses the need for a global library of underwater sound sources, to expand our understanding of underwater soundscapes and biotic sound-sources. This would enable a

better use of acoustic monitoring for the investigations of ecosystem health and the delineation of important biological areas. This platform would include among others: an overview of biotic sounds, both from known and unknown sources and a library of audio recordings of single sources and of soundscapes. The soundscape and fish signatures identified in this research can thus be an addition to the growing data on underwater sound sources and soundscapes.

Previously, the soundscape has been used to investigate the quality of an MPA in terms of noise pollution (Buscaino et al., 2016). In this research it was observed that the recordings taken in Las Cruces contained a lot of anthropogenic activity. This likely has serious implications for the quality of the MPA, as it has already been observed that excess noise has physiological and behavioural impacts on marine animals, affecting cognitive capabilities, hormone levels and disturbing interspecific interactions (Duarte et al., 2021; Mills et al., 2020; Nedelec et al., 2017). Since acoustic energy propagates far in the water, small MPAs are especially vulnerable to this kind of pollution. Thus, further research on the anthropogenic activity within the dataset or research area, and the impact of this on organisms, would be extremely valuable.

The comparison of acoustic indices with eDNA showed that they can be used reliably to compare the diversity of different sites, especially the combination of ACI and H, AEI and BI. However, these indices seem to be strongly impacted by anthropogenic noise, and do not accurately reflect the diversity of fish signals observed. Therefore, repeated studies using a combination of methods is needed to better understand which part of the soundscape is highlighted by different indices. In addition, it would be relevant to investigate how best to combine several indices, which may represent diversity better. Further research should be conducted using a higher number of sites, which would be able to give a better indication of the spatial variability of eDNA and acoustic diversity and how these relate to each other, as well as improving the evaluation of the relevance of acoustic data for more granular analysis of temporal effects of variables on populations.

5. CONCLUSION

Both eDNA and Passive Acoustic Monitoring have been used in a variety of ecosystems, terrestrial as well as aquatic. The possibilities that these methods offer are expanding rapidly, with new research designs and tools being developed to be deployed in a variety of contexts. The relevance of these techniques is not restricted to the fact that they allow the monitoring of ecosystems and species that are difficult to access with traditional methods, but they also enable studying systems in a non-intrusive and continuous manner. In this study we showed that both methods can be used together in an effective way, providing comparable results. In addition, despite limited data on sound-producing fish in the area, it seems possible to recognise fish species based on the occurrence of their vocal signatures in the soundscape. This could be a cost-effective way of monitoring species. This study has also shown that acoustic indices are useful in assessing diversity in marine ecosystems and can be used to compare sites, as well as the efficacy of marine protected areas. There is not yet an indication that these are also able to evaluate the impact of disturbances and changes in abiotic factors on the ecosystem. However, more research is necessary to understand these processes better.

Through the Sustainable Development Goals, we have pledged to protect life on our planet living below the water. As growing evidence is coming to light of the impact human life has had on the planet, it is imperative that we understand and protect ecosystems that are vulnerable to the many anthropogenic and environmental threats. Simply by listening to our oceans, we are now able to study what is taking place beneath the waves. As such, we come closer to bringing mesophotic reefs to the light, understanding their link with shallow ecosystems and moving towards better protection strategies. Still, there is a long way to go yet before these methods are ready to be used on a large scale, as indices need to be better tested and adjusted for marine ecosystems, taking into account the anthropogenic noise polluting our oceans, and working through the strengths, weaknesses and possibilities which are presented in this study. Despite the limitations at this time, it is promising to conclude that these tools can be easily deployed in new ecosystems, offering fast and reliable measures of diversity.

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APPENDIX A: SAMPLING DETAILS

Supplementary table 1. Details for the deployment and retrieval of hydrophones.

Series number	Site	Site coordinate	Date & time deployment	Date & time retrieval	Depth
6315	Algarrobo	-33.348236°, -71.701469°	16-02-2022 17:21	02-05-2022 11:31	33m
6318	Las Cruces	-33.489661°, -71.665048°	16 February 2022 13:51	2 Mei 2022 13:57	33m

Supplementary table 2. Details for the eDNA samples taken on the 11^{1h} and 12th of January in Algarrobo and Las Cruces.

Sample number	Latitude	Longitude	Depth	Time	Sampling site
1	33°20.932	71°42.089	30	10:25	Algarrobo
2	33°20.934	71°42.080	30	10:48	Algarrobo
3	33°20/930	71°42.076	30	11:11	Algarrobo
1	33°29.366	71°39.917	31	12:41	Las Cruces
2	33°29.378	71°39.892	33	13:02	Las Cruces
3	33°29.374	71°39.918	30	13:36	Las Cruces

Supplementary table 3. Details for the eDNA samples taken on the 13th and 14th of March in Algarrobo and Las Cruces.

Sample number	**Latitude**	**Longitude**	**Depth**	**Time**	**Sampling site**
1	-33,348989	-71,701329	30	11:37	Algarrobo
2	-33,349116	-71,701462	30	12:18	Algarrobo
3	-33,348991	-71,701387	30	12:53	Algarrobo
1	-33,489556	-71,664751	30	13:09	Las Cruces
2	-33,489517	-71,664781	31	14:14	Las Cruces
3	-33,489617	-71,664669	28	14:33	Las Cruces

APPENDIX B: RESULTS OF THE EDNA

Supplementary table 4. List of fish and invertebrate species identified with eDNA per season and site.

Genus	Species	Phylum	Site	Season
		Fish		
Chromis	Chromis enchrysura	Chordata	Both	Both
Engraulis	Engraulis ringens	Chordata	Both	Both
Genypterus		Chordata	ALG	Summer
Isacia	Isacia conceptionis	Chordata	LC	Summer
Isacia	Isacia conceptionis	Chordata	ALG	Autumn
Merluccius	Merluccius gayi	Chordata	LC	Summer
Merluccius		Chordata	Both	Summer
Merluccius	Merluccius productus	Chordata	ALG	Summer
Sebastes	Sebastes oculatus	Chordata	ALG	Summer
Seriolella		Chordata	Both	Summer
Trachurus	Trachurus symmetricus	Chordata	Both	Summer
		Invertebrates		
Adaliopsis	Adaliopsis alpina	Arthropoda	ALG	Summer
Aequorea	Aequorea sp. MW-2012	Cnidaria	ALG	Summer
Agraphydrus	Agraphydrus sp. 3 MTM-2009	Arthropoda	LC	Both
Austromegabalanus	Austromegabalanus psittacus	Arthropoda	ALG	Autumn
Axiopsis	Axiopsis serratifrons	Arthropoda	LC	Summer
Bicellariella	Bicellariella ciliata	Bryozoa	ALG	Summer
Calanoides	Calanoides patagoniensis	Arthropoda	Both	Autumn
Calanoides	Calanoides acutus	Arthropoda	Both	Both
Calanoides	Calanoides patagoniensis	Arthropoda	Both	Summer
Calanus	Calanus chilensis	Arthropoda	LC	Autumn
Calanus		Arthropoda	Both	Both
Calocalanus	Calocalanus tenuis	Arthropoda	LC	Autumn

Calyptraeotheres	Calyptraeotheres politus	Arthropoda	ALG	Autumn
Carinoma	Carinoma tremaphoros	Nemertea	LC	Summer
Centropages	Centropages typicus	Arthropoda	Both	Both
Chaetopterus		Annelida	ALG	Summer
Chrysaora		Cnidaria	ALG	Summer
Chrysaora	Chrysaora plocamia	Cnidaria	ALG	Summer
Cicadula	Cicadula ornata	Arthropoda	ALG	Summer
Clytia		Cnidaria	Both	Autumn
Clytia	Clytia sp. 2 SL-2013	Cnidaria	LC	Autumn
Clytia	Clytia gracilis	Cnidaria	Both	Summer
Clytia		Cnidaria	Both	Summer
Ctenocalanus		Arthropoda	ALG	Summer
Dendrobaena	Dendrobaena veneta	Annelida	LC	Autumn
Dipolydora		Annelida	ALG	Autumn
Eurytemora	Eurytemora carolleeae	Arthropoda	ALG	Autumn
Lamprigera	Lamprigera sp. 1 GL-2020	Arthropoda	Both	Summer
Laonice	Laonice irinae	Annelida	LC	Autumn
Leptopeza	Leptopeza sp. BBDED765-10	Arthropoda	LC	Summer
Lordithon	Lordithon sp. CHU1	Arthropoda	ALG	Summer
Muggiaea	Muggiaea atlantica	Cnidaria	Both	Both
NA	Cyclopidae sp. BIOUG10171-D02	Arthropoda	LC	Autumn
NA	Dolichopodidae sp. BIOUG01475-A07	Arthropoda	LC	Autumn
Nassarius		Mollusca	LC	Autumn
Neocalanus		Arthropoda	Both	Summer
Nephtys		Annelida	LC	Summer
Oithona	Oithona sp. 1 New Caledonia-RJH-2001	Arthropoda	Both	Autumn
Paracalanus	Paracalanus sp. D AC-2013	Arthropoda	Both	Autumn

Paracalanus	Paracalanus parvus	Arthropoda	Both	Both
Paracalanus	Paracalanus sp. D AC-2013	Arthropoda	Both	Summer
Paraprionospio	Paraprionospio sp. EPK-2019	Annelida	Both	Autumn
Peltogasterella	Peltogasterella gracilis	Arthropoda	ALG	Summer
Phalacrostemma	Phalacrostemma sp. AM_W50676	Annelida	ALG	Autumn
Phragmatopoma	Phragmatopoma virgini	Annelida	Both	Both
Phragmatopoma	Phragmatopoma sp. VR-2004	Annelida	LC	Summer
Polydora	Polydora websteri	Annelida	ALG	Autumn
Polydora		Annelida	Both	Autumn
Polydora	Polydora cornuta	Annelida	ALG	Autumn
Polydora	Polydora hoplura	Annelida	ALG	Autumn
Protaphorura	Protaphorura sp. GHLYST_4	Arthropoda	LC	Autumn
Rhincalanus	Rhincalanus nasutus	Arthropoda	ALG	Summer
Scolelepis		Annelida	LC	Summer
Sinocalanus	Sinocalanus sinensis	Arthropoda	Both	Both
Spiophanes	Spiophanes kroyeri	Annelida	Both	Autumn
Spiophanes		Annelida	LC	Summer
Triconia		Arthropoda	LC	Summer
Triticella	Triticella pedicellata	Bryozoa	ALG	Summer
Verruca	Verruca laevigata	Arthropoda	Both	Both
Xylotrechus	Xylotrechus grayii	Arthropoda	LC	Summer
	Palpata sp. PO2	Annelida	ALG	Summer
	Sagittoidea sp. USNM IZ 1448441	Chaetognatha	ALG	Summer

APPENDIX C: RESULTS OF THE GLM

Supplementary table 5. LM with SPL as dependent factor and oxygen, site, and day/night as independent variables. Pr(>|t|) in red indicate a significant result ($p < 0.05$).

	Estimate	Std. Error	t value	Pr(>|t|)
High frequencies				
(Intercept)	93.5038	15.6133	5.989	7.59E-08
Oxygen.catNormal	21.0919	16.2452	1.298	0.198
Oxygen.catSevere hypoxia	-11.2035	18.553	-0.604	0.548
Temperature	2.6481	1.6252	1.629	0.108
SiteLC	-2.427	0.3246	-7.477	1.44E-10
Oxygen.catNormal:Temperature	-2.1549	1.687	-1.277	0.206
Oxygen.catSevere hypoxia:Temperature	1.2002	1.9297	0.622	0.536
Oxygen.catNormal:SiteLC	-0.574	0.4585	-1.252	0.215
Oxygen.catSevere hypoxia:SiteLC	-0.6895	0.4732	-1.457	0.149
Low frequencies				
(Intercept)	111.5416	22.8071	4.891	5.92E-06
Oxygen.catNormal	16.3476	23.7301	0.689	0.493
Oxygen.catSevere hypoxia	22.4243	27.1013	0.827	0.411
Temperature	2.2794	2.374	0.96	0.34
SiteLC	-0.2319	0.4741	-0.489	0.626
Oxygen.catNormal:Temperature	-1.6729	2.4643	-0.679	0.499
Oxygen.catSevere hypoxia:Temperature	-2.3691	2.8187	-0.84	0.403
Oxygen.catNormal:SiteLC	-0.476	0.6698	-0.711	0.48
Oxygen.catSevere hypoxia:SiteLC	0.6417	0.6912	0.928	0.356

Supplementary table 6. LM with ACI values as dependent factor and oxygen, site, and day/night as independent variables. F-values in red indicate a significant result ($p < 0.05$).

	Estimate	Std. Error	t value	Pr(>|t|)
High frequencies				
(Intercept)	200.5319	18.6865	10.731	<2e-16
Oxygen.catNormal	-17.6237	22.4619	-0.785	0.435
Oxygen.catSevere hypoxia	-26.7466	31.6487	-0.845	0.401

Temperature	-2.5988	1.9224	-1.352	0.181
SiteLC	18.6979	0.8523	21.937	<2e-16
Oxygen.catNormal:Temperature	1.8405	2.3007	0.8	0.426
Oxygen.catSevere hypoxia:Temperature	2.7509	3.2772	0.839	0.404
Oxygen.catNormal:SiteLC	-0.9334	1.2382	-0.754	0.453
Oxygen.catSevere hypoxia:SiteLC	1.3324	1.2127	1.099	0.276
Low frequencies				
(Intercept)	284.898	157.589	1.808	0.0748
Oxygen.catNormal	-180.682	189.428	-0.954	0.3434
Oxygen.catSevere hypoxia	-227.893	266.903	-0.854	0.396
Temperature	-8.717	16.213	-0.538	0.5925
SiteLC	-7.043	7.188	-0.98	0.3304
Oxygen.catNormal:Temperature	18.539	19.402	0.955	0.3425
Oxygen.catSevere hypoxia:Temperature	23.981	27.638	0.868	0.3884
Oxygen.catNormal:SiteLC	17.657	10.442	1.691	0.0952
Oxygen.catSevere hypoxia:SiteLC	4.442	10.227	0.434	0.6653

Supplementary table 7. LM with H values as dependent factor and oxygen, site, and day/night as independent variables. F-values in red indicate a significant result ($p < 0.05$).

	Estimate	Std. Error	t value	Pr(>|t|)
High frequencies				
(Intercept)	0.860366	0.0457606	18.801	< 2e-16
Oxygen.catNormal	0.0756291	0.0476126	1.588	0.1166
Oxygen.catSevere hypoxia	0.0739605	0.0543767	1.36	0.178
Temperature	0.0080738	0.0047632	1.695	0.0944
SiteLC	-0.0071256	0.0009513	-7.49	1.37E-10
Oxygen.catNormal:Temperature	-0.0079194	0.0049444	-1.602	0.1136
Oxygen.catSevere hypoxia:Temperature	-0.0077706	0.0056556	-1.374	0.1737
Oxygen.catNormal:SiteLC	0.0017156	0.0013439	1.277	0.2059
Oxygen.catSevere hypoxia:SiteLC	0.0001694	0.0013869	0.122	0.9031
Low frequencies				
(Intercept)	0.112667	0.403039	0.28	0.781
Oxygen.catNormal	0.136955	0.419351	0.327	0.745

Oxygen.catSevere hypoxia	0.327012	0.478926	0.683	0.497
Temperature	0.008476	0.041952	0.202	0.84
SiteLC	0.007162	0.008379	0.855	0.395
Oxygen.catNormal:Temperature	-0.013746	0.043548	-0.316	0.753
Oxygen.catSevere hypoxia:Temperature	-0.035134	0.049812	-0.705	0.483
Oxygen.catNormal:SiteLC	-0.001492	0.011837	-0.126	0.9
Oxygen.catSevere hypoxia:SiteLC	0.017114	0.012215	1.401	0.165

Supplementary table 8. LM with PC1 values as dependent factor and oxygen, site, and day/night as independent variables. F-values in red indicate a significant result ($p < 0.05$).

	Estimate	Std. Error	t value	Pr(>|t|)
High frequencies				
(Intercept)	25.0276	14.9424	1.675	0.0983
Oxygen.catNormal	-22.7813	15.5471	-1.465	0.1472
Oxygen.catSevere hypoxia	-8.2668	17.7558	-0.466	0.6429
Temperature	-2.8517	1.5553	-1.834	0.0709
SiteLC	3.8624	0.3106	12.434	<2e-16
Oxygen.catNormal:Temperature	2.3623	1.6145	1.463	0.1478
Oxygen.catSevere hypoxia:Temperature	0.8438	1.8467	0.457	0.6491
Oxygen.catNormal:SiteLC	-0.1345	0.4388	-0.307	0.76
Oxygen.catSevere hypoxia:SiteLC	0.1065	0.4529	0.235	0.8148
Low frequencies				
(Intercept)	3.5996	17.8268	0.202	0.8405
Oxygen.catNormal	-4.1028	18.5483	-0.221	0.8256
Oxygen.catSevere hypoxia	-17.9056	21.1833	-0.845	0.4008
Temperature	-0.2558	1.8556	-0.138	0.8907
SiteLC	-1.9683	0.3706	-5.311	1.16E-06
Oxygen.catNormal:Temperature	0.4018	1.9262	0.209	0.8353
Oxygen.catSevere hypoxia:Temperature	1.9427	2.2032	0.882	0.3808
Oxygen.catNormal:SiteLC	0.1617	0.5235	0.309	0.7583
Oxygen.catSevere hypoxia:SiteLC	-1.1604	0.5403	-2.148	0.0351

APPENDIX D: RESULTS OF THE KRUSKAL-WALLIS RANK SUM TESTS

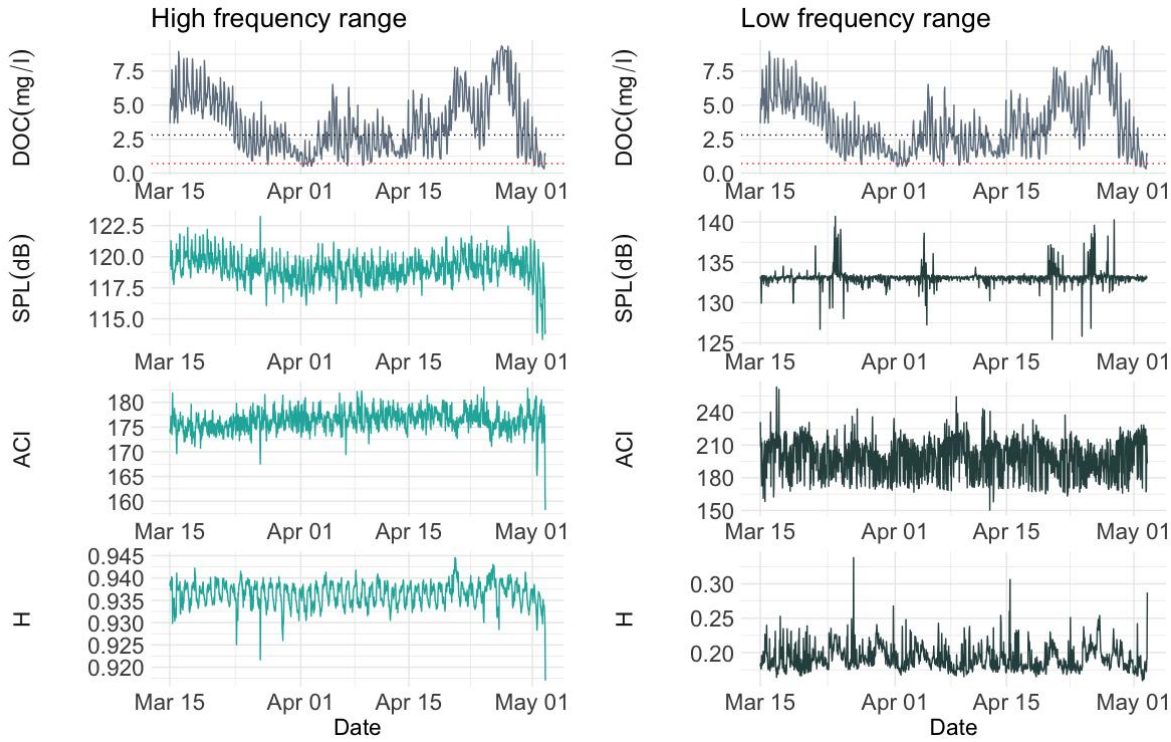
Supplementary table 9. Results of the Kruskal-Wallis test on oxygen categories for SPL, ACI, PC1 and H for both frequency ranges. The significative correlations are in red ($p < 0.05$).

Acoustic index and frequency range	**H**	**df**	**p-value**
SPL low	1.7875	2	0.4091
SPL high	1.0491	2	0.5918
PC low	0.98458	2	0.6112
PC high	1.0745	2	0.5844
ACI low	2.337	2	0.3108
ACI high	0.27475	2	0.8716
H low	2.1465	2	0.3419
H high	0.48192	2	0.7859

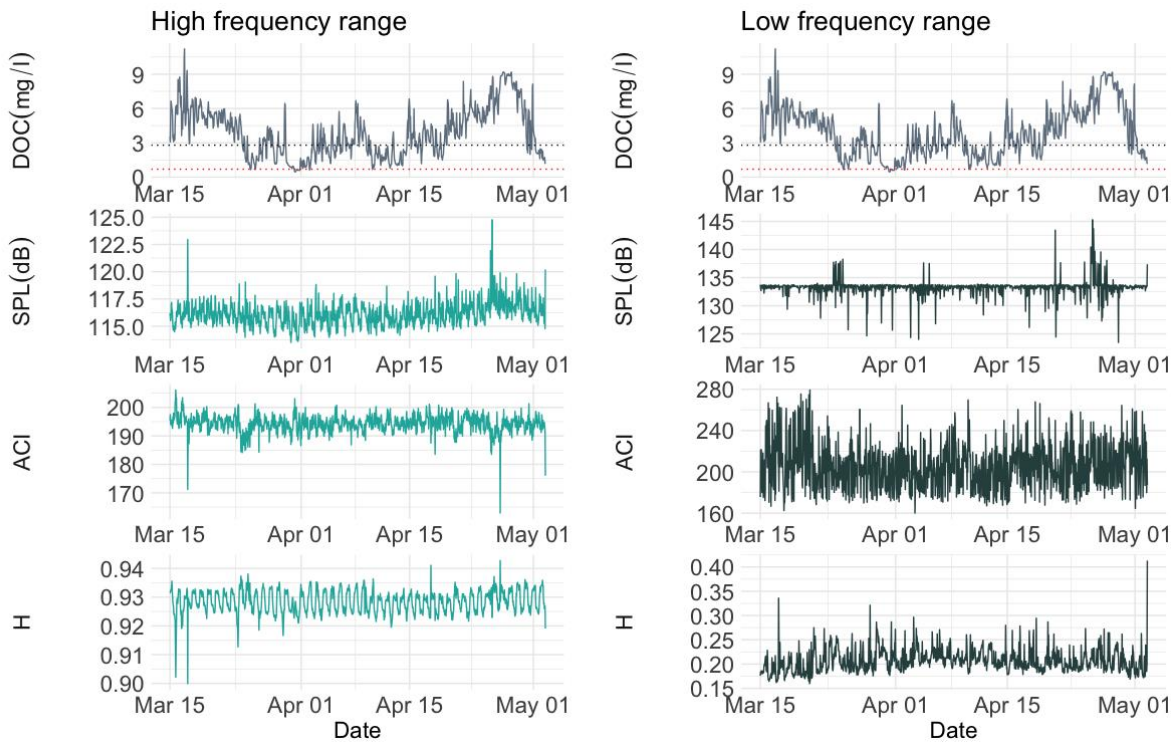
Supplementary table 10. Results of the Kruskal-Wallis test on site for SPL, ACI, PC1 and H for both frequency ranges. The significative correlations are in red ($p < 0.05$).

Acoustic index and frequency range	**H**	**df**	**p-value**
SPL low	275.38	1	< 2.2e-16
SPL high	1529.8	1	< 2.2e-16
PC low	1517.1	1	< 2.2e-16
PC high	1715.4	1	< 2.2e-16
ACI low	47.287	1	6.13E-12
ACI high	1734.5	1	< 2.2e-16
H low	305.7	1	< 2.2e-16
H high	1492.2	1	< 2.2e-16

APPENDIX E: TIME SERIES



Supplementary figure 1. Time series of DOC, SPL, ACI and H for high and low frequency ranges in Algarrobo.



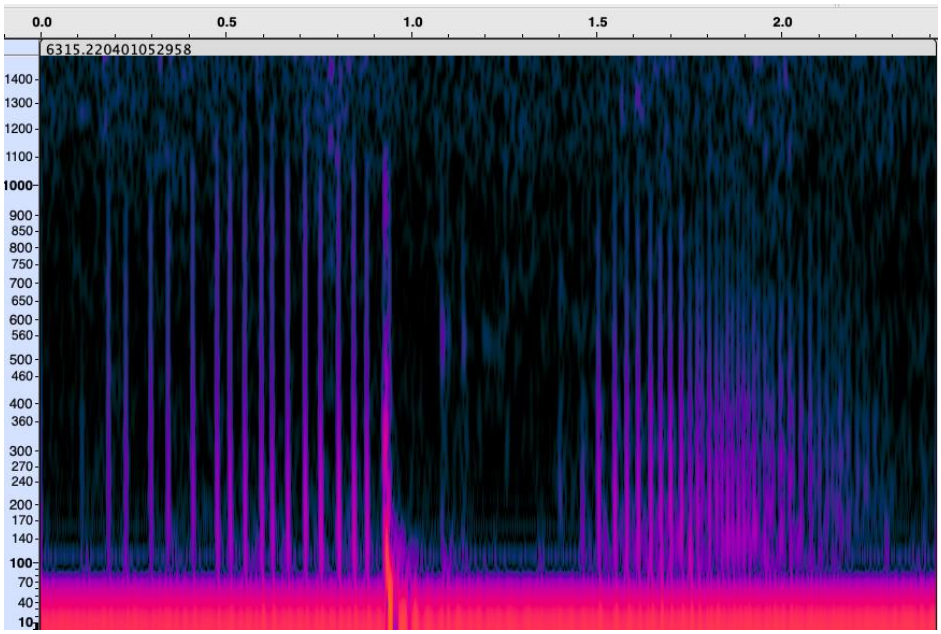
Supplementary figure 2. Time series of DOC, SPL, ACI and H for high and low frequency ranges in Las Cruces.

APPENDIX F: OCCURRENCE OF FISH SIGNATURES WITHIN AND OUTSIDE OF OMZS

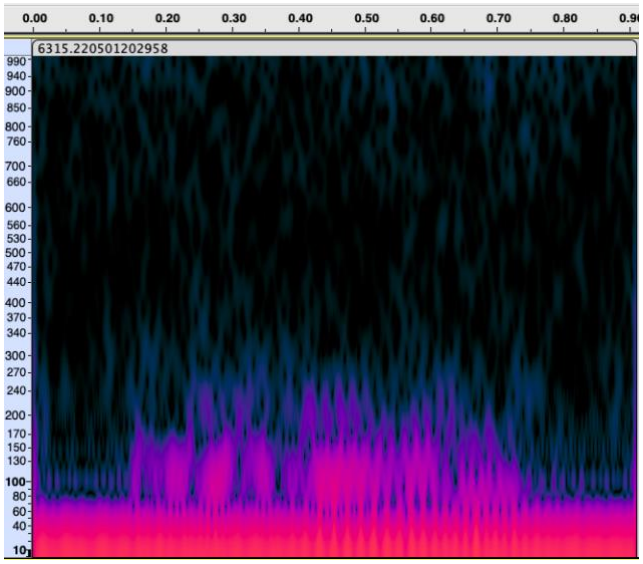
Supplementary 11. Occurrence of fish signatures within and outside of OMZs. The occurrence of the signatures is conveyed in percentage of recordings they appeared in, for samples of recordings taken within and outside of OMZs. The total number of recordings is 16 for Algarrobo and 11 for Las Cruces.

Name	**Algarrobo**		**Las Cruces**	
	OMZ	***No OMZ***	***OMZ***	***No OMZ***
Stridulation	100	93.75	100	81.8
Grunt	100	100	100	100
Croak	87.5	81.25	45.5	81.8
Tapping	81.25	68.75	90.9	90.9
Rattling	81.25	87.5	36.4	54.5
Trilling	81.25	87.5	18.2	27.3
High Tap	75	37.5	0	18.2
Clac	25	18.75	9.1	9.1
Growl	81.25	93.75	100	100
Laugh	6.3	0	0	0
Cackling	37.5	12.5	9.1	27.3
Wheezing	6.25	6.25	0	0
Woosh	0	12.5	18.2	0
Rumbling	0	0	18.2	63.6

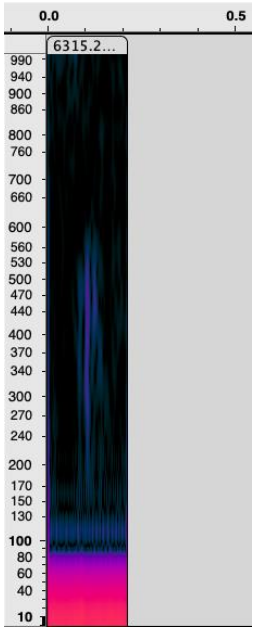
APPENDIX G: FISH SIGNATURES



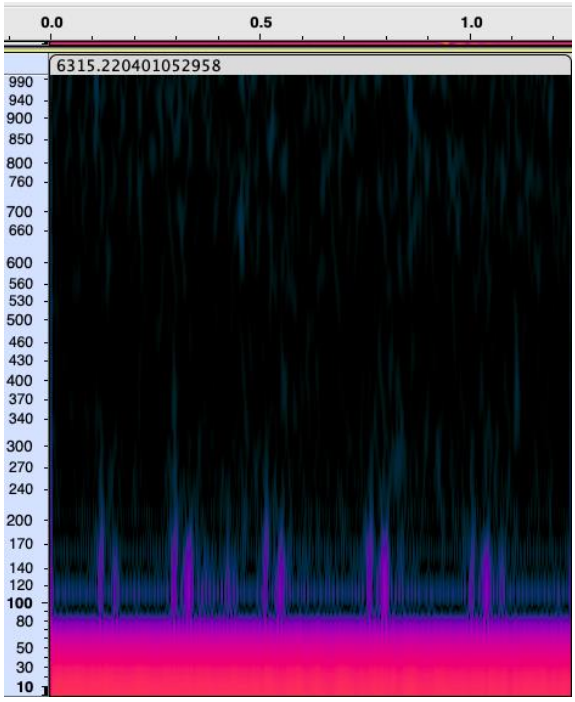
Supplementary figure 3. Spectrogram depicting stridulation. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



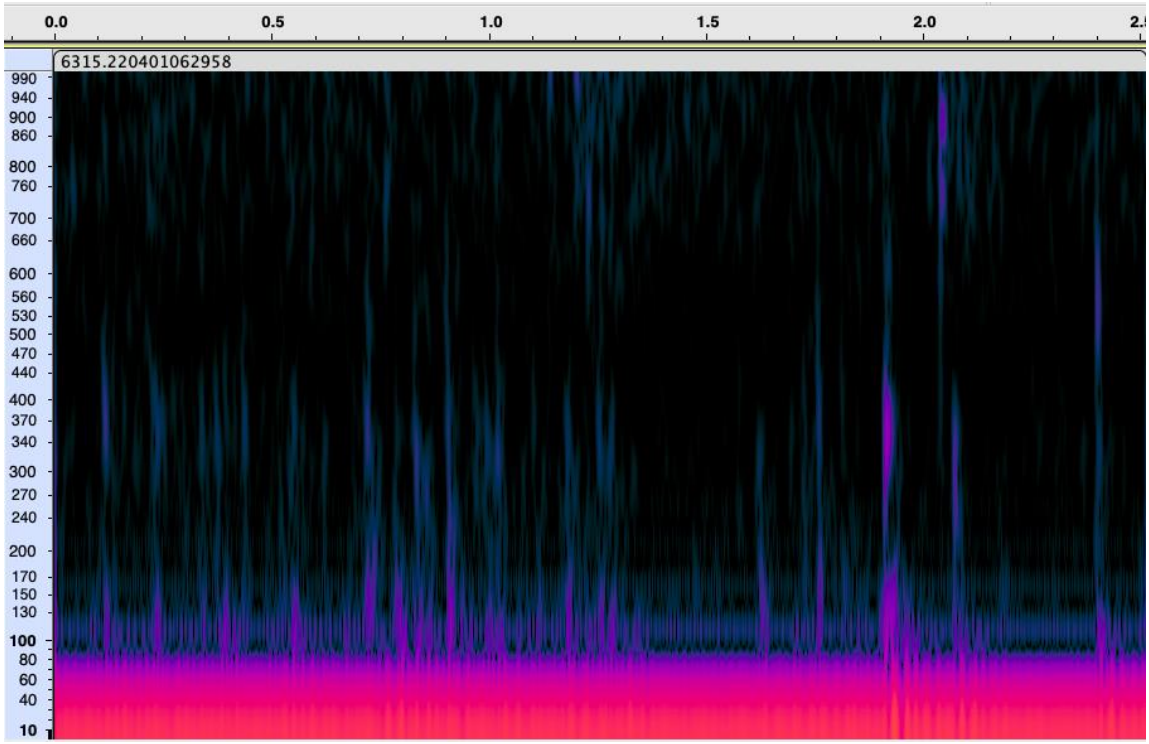
Supplementary figure 4. Spectrogram depicting a fish grunt. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



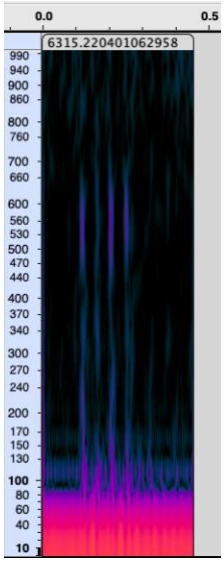
Supplementary figure 5. Spectrogram depicting a fish croak. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



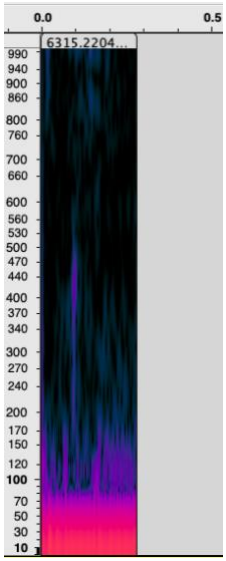
Supplementary figure 6. Spectrogram depicting a tapping signal. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



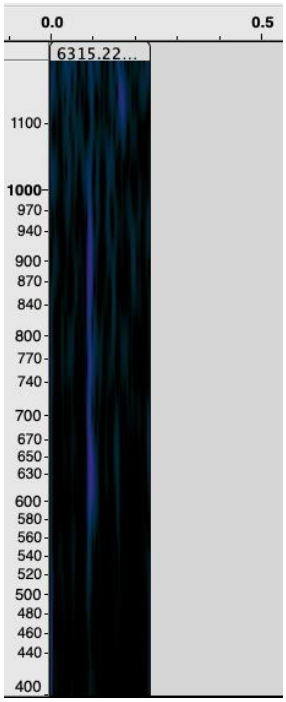
Supplementary figure 7. Spectrogram depicting a rattling signal. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



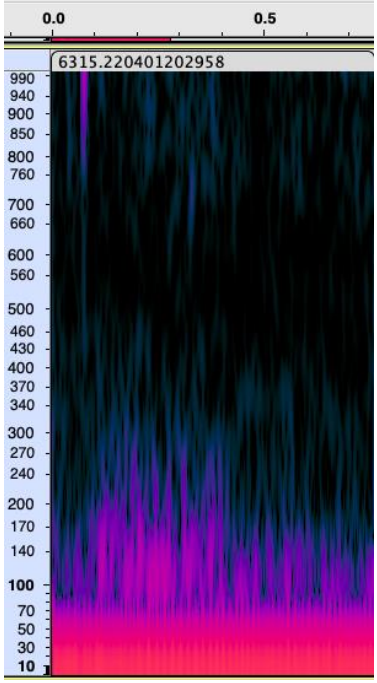
Supplementary figure 8. Spectrogram depicting a trill. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



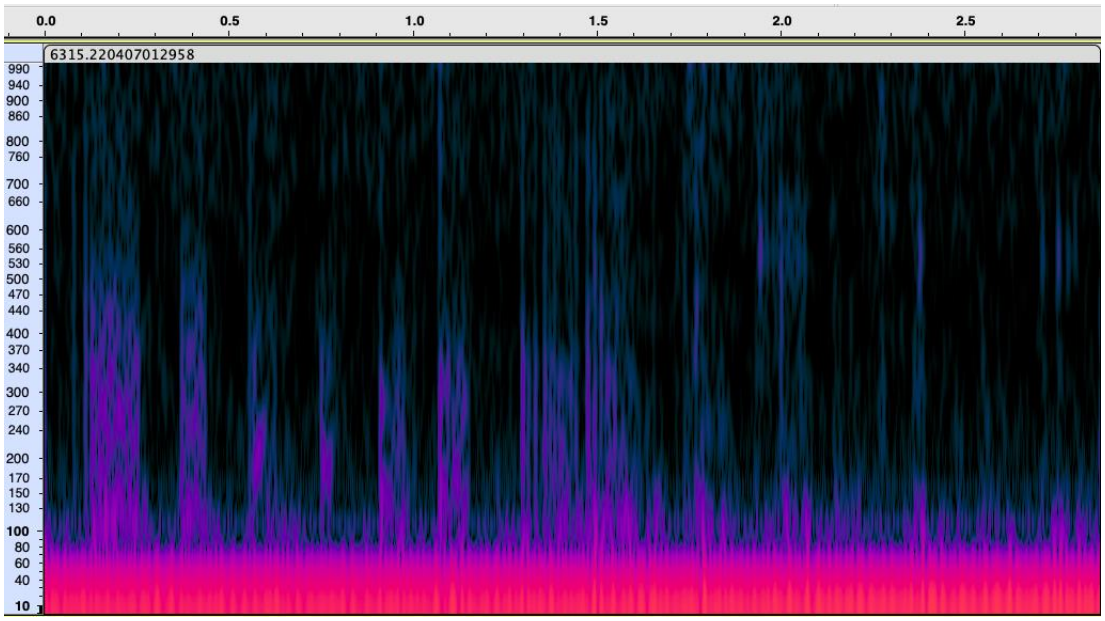
Supplementary figure 9. Spectrogram depicting a high tap. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



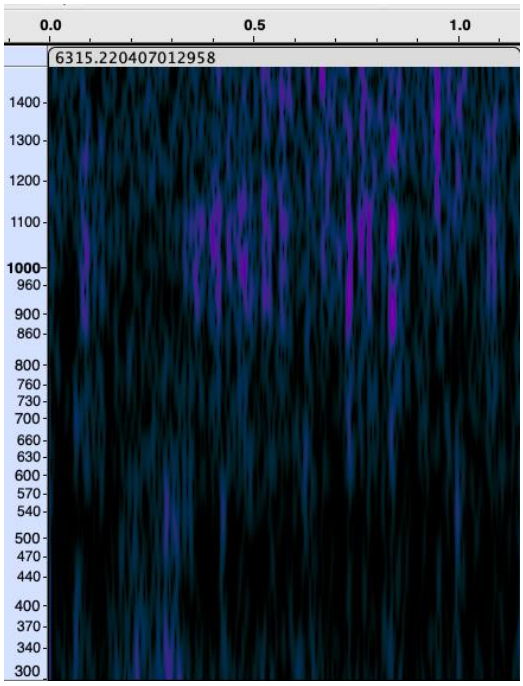
Supplementary figure 10. Spectrogram depicting a “clac” sound. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



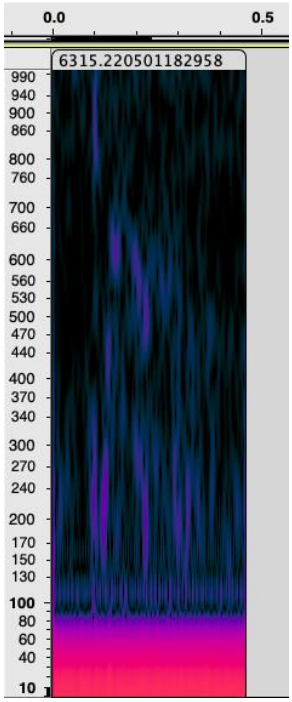
Supplementary figure 11. Spectrogram depicting a fish growl. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



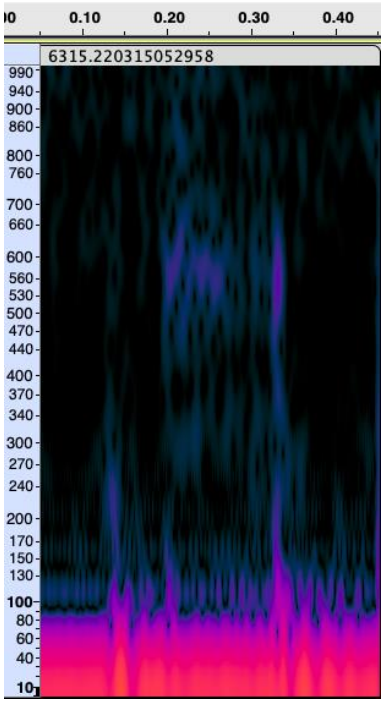
Supplementary figure 12. Spectrogram depicting a laughing sound produced by a fish. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



Supplementary figure 13. Spectrogram depicting a cackling sound. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



Supplementary figure 14. Spectrogram depicting a wheezing sound. The y-axis represent time in seconds and the x-axis represents frequency in Hz.



Supplementary figure 15. Spectrogram depicting a wooshing sound. The y-axis represent time in seconds and the x-axis represents frequency in Hz.