Ispa
Instituto Universitário
de Ciências Psicológicas,
Sociais e da Vida

INTEGRATING VIDEO AND ACOUSTIC NON-INVASIVE TECHNIQUES TO MONITOR THE PROFESSOR LUIZ SALDANHA MARINE PARK (PLSMP)

Noelia Ríos Ruiz

Tese submetida como requisito principal para obtenção do grau de
Doutoramento em Biologia do Comportamento

Lisboa 2025

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ISPA Instituto Universitário de Ciências Psicológicas Sociais e da Vida- MARE - Marine and Environmental Sciences Centre/ARNET - Aquatic Research Network.

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Orientadores:

Doutor Gonçalo Silva
ISPA Instituto Universitário de Ciências Psicológicas Sociais e da Vida- MARE - Marine and Environmental Sciences Centre/ARNET - Aquatic Research Network

Doutor Miguel Pessanha Pais
Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal

Doutora Maria Clara Amorim
Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal

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“Love for the ocean is the first step towards its conservation. We need more people to fall in love with its beauty and take action to protect it.” Sylvia Earle

RESUMO

Com o declínio global da biodiversidade, há uma necessidade urgente de tecnologias rentáveis e acessíveis para monitorizar os impactos nos ambientes marinhos. A monitorização a longo prazo é essencial para preservar o funcionamento dos ecossistemas, através da avaliação da biodiversidade, das dinâmicas espaciais e temporais, e dos efeitos das atividades humanas nos padrões de comportamento. Os organismos marinhos dependem do som para comunicar, navegar e encontrar alimento, tornando a Monitorização Acústica Passiva (PAM) um método não invasivo e eficaz para estudar a biodiversidade e os impactos antropogênicos. Desde o seu desenvolvimento em meados do século XX, a monitorização acústica tornou-se uma ferramenta valiosa na ecologia e conservação, especialmente quando combinada com métodos visuais ou genéticos.

Esta tese explora a integração da monitorização acústica e visual no Parque Marinho Professor Luiz Saldanha (PMPLS), para estudar comunidades acústicas de peixes e o impacto dos sons antropogênicos nos comportamentos. Os principais objetivos são compilar sons de peixes através do uso de PAM, identificar fontes sonoras usando sistemas de câmaras iscadas (acrónimo inglês BRUVS), analisar variações temporais e entre diferentes níveis de proteção/habitats, comparar métodos de monitorização e validar índices acústicos com índices tradicionais de biodiversidade. Estas abordagens visual e acústica ajudam a avaliar padrões comportamentais e os impactos da atividade antropogênica em espécies marinhas chave.

Hidrofones foram instalados em três locais com diferentes tipos de habitat durante 15 meses. Uma revisão da literatura identificou 28 espécies sonoras e 71 potencialmente sonoras, representando 18,6% e 52,6% das espécies registradas na área. As análises acústicas revelaram 33 tipos de sons putativos de peixes, classificados como contínuos ou pulsados. A combinação de registros acústicos com BRUVS evidenciou limitações na identificação de fontes sonoras. A comparação com sons descritos na literatura permitiu associar seis sons a famílias de peixes específicas: os sons #4, #8 e #33 aos Serranidae; o som #10 aos Triglidae; o #15 aos Sciaenidae; o #42 aos Pomacentridae; e o #43 aos Scorpaenidae. A ocorrência dos sons foi analisada sazonalmente, ao longo do dia e entre áreas de proteção. Embora a variação sazonal fosse limitada, a produção e diversidade sonora aumentaram ao pôr-do-sol e à noite. As diferenças espaciais sugerem que a composição rochosa e a distância ao estuário influenciam a presença e diversidade sonora. Os índices de biodiversidade e abundância obtidos por três métodos (UVC, BRUVS e PAM) foram comparados para avaliar a sua eficácia, revelando complementaridade nestes métodos na avaliação da biodiversidade em peixes.

Foi também realizado um estudo com BRUVS pelágicos à deriva, focado na presença e comportamento alimentar do tubarão-azul. Juvenis foram mais observados na primavera e em zonas costeiras, sugerindo que a área pode servir de berçário. Adultos ocorreram mais ao largo, sobretudo sobre canhões submarinos. A temperatura, a visibilidade e a distância à costa influenciaram o comportamento. A análise acústica indicou que o ruído de embarcações reduziu o tempo de interação dos tubarões com os BRUVS e o número de comportamentos registados.

Este estudo demonstra que a combinação de métodos visuais e acústicos são ferramentas eficazes para monitorizar comunidades acústicas de peixes e espécies elusivas como o tubarão-azul. A combinação mostra-se promissora para avaliar a biodiversidade e os impactos de alterações ambientais, apoiando a gestão e conservação dos ecossistemas marinhos.

Palavras-chave: Monitorização Acústica Passiva, Áreas Marinhas Protegidas, Paisagens sonoros, comunidades de peixes

ABSTRACT

With global biodiversity in decline, cost-effective and affordable technologies are urgently needed to monitor the impacts over marine environments. Long-term monitoring is essential for preserving ecosystem functions by assessing biodiversity, spatial and temporal dynamics, and the effects of human activities on behaviour. Marine organisms depend on sound for communication, navigation, and foraging, making Passive Acoustic Monitoring (PAM) a non-invasive and effective method for studying biodiversity and anthropogenic impacts. Since its development in the mid-20th century, acoustic monitoring has become a valuable tool in ecology and conservation, particularly when used alongside visual or genetic methods.

This thesis explores the integration of video and acoustic monitoring in the Professor Luiz Saldanha Marine Park (PLSMP) to study fish acoustic communities and the impact of anthropogenic sounds on their behaviours. The main aims are to compile underwater fish sounds using PAM, identify sound sources through Baited Remote Underwater Visual Systems (BRUVS), examine temporal variations across protection levels/habitats, compare monitoring techniques, and validate acoustic indices against traditional biodiversity indices. These visual and acoustic approaches help assess behaviour patterns and the impact of anthropogenic activity on key marine species.

Acoustic recorders were deployed at three locations with varying habitat types for 15 months. A literature review of species in the area identified 28 soniferous and 71 potentially soniferous fish, representing 18.6% and 52.6% of the reported species, respectively. Acoustic analyses revealed 33 putative fish sound types, categorised as either continuous or pulsed. Combining hydrophone data with BRUVS showed some limitations in identifying sound sources. By comparing recorded sounds with those in the literature, six sound types were tentatively linked to specific fish families. Sounds #4, #8, and #33 resembled those of the Serranidae family; #10 matched Triglidae; #15 was similar to Sciaenidae; #42 to Pomacentridae; and #43 to Scorpaenidae.

Fish sound occurrence was analysed across seasons, times of day, and protection areas. While seasonal variation was limited, increased sound production and diversity occurred at sunset and night. Spatial differences suggested that rock substrate and distance to the estuary influenced the presence and variety of fish sounds. The biodiversity and abundance indices from three methods (UVC, BRUVS, and PAM) were also compared to assess their relative effectiveness, highlighting their complementarity in assessing fish biodiversity.

Additionally, a pelagic drift BRUVS study focused on blue shark presence and foraging behaviour. Juveniles were mostly observed in spring, closer to shore, especially in the epipelagic and mesopelagic zones, indicating the area may serve as a nursery. Adults were more commonly sighted offshore, particularly near submarine canyons. Environmental factors such as temperature, visibility, and proximity to the coast influenced shark behaviour. Notably, acoustic analysis showed that boat noise reduced both the duration and frequency of shark interactions with the BRUVS.

This study shows that the combination of visual and acoustic methods are effective tools for monitoring fish acoustic communities and elusive species like the blue shark. The combination holds great promise for assessing fish biodiversity and the impacts of environmental change, supporting improved marine management and conservation.

Keywords:

Passive Acoustic Monitoring, Marine Protected Areas, Soundscapes, Fish Communities

List of research articles

This thesis dissertation is composed of four scientific outputs, each corresponding to a chapter (2 to 5). Two of these outputs are published in peer-reviewed international journals, one is under review and one in preparation.

Chapter II: ***Acoustic fish community in a biogeographic transition zone of the Northeast Atlantic***

Noelia Ríos, Jodanne Pereira, Sebastian Muñoz-Duque, Gonçalo Silva, Miguel Pessanha Pais, Paulo J. Fonseca, Manuel Vieira, Maria Clara P. Amorim

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Chapter III: ***Assessing temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area***

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Chapter IV: ***Monitoring Coastal fish communities: Visual vs. Acoustic Methods***

Noelia Ríos, Mariana Coxey, María Jimenez, Gustavo Franco, Miguel Pessanha Pais, Emanuel J. Gonçalves, Manuel Vieira, Maria Clara P. Amorim, Gonçalo Silva

In prep.

Chapter V: ***Characterising the behaviour of bait-attracted blue sharks Prionace glauca using pelagic drift video***

Noelia Ríos, María Jimenez, Gustavo Franco, Gonçalo Ramos, Miguel Pessanha Pais, Emanuel J. Gonçalves, Maria Clara P. Amorim, Gonçalo Silva

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The data from this study was also used in two other scientific articles.

Cross-referencing unidentified fish sound data sets to unravel sound sources: a case study from the Temperate Northern Atlantic

Vieira, M., Ríos, N., Muñoz-Duque, S., Pereira, J., Carriço, R., Fernandez, M., Monteiro, J. G., Pais, M. P., Quintella, B. R., Silva, G., Silva, R. P., Fonseca, P. J., & Amorim, M. C. P.

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Worldwide Soundscapes: A Synthesis of Passive Acoustic Monitoring Across Realms

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List of figures

List of tables

Supplementary Material

Figures

Tables

Video

Link to behaviour video: https://drive.google.com/file/d/1HalwvO_onmGhmAhHq9nZOIzUlRTB_yjK/view?usp=drive_link

ABBREVIATIONS

MPAs – Marine Protected Area
PLSMP- Professor Luiz Saldanha Marine Park
UNEP – United Nations Environment Program
IUCN – International Union for Conservation of Nature
BRUVS – Baited Remote Underwater Video Systems
UVC- Underwater Visual Census
PAM -Passive Acoustic Monitoring
ROV-Remotely Operated Vehicles
MSFD- Marine Strategy Framework Directive
LC – Least Concern
NT – Near Threaten
VU – Vulnerable
EN – Endangered
CR – Critically Endangered
DD – Data Deficient
CNNs–Convolutional neural networks
H- acoustic entropy index
SPL- Sound Pressure Level
BI- Bioacoustic Index
AI- Amplitude Index
AR- Acoustic Richness
AEI- Acoustic Evenness Index
ACI -Acoustic Complexity Index
MaxN – Maximum number of individuals for a certain species identified/counted in a single video frame.
AI- Artificial intelligence

CHAPTER I

General introduction

Chapter I: General Introduction

1.1 Threatened oceans and Marine Protected Areas

The ocean covers two-thirds of the planet, regulating climate systems and hosting a high biodiversity of flora and fauna. Besides, marine environments are essential for supporting life and providing ecosystem resources, including food and water, and cultural value (Strong et al. 2015, Buonocore et al. 2021). Most coastal communities worldwide rely on marine resources for their livelihoods, with fishing being the primary source of income in many developing countries (Lam et al. 2020). Small-scale fisheries provide at least 40% of global fish catches highlighting their importance supplying food security to these communities (Basurto et al. 2025). However, anthropogenic activities have a significant direct or indirect impact on all marine ecosystems, with overfishing, tourism, deep sea mining, pollution (plastic, chemical and noise), invasive species, habitat degradation, and climate change posing major threats (Halpern et al. 2008, Swartz et al. 2010). Research has shown that global climate change has a significant impact on ecosystems, societies, and economies (Feliciano et al. 2022). Considering these increasing threats, it is essential to prioritise the protection and management of marine ecosystems (Cooley et al. 2022).

Marine Protected Areas (MPAs) are essential tools for restoring and preserving ocean health and protecting ecological service (IPBES 2019). When protection is properly enforced, MPAs can lead to improvement in biodiversity and habitat conservation (Zupan et al. 2018, Grorud-Colvert et al. 2021), and increasing resilience through the reduction of additional stressors (Jacquemont et al. 2022). MPAs can thus bring social and economic benefits for local communities (Grorud-Colvert et al. 2021, Georgian et al. 2022). Achieving these outcomes requires careful consideration of how MPAs are designed, established, and managed across their different stages (Benedetti-Cecchi et al. 2024).

The MPA success depends on basic conditions being met throughout planning, design, implementation, and governance (Grorud-Colvert et al. 2021). The process involves four main stages: proposal, designation, implementation, and active management. In the proposed stage, the intent to create an MPA is made public, with clear goals and stakeholder participation. At the designated stage, the MPA is established through legal means with defined boundaries and goals. The implemented stage involves transitioning the MPA from paper to operational status, with the choice of the governance model and management plans. Finally, in the actively managed stage, ongoing monitoring and adjustments are made to achieve conservation goals and promote commitment between biodiversity and human activities (Grorud-Colvert et al. 2021, Gonçalves et al. 2023). This thesis comes into play in the active management stage. It involves utilizing a combination of established and innovative monitoring techniques, including video and acoustic methods, to integrate new monitoring approaches in the Professor Luiz Saldanha Marine Park (Benedetti-Cecchi et al. 2024).

1.2. The significance of monitoring in marine conservation

Monitoring is fundamental to marine conservation, providing data to inform decision-making and adaptive management (Fortuna et al. 2024). Effective monitoring includes both ecological and human factors to fully capture ecosystem dynamics and resilience (Grorud-Colvert et al. 2021, Benedetti-Cecchi et al. 2024). Long-term monitoring supports management by tracking biodiversity trends, habitat changes, and emerging threats, guiding timely conservation actions to sustain ecosystem resilience under environmental and human pressures (Queiroz et al. 2019).

However, underwater monitoring presents significant challenges, including limited visibility, weather conditions, restricted accessibility, and high costs. Poor visibility due to low light and suspended particles can hinder data collection, while deep or remote areas are difficult to access, often requiring costly specialized equipment (Li et al. 2024). Additionally, financial constraints limit the frequency and scale of monitoring efforts, affecting long-term data collection and management strategies (Abdillah et al. 2017). Every method has its own limitations regarding implementation, costs, and the type and quality of data they produce. To address these limitations, integration and complementary methods is essential for improving the accuracy and effectiveness of marine monitoring programs (Danovaro et al. 2016, Matley et al. 2023).

1.2.1 Traditional and emerging monitoring techniques

With marine threats increasing, there is an urgent need for cost-effective and scalable monitoring technologies to assess the impact of global change on wildlife and ecosystems. Efficient monitoring of MPAs requires non-invasive techniques due to their protected status and ethical considerations. These techniques are crucial for evaluating the abundance, biomass, density, and biodiversity within MPAs (Hardinge et al. 2013, Grane-Feliu et al. 2019) and to evaluate behaviour patterns (Chapuis et al. 2019). This information can help quantify natural variations and detect the impacts of anthropogenic activities (Schram et al. 2019). Furthermore, long-term monitoring is essential for examining ecological responses to management actions (Rhodes et al. 2020). Some techniques used for studying marine environments include experimental fishing (Priester et al. 2021) and telemetry (Garel et al. 2024), which are considered invasive methods. Additionally, non-invasive fishery-independent techniques such as Underwater Visual Census (UVC) (Castro et al. 2023), Baited Remote Underwater Video Systems (BRUVS) (Letessier et al. 2022), and Remotely Operated Vehicles (ROV) (Gómez-Bravo et al. 2024) are commonly employed. More recently, emerging tools like environmental DNA (Brodnicke et al. 2024) and Passive Acoustic Monitoring (PAM) (Carriço et al. 2019) have been applied to monitor marine environments.

1.2.2 Underwater Visual Census (UVC)

For more than 60 years, Underwater Visual Census (UVC) techniques have been widely used to monitor fish assemblages and habitats in shallow marine and freshwater environments (Jones et al. 2015, Prato et al. 2017, Pais & Cabral 2017). These methods are non-destructive, making them suitable for use in MPAs and for long-term monitoring programs (Davis et al. 2014, Grane-Feliu et al. 2019). This technique is commonly employed to generate species inventories and assess abundance, biodiversity, and biomass (Pais & Cabral 2017). However, there are limitations to underwater visual surveys, including the requirement for trained divers, potential inconsistencies in species identification among observers, and constraints on scuba divers due to depth and bottom time limits. These limitations can restrict the survey's spatial coverage and reproducibility (Colton & Swearer 2010, Mallet & Pelletier 2014). Moreover, fish behaviour can be influenced by human presence, affecting the accuracy of UVC estimates: schooling or bold species may be overestimated, while cryptic or shy species are often underestimated (Pais & Cabral 2017).

1.2.3 Baited Remote Underwater Videos System (BRUVS)

Baited Remote Underwater Video Systems (BRUVS) technique allows for non-invasive and cost-effective data collection, providing data of abundance, species composition, biomass, habitat use and behaviour (Grane-Feliu et al. 2019, Jones et al. 2019). This could be applied to study the assemblage of different taxonomic groups, mainly fish (Loyola da Cruz et al. 2022), and elasmobranchs (Prat-Varela et al. 2023, Leonetti et al. 2024). Studies utilising BRUVS help to enhance our knowledge of population dynamics, and the efficacy of MPA management strategies (Harvey et al. 2012, Schram et al. 2019).

Baited videos offer significant advantages over UVC for assessing marine biodiversity. They minimise observer bias by recording footage that can be reviewed multiple times, ensuring accurate species identification and abundance estimates (Whitmarsh et al. 2017). Baited videos can be deployed in deeper waters and challenging conditions where diver surveys may be unsafe or impractical. Additionally, BRUVS allow sampling across a wider range of depths and habitats, capture a greater diversity of species, and provide stronger statistical power (Cundy et al. 2017). BRUVS have been used to monitor different habitats like seagrass (French et al. 2021), coral reefs (Salvador et al. 2024), and seamounts (Baletaud et al. 2023). Various types of BRUVS are used across aquatic environments to collect data. Benthic BRUVS are designed to study species living on the ocean floor, while pelagic BRUVS focuses on species in the water column (Bouchet et al. 2018). Pelagic BRUVS can be static or drifting, covering different study areas (Prat-Varela et al. 2023). In addition, the arrangement of cameras, whether mono or stereo, affects the data collected. Stereo BRUVS allow for measurements of animals to determine size, age, and biomass, whereas mono-BRUVS require additional tools such as lasers for size calculations (Collins et al. 2017, Díaz-Gil et al. 2017).

Similar to every monitoring approach, there are limitations in the use of BRUVS. The bait attracts different fish species based on factors such as smell, hunger, behaviour and schooling tendencies (Santana-Garcon et al. 2014). Bait plume attracts fish to video systems, influencing their approach distance (Rhodes et al. 2020). This method could attract carnivorous species and may not be effective in identifying cryptic species, which could result in a misinterpretation of biodiversity (Langlois et al. 2010). Also, the presence of nearby predators may affect the behaviour of smaller fish (Goetze et al. 2019). The optimal deployment time and sample size for BRUVS and the bait may vary depending on the species and study objectives (De Vos et al. 2014). Other limitations include currents, poor visibility, depth constraints caused by low light levels, and the diet preferences of attracted species (Harvey et al. 2013).

BRUVS have been used to study, behaviour, vertical distribution and diel migration patterns of pelagic and demersal species, distinguishing fish communities at different depths (Santana-Garcon et al. 2014). This technique is useful for examining predator-prey interactions, territoriality, and habitat preferences (Whitmarsh et al. 2017). They were used to assess social behaviours such as mating, and species-specific responses to bait and dominance hierarchies (Bond et al. 2012). Additionally, they have been used to evaluate site fidelity, and movement patterns, providing insights into habitat connectivity and species distribution within MPAs (Espinoza et al. 2014). For example, studies on reef-associated sharks, such as the grey reef shark (*Carcharhinus amblyrhynchos*), have revealed patterns of site fidelity and habitat use within MPA (Bond et al. 2012). Video systems have also been used to study the impact of anthropogenic noise, including boat sound, on fish behaviour. Research has shown that boat noise can influence fish activity, causing changes in feeding behaviour, schooling patterns, and predator avoidance strategies (Mensinger et al. 2016, Chapuis et al. 2019). BRUVS can assess how marine species are affected by noise or other human related stressors. In conclusion, BRUVS can help to monitor behaviours of different species, aiding in fisheries management and conservation efforts.

1.2.4 Acoustics monitoring

Given the need for novel and accessible combinations of techniques to assess long-term fish biodiversity, acoustic methodologies have been increasingly adopted in aquatic environments as a complementary approach to traditional monitoring methods. Due to the specific concepts and techniques involved in monitoring marine environments through sound, the next section will outline the fundamentals of underwater acoustics, bioacoustics, and the associated monitoring methods.

1.3. Acoustics monitoring techniques

1.3.1 Underwater acoustics

Sound is a vibration that propagates through a medium (such as air, water, or solid substrate) as a wave. It has two components, sound pressure and particle motion. Sound pressure is a scalar quantity representing pressure fluctuations caused by sound waves, providing information on temporal and frequency characteristics but not on direction. In contrast, particle motion inherently conveys direction through oscillatory movement (Hawkins & Popper 2017). Sound is a type of compressional or longitudinal wave that travels through mediums, causing particles to move back and forth around a fixed point called the equilibrium position. It is created when a sound-producing object, such as an animal's larynx or a loudspeaker, vibrates and compresses and rarefies the medium, generating waves of alternating high-pressure (compression) and low-pressure (rarefaction) regions that spread outward in all directions (Bradbury & Vehrencamp 1998). Sound can be described by measurable characteristics like amplitude (relates to how loud or soft a sound), and frequency (relates to pitch).

Sound travels faster and farther in water than in air due to its density and low attenuation, with speed in marine environments mainly determined by temperature, salinity, and pressure (Ladich & Winkler 2017). Sound detection is influenced by the receptor’s hearing sensitivity, source level and propagation characteristics of the environment (Popper et al. 2014).

1.3.2 Soundscapes

Soundscapes are defined as “an acoustical composition that results from the voluntary or involuntary overlap of different sounds of physical or biological origin” (Farina et al. 2014). Marine soundscapes are composed of three different sound sources; **biophony** (sounds originating from marine organisms), **geophony** (the sounds of weather and other natural elements, such as rain or waves) and **anthrophony** (sounds created by humans) (Erbe et al. 2011). The first marine soundscape study was conducted in 1978, but there was a significant gap until 2006 due to limited technology (Havlik et al. 2022). The number of studies increased after 2010, driven by technological advancements and improved sound analysis capabilities (Havlik et al. 2022). These advances in soundscape analysis have provided new ecological insights into ecosystem structure and function, offering valuable support for monitoring efforts. Soundscapes are now recognized as indicators of ecosystem health and human impacts, driving new avenues of research (Pijanowski et al. 2011, Bohnenstiehl et al. 2018). The area of soundscape research dedicated to studying animal communication, behaviour, and the acoustic physics of sound production is defined as bioacoustics (Pijanowski et al. 2011). Ecoacoustics, an emerging interdisciplinary field, studies natural and anthropogenic sounds and their relationships with the environment across multiple spatial and temporal scales (Farina et al. 2014).

1.3.3 Biophony

Sound production in different taxa

Sound produced for marine organisms is diverse and encompasses a wide range of taxa. Vocalizations vary among species but are regulated by similar neural pathways in all vertebrates (Bro-Jørgensen 2009). Research suggests that vertebrates share an evolutionary history for vocal communication (Bass et al. 2008).

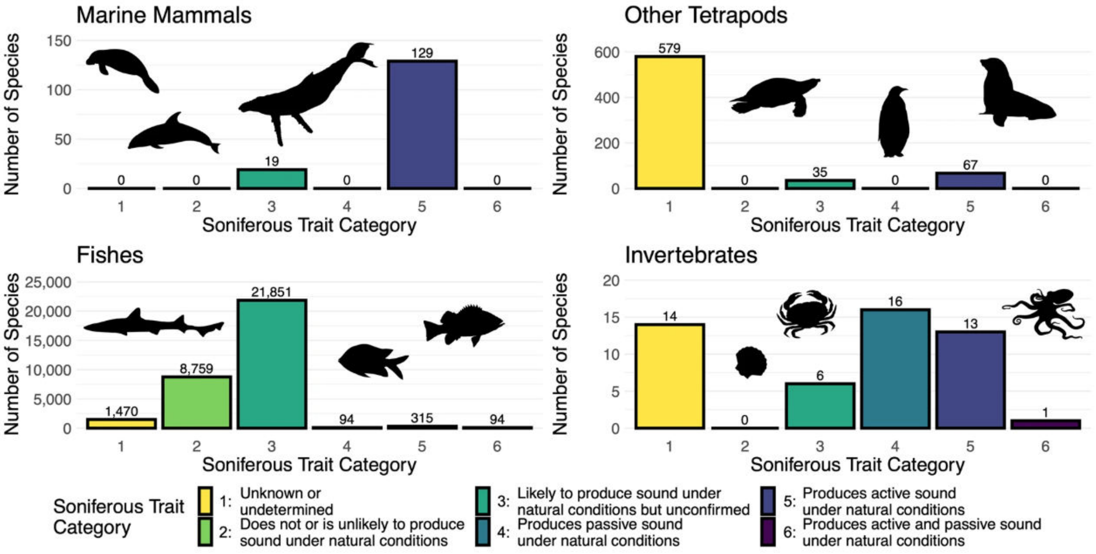
Cetaceans use vocalizations for echolocation and communication, emitting both low-frequency sounds for communication that can travel long distances (e.g., fin whales at 0.02 kHz, Miller et al. 2021) and high-frequency sounds for short-distance communication (e.g., bottlenose dolphins up to 150 kHz, Jones et al. 2019). Peak frequencies of echolocation clicks are about 40 to 130 kHz (Jones et al. 2019).

Pinnipeds, such as seals and sea lions, use vocalizations for communication purposes with signals ranging in frequency from a few Hz to 150 kHz. Sirenians, like manatees and dugongs, emit sounds with frequencies of 2.5 to 5 kHz in various behavioural contexts. Dugongs produce similar sounds with frequencies between 1-8 kHz (Au & Hastings 2008). Sea turtles also produce sound between 0.2 to 0.4 kHz (De Melo et al. 2023).

While marine mammals are the most studied species, marine soundscapes are typically dominated by invertebrates and fish sounds. **Figure 1.1** illustrates the distribution of aquatic and semi-aquatic species and subspecies based on different soniferous trait categories. Invertebrates is the group of marine animals less studied in terms of biophonies. Marine invertebrates create sound through stridulation, muscle contractions, cavitation, and rapping (Coquereau et al. 2016). Stridulation, the most common method, involves rubbing body parts together at a frequency of 6 to 8 kHz (Coquereau et al. 2016). Perhaps one of the most ubiquitous sounds is produced by snapping shrimp, which create distinctive crackling sounds in marine environments by rapidly closing their claws to produce cavitation bubbles with frequencies between 20-200 kHz (Lillis & Mooney 2018, Jakobsen et al. 2021) (see more details below, section b).

Sound production is widespread in bony fishes. Bony fish, comprising the larger group of vocal vertebrates employing acoustic signals for communication, play a substantial role in shaping marine soundscapes (Ladich 2004). To date, researchers have identified over 980 fish species capable of producing sounds, belonging to nearly two-thirds of actinopterygian families (Looby et al. 2022, Rice et al. 2022). While evidence of active sound production in elasmobranchs such as sharks, rays, and skates is still limited (Looby et al. 2022), recent studies have reported sound production in rays and skates with a frequency sound between 1-700 kHz (Fetterplace et al. 2022, Almagro & Barria 2024, Barroil et al. 2024). Another study documents the first case of deliberate sound production by a shark, *Mustelus lenticulatus*, which produces broadband clicks between 2.4–18.5 kHz when handled underwater (Nieder et al. 2025).

From: Global inventory of species categorized by known underwater sonifery



Bar graphs showing the number of aquatic and semi-aquatic species and subspecies placed in each soniferous trait category. The panels separate each taxa grouping, with images included to represent them.

Figure 1.1. Bar graphs illustrate the number of aquatic and semi-aquatic species and subspecies across different soniferous trait categories. Each taxa grouping is visually represented with corresponding images. From Looby et al. 2023).

Sound production in fish

Fish produce sounds through both active and passive mechanisms, each serving important ecological functions (Amorim 2006, Kasumyan 2008). Active sounds are intentional, communicative sounds linked to specific behaviours (Kasumyan 2008), while passive sounds are general sounds produced inadvertently during activities like feeding, moving, or even breathing (Radford et al. 2014).

Fish sounds are typically simple pulses or pulse trains with frequencies below 2 kHz (Ladich & Fine 2006), but tonal sounds and frequencies up to 4 kHz have also been reported (Desiderà et al. 2019, Mooney et al. 2020, Muñoz-Duque et al. 2024). Fish vocalizations are often described using onomatopoeic terms like grunts, growls, knocks, and pops (Amorim 2006, Kasumyan 2008). Nonetheless, these descriptions can be ambiguous and not easily compared between species. Despite this, aural descriptions can help in identifying fish vocalizations in underwater recordings (Anderson et al. 2008). Fish sounds can vary between and within a species, helping to distinguish between species, individuals, and behaviours. These variations can indicate the identity or motivation of the sound emitter (Amorim 2023). Factors like motivation, context, sexual dimorphism, social status, season, and time of day can influence the sounds produced by fish (Amorim 2006).

Fish show diverse sound production mechanisms, developed independently in distantly related species, highlighting the importance of sound in social communication and reproduction (Parmentier et al. 2017). Fish vocalizations are produced by sonic organs, evolved specifically for acoustic signalling (Ladich & Fine 2006, Kasumyan 2009).

Sonic organs generally consist of:

- **Rigid or bony structure**s. (e.g., pharyngeal teeth or pectoral spines) that produce sounds by vibration (Parmentier et al. 2008, Fine et al. 2009) or stridulation (Lechner et al. 2010).
- **Sonic muscles connected to the swim bladder, either intrinsic or extrinsic**. Extrinsic muscles attach to skeletal structures and cause vibrations in the swim bladder indirectly (Fine & Parmentier 2015, 2022), while intrinsic muscles vibrate the swim bladder wall directly (Bass et al. 2008, Chagnaud et al. 2011).

Fish emit sounds in a **variety of contexts**, mostly during social interactions, including courting, spawning, advertising calls, territory defence, predator avoidance, competition, and group cohesion (Amorim 2006, 2015, Larsson 2009, Van Oosterom et al. 2016).

Courtship signals are species-specific and dynamic, aiding mate assessment and synchronization during close interactions. These signals should be short-range and high frequency to reduce detection by predators and could be used as advertisement calls (Amorim et al. 2015). Besides, males use low-frequency sounds to attract mates or signal breeding sites, with ranges varying from centimetres in Gobiidae (short-range) to 100 meters in Sciaenidae (long-range) (Amorim et al. 2015).

Advertising calls– advertising calls in fish species serve as important signals for mate attraction and reproductive success. These are typically loud and low frequency, enhancing detection over long distances for species recognition (Laidre 2012). Advertising calls are key to reproduction in many fishes. For example, in the plainfin midshipman (*Porichthys notatus*), nesting males produce long, multi-harmonic calls that attract gravid females and signal their size and condition (Balebail & Cisneros 2022).

Agonistic interactions– fish produce sounds during competitive interactions to establish dominance, defend territories, and resolve conflicts. These acoustic signals often vary in frequency, duration, and intensity, reflecting aggression or submission. For example, damselfish (*Abudefduf abdominalis*) use short, low-frequency sounds to defend nests and deter intruders (Maruska et al. 2007).

Phenology of sound production on fish

Studying marine bioacoustics provides insights into the phenology and behaviour of marine species by analysing vocalization patterns (Sueur et al. 2019) and identifying daily, lunar, and seasonal rhythms (Ruppé et al. 2015). Fish sound production can vary, timing their calls to specific biological and environmental contexts, such as reproductive periods or habitat conditions (Desiderà et al. 2019, Bertucci et al. 2020, Vieira et al. 2022) These calls are normally associated with spawning cycles and daily times (Amorim et al. 2006, Buscaino et al. 2020).

For example, nocturnal sound production, often linked to predator avoidance and reproductive behaviours, has been recorded on Atlantic seamounts. Vocal activity peaks during dusk and night periods, indicate potential continuous reproductive behaviour across some species (Carriço et al. 2020). Nevertheless, some species are active during the day, showing diurnal vocalisation patterns associated with their ecological roles (Muñoz-Duque et al. 2024). Lunar phases also may influence fish acoustic activity; for example, Serranidae exhibit reduced spawning sounds during full moons, potentially adjusting to changes in light levels or predator activity (Mann et al. 2009). The diversity and abundance of fish sounds vary across habitats and time, composing a dynamic and complex marine soundscape influenced by biotic and abiotic factors (Radford et al. 2008). Understanding these patterns is crucial for assessing ecosystem state, protecting critical habitats, and mitigating the impacts of noise pollution on fish behaviour and reproduction.

1.3.4 Anthrophony

Coastal soundscapes are frequently impacted by human activities, with artificial noise being a significant source of disturbance (Duarte et al. 2021). Anthropogenic noise in the marine environment comes from fishing, vessel propeller cavitation, industrial activities, seismic exploration, echo sounders, construction (dredging), tourism (recreational boats), infrastructure machinery (pumps, turbines and wind farms) (Miksis-Olds et al. 2018). Vessel traffic is the primary source of constant noise in these areas (Clark et al. 2009), affecting underwater noise levels temporally and spatially (Rako et al. 2013). Shipping industries have increased their activity in the last few decades, leading to a rise in low and mid frequency noise in major shipping routes (Burnham et al. 2021). Coastal areas experience increased noise from smaller boats, coastal development, fisheries, and transportation, contributing to anthropogenic sounds (Vieira et al. 2021).

Research has demonstrated that constant exposure to anthropogenic noise can have considerable impact on the ecology of marine fauna (Berkhout et al. 2023, Carlucci et al. 2024). Chronic exposure to anthropogenic noise can be detrimental for fish by affecting their hearing ability, damaging sensory cells (Popper & Hawkins 2018), increasing stress, metabolic costs, and impacting reproduction and larval development (De Jong et al. 2020, Faria et al. 2022). Furthermore, the masking effect can contribute to important sounds not being detected, hindering communication which can in turn affect feeding and reproduction (Vieira et al. 2021). The impact differs between species, with fish that have more sensitive hearing thresholds being more susceptible to hearing loss (Smith 2016). **Figure 1.2** displays the hearing capacity and sound-producing frequencies of the different taxa, along with the frequencies of human activities, to demonstrate their overlap.

Scientific and technical advancements are needed to establish criteria for the acoustic impact on the marine environment, including energy effects on marine life and noise levels. Two indicators were introduced in the Descriptor 11 (Noise/energy) of the Marine Strategy Framework Directive (MSFD EU, 2008) in the EC Decision 2010/477/EU for achieving Good Environmental Status (GES) in marine waters (Dekeling et al. 2014). The threshold limits to follow these indicators are currently being proposed considering impulsive and continuous sources (Borsani et al. 2023).

Using PAM to monitor anthropogenic activities can help assess their impact on fauna by assessing changes on phenology and behaviour patterns but also to evaluate the effectiveness of enforcement and conservation efforts.

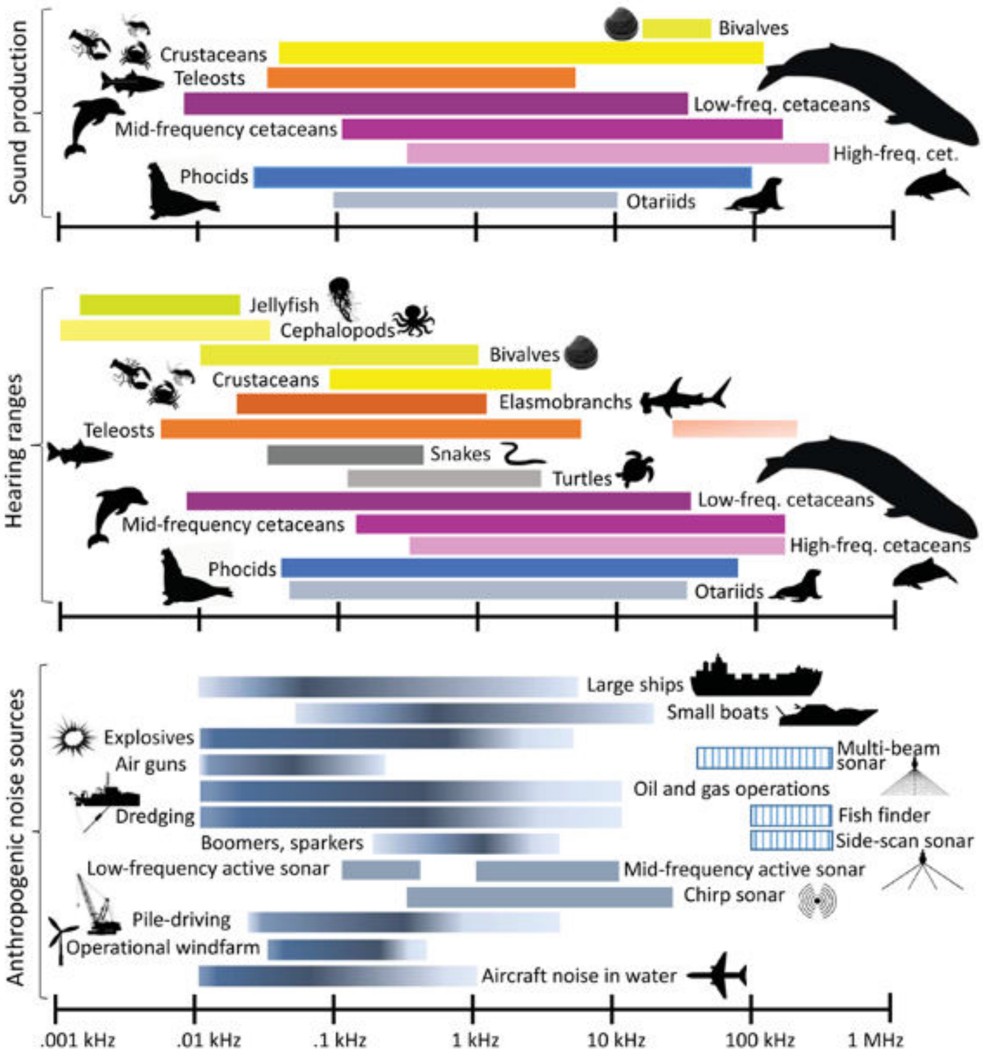


Figure 1.2 Sources and animal receivers of sound in the ocean soundscape.

Approximate sound production and hearing abilities of different marine taxa are shown alongside the frequency bands of selected anthropogenic sources. These illustrate where most acoustic energy is concentrated for each type of sound. Colour shading indicates the dominant frequency band associated with each source. Dashed lines represent sonars to depict the multifrequency nature of these sounds. From *Duarte et al. 2021*.

1.3.5 Sound perception by marine fauna

As mentioned above, sound consists of both pressure and particle motion components (Rogers & Cox 1988). While terrestrial animals and marine mammals primarily detect sound pressure, fish and aquatic invertebrates are more attuned to particle motion (Popper & Hawkins 2018). Among these, smaller marine organisms, such as fish, molluscs, crustaceans, and larvae rely on sound as an early sensory cue to identify suitable habitats, often before responding to other environmental signals (Gordon et al. 2019, Butler et al. 2022).

Marine mammals show diverse, ecology-driven hearing adaptations: baleen whales (mysticetes) hear best at low frequencies suited for long-distance communication, whereas toothed whales (odontocetes) are specialized for high-frequency hearing and echolocation (Southall et al. 2019). The inner ear is the primary auditory organ in **fish**, allowing most species to detect sounds from below 50 Hz to over 800 Hz. Specifically, this ability relies on the otolith organs within the inner ear, which sense particle motion through sensory hair cells (Popper & Hawkins, 2018). All fishes detect particle motion, but some have adaptations like Weberian ossicles or swim bladder extensions that also enable detection of sound pressure, improving sensitivity and frequency range (Popper & Hawkins, 2018). **Elasmobranchs** are thought to detect only particle motion, resulting in a narrower hearing range and lower sensitivity compared to fish with hearing adaptations (Popper & Hawkins 2018, Popper et al. 2022). Their hearing range spans 40 Hz to 1500 Hz (Chapuis & Collin 2022, Nieder et al. 2023). They have a complex inner ear with three semicircular canals, otolith organs, and a macula neglecta. While the inner ear structure is similar across species, there are variations in size and shape (Maisey & Lane 2010). Sharks can detect sounds from all directions (Casper & Mann 2007b), with pelagic sharks being more sensitive to vertical motions below 200 Hz, improving sound localization (Nieder 2023).

Invertebrates are mainly sensitive to particle motion rather than sound pressure (Hawkins et al. 2021). They perceive underwater sound through three sensory systems: superficial receptors, statocyst receptors, and chordotonal organs (Budelmann 1992).

1.3.6 Passive Acoustic Monitorization (PAM)

Because sound is a particularly important way of communication in the marine environment, monitoring marine animal sounds can provide valuable insights into their distribution and behaviour, complementing other biodiversity assessment methods (Mooney et al. 2020, Pijanowski et al. 2011). This technique provides a non-invasive, cost-effective, and continuous monitoring of vocal marine species, as well as anthropogenic noise (Van Parijs et al. 2009). Acoustic monitoring allows for diversity data collection over extended periods, facilitating in-depth analysis of species behaviour of remote environments, such as the deep sea, and in extreme weather and poor visibility conditions (Desjonquères et al. 2022, Ross et al. 2023). It also provides valuable insights for ecological studies on chronobiology and temporal ecology, including species spawning events and the influence of factors such as diel, the lunar cycle, and seasonal variations (Ruppé et al. 2015, Buscaino et al. 2016, Rice et al. 2017). PAM involves using autonomous recording equipped with hydrophones to capture audio data in the field (Gibb et al. 2019). Acoustic monitoring can simultaneously study multiple taxonomic groups, facilitating ecosystem analysis across different ecological levels, from individual organisms to landscapes and soundscapes (Sueur & Farina 2015).

In PAM, to record sound, sound waves are converted into electrical signals through transduction. This process typically involves vibrating a membrane, such as a hydrophone's piezoelectric transducer. Each hydrophone has a specific frequency sensitivity curve, optimising detection within its range (Browning et al. 2017). Digital recorders in bioacoustics provide advantages over analogue systems, including longer recording times and programmable schedules. During digital recording, the signal amplitude is sampled at a defined rate and bit-depth. A sampling rate at least twice the highest frequency of interest is required for full frequency resolution, with audible-range recordings adjustable to necessities (Zimmer 2011).

Acoustic data are analysed using software designed to visualise and interpret animal vocalisations. The software employs spectrograms—displaying frequency over time—and waveforms—showing amplitude over time—to represent sound. In spectrograms, time is plotted along the horizontal axis, frequency along the vertical axis, and the relative intensity of each frequency is depicted as a gradient of colour or greyscale.

A typical workflow for classifying wildlife sounds in PAM involves detecting signals, by first converting recordings into spectrograms, and then defining a survey method to extract features, and identifying vocalisations through spectrogram and aural inspection. This can be done manually or in an automated way, using detection and classification algorithms, such as machine learning tools (Barroso et al. 2023, Huang et al. 2025). Data processing then includes call type detection, soundscape metrics, and statistical analysis (Browning et al. 2017).

There are some **challenges** associated with this technique such as variation in signal strength, occurrence of calls, and detectability both between and within species. Furthermore, some species do not produce sounds, and vocalizing species do not produce sounds in all life history stages either all year around (Amorim & Hawkins 2005). Also, some species may emit different sound types while multiple species can have similar vocalizations, hindering species recognition (Raick et al. 2022). Additionally, signals propagate non-uniformly depending on habitat structure, weather, and signal type, making accurate estimation difficult (Tonolla et al. 2010, Ladich & Winkler 2017). Longer studies may also face challenges in extracting biodiversity information from recordings due to the presence of non-target sounds like geophonies (Ross et al. 2021). The effectiveness of PAM is also hindered by additional obstacles, including sounds from unknown origins (Vieria et al. 2024).

Despite its challenges, PAM has proven to be an effective method for monitoring aquatic biodiversity at scale. Indeed, recent research has shifted from traditional bioacoustics toward analysing large acoustic datasets for conservation and biodiversity monitoring, taking advantage of advancing computational techniques (Figure 1.3) (Bolgan et al. 2025). This moves towards data-driven, non-destructive, and scalable approaches positioning PAM as a necessary tool in today's aquatic monitoring, aligned with the global conservation agenda and the growing need for efficient ecosystem management (Bolgan et al. 2025).

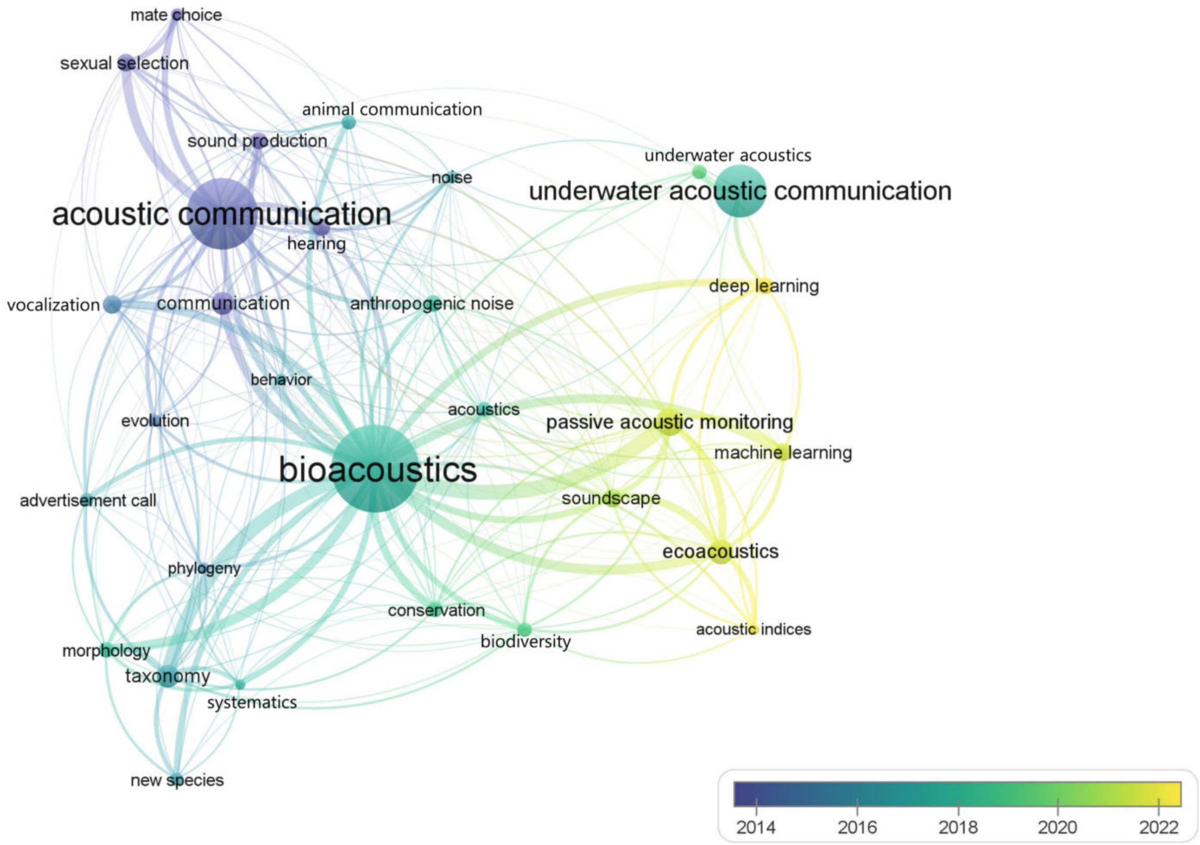


Figure 1. 3. Analysis of the evolution of the use of keywords in bioacoustic and eco-acoustic related research. The diagram represents a thematic network map of authors' keywords used in papers over time. Node size represents the frequency of each keyword's occurrence, while the thickness of connecting lines indicates the strength of co-occurrence between keywords. Thematic clusters are color-coded based on their association strength: (1) yellow, (2) blue, and (3) green. From *Bolgan et al. (2025)*.

1. 4. Acoustics in biodiversity monitoring and conservation: Methods integration

Marine acoustic monitoring had been applied with multiple objectives in the last decade. From biodiversity assessment to the impact of anthropogenic activities like underwater noise pollution, (Duarte et al. 2021).

Over the years, acoustic monitoring has become a powerful ally in marine conservation, especially when it comes to protecting endangered whales. For instance, researchers have been able to track North Atlantic whales by tracking their calls and mapping their migration routes (Davis et al. 2017). These insights have led to measurement, such as changing shipping routes and enforcing speed restrictions to reduce the risk of collisions (Erbe et al. 2019). Beyond whales, acoustic tools have also helped researchers understand how anthropogenic activities like offshore wind farms and seismic surveys for oil exploration, affect marine life (Todd et al. 2016).

In coral reef ecosystems, PAM has shown that healthy reefs tend to be louder and more acoustically diverse. These rich soundscapes are not just noise—they actually help draw in reef organisms (Lamont et al. 2021). By comparing degraded, restored, and healthy reefs, studies have revealed that restored areas can recover a similar level of biotic sound to that of untouched reefs (Lamont et al. 2021). Interestingly, researchers have even used sound recordings from healthy reefs to "call back" marine life. Playing these recordings underwater has helped guide juvenile fish and invertebrates to settle in suitable habitats (Gordon et al. 2019). Furthermore, PAM is a valuable tool for assessing coral reef responses to extreme weather. Following typhoons, reductions in fish vocalizations and acoustic richness have been observed, indicating changes in fish activity and presence. Kimura et al. (2024) demonstrated that declines in biophonic components can signal reduced ecosystem function, providing a non-invasive means to monitor reef resilience and post-disturbance recovery.

Acoustic techniques are now being extended into the deep sea, a region traditionally difficult to study. In waters around the Hawaiian archipelago, Merkens et al. (2021) used PAM to document deep-sea soundscapes, uncovering clear spatial and seasonal patterns in their calling behaviour. These findings have shown that even in remote environments PAM can reveal vital clues about species distribution, behaviour, and ecosystem health. Long-term acoustic recordings in these settings are especially valuable for informing conservation and management plans (Bolgan & Parmentier 2020).

PAM also captures broader ecosystem conditions (Sueur & Farina 2015). In French Polynesia, for example, researchers found that MPAs had more acoustically diverse and complex soundscapes than surrounding non-protected zones suggesting higher biodiversity levels (Bertucci et al. 2016). Long-term acoustic data has also proven useful for evaluating how well MPAs are performing in terms of conservation goals (Fox et al. 2014).

In terms of reproduction, PAM has helped scientists track spawning behaviour in vocal fish species like those from the Sciaenidae family (Bolgan et al. 2020, Picciulin et al. 2020). It has also been used to pinpoint spawning sites for commercially and ecologically important species, such as Gadidae and Serranidae, offering critical information for their protection (Caiger et al. 2020, Wilson et al. 2020). Moreover, acoustic tools have provided knowledge about how anthropogenic noise can disrupt fish behaviour. Studies on species like the Lusitanian toadfish (*Halobatrachus didactylus*), painted goby (*Pomatoschistus pictus*), and meagre (*Argyrosomus regius*) have revealed behavioural shifts linked to underwater noise pollution and other anthropogenic sounds (De Jong et al. 2018, Vieira et al. 2024).

Through these applications, PAM has enhanced the protection of marine species by providing robust scientific evidence to inform conservation policy and sustainable ocean management. It contributes to the development of strategies for offshore energy and fisheries management, supporting a balance between anthropogenic activities and ecosystem protection (Kark et al. 2015). Integrating acoustics into biodiversity monitoring and conservation enhances the ability to assess species presence and ecosystem health across diverse habitats.

1.4.1 Combination of Video and PAM

To further enhance the potential of PAM in biodiversity assessment and conservation, it can be combined with other techniques. One effective approach is the combination of video with acoustic. This integration of methods not only strengthens data reliability but also facilitates more comprehensive assessments of biodiversity, enabling targeted conservation strategies and long-term ecosystem management (Buscaino et al. 2025). Integrating BRUVS with PAM provides a comprehensive approach to studying marine fish communities and assessing anthropogenic impacts (Landero Figueroa et al. 2022). While BRUVS offers visual data on species presence, abundance, and behaviour, particularly for mobile and benthic species, PAM provides continuous acoustic data that reveal temporal patterns of biological activity and human use. For instance, La Manna et al. (2021) combined BRUVS and PAM in the Capo Caccia–Isola Piana MPA, showing that acoustic diversity reflects local fish biodiversity and can detect cryptic species. Similarly, Landero Figueroa et al. (2022) applied both methods in Western Australia, demonstrating that their integration improves species detection and reveals the effects of anthropogenic noise over these communities. These studies underscore the value of integrating BRUVS and PAM to achieve a more holistic understanding of marine ecosystems.

The combination of PAM and video techniques addresses the lack of data on sound sources in freshwater and marine ecosystems. For example, a portable 6-hydrophone array with a video camera successfully captured fish sounds in the wild, including those from tautog *Tautoga onitis* (Mouy et al. 2018). An Renited Underwater Video (RUV) has also been successful in identifying sound-producing fish species in coral reefs, such as *Chromis weberi* and *Amphiprion akallopisos*, contributing to the development of fish sound libraries for African regions (Puebla-Aparicio et al. 2024). Action cameras, such as GoPros, could be used to analyse acoustic data providing ecoacoustic indices like temporal variability, the acoustic complexity index, and acoustic richness in shallow tropical sea environments offering a low-cost alternative for expanding PAM efforts (Chapuis et al. 2021). A recent study combining spatial audio with 360° video, this study identifies fish-specific sounds on coral reefs, providing a validated resource that enhances PAM and enables species-level analysis of reef soundscapes (Dantzker et al. 2025).

Furthermore, combining video with PAM facilitates the assessment of noise pollution effects. For example, Video-PAM has been used to study the swimming behaviour of southern stingrays *Hypanus americanus* in response to low frequency sounds (Mickle et al. 2020). Baited video-PAM has also been used to study fish responses to boat noise, with baited underwater video revealing behavioural shifts in snapper (*Pagrus auratus*) and other reef species during motorboat transits (Mensinger et al. 2016).

Combining methods can be valuable for evaluating habitat restoration. Van Hoeck et al. (2021) applied both PAM and time-lapse video on artificial reef structures, demonstrating that acoustic monitoring and video-based observations of fish colonization offer complementary insights into ecological recovery. Video revealed that reef colonization followed a predictable temporal sequence, while early vocalisations from cryptic species like toadfish were captured acoustically, even before visual detection. These findings highlight that video-PAM monitoring can capture both overt and cryptic behavioural responses—information that is critical for understanding the impact of anthropogenic noise and for informing noise-management and restoration strategies in line with the EU Marine Strategy Framework Directive (Robert et al. 2016).

1.5. Biodiversity assessment

Marine biodiversity conservation initiatives like OSPAR, EU Marine Strategy, US National Ocean Policy, and UN Convention on Biological Diversity emphasize the need for comprehensive environmental assessments and offer conservation guidelines (Geijzendorffer et al. 2015). The EU Marine Strategy Framework Directive uses an ecosystem-based approach with 11 descriptors, with biological diversity (D1) as the priority.

The assessment of environmental assemblages is primarily based on the concentration of environmental information, biodiversity, and the abundance of marine fauna, which are considered key priority indices (Pendred et al. 2016, Cardoso-Andrade et al. 2022). Biological diversity encompasses the variety of life types across different levels of interpretation, including population, morphology, taxonomy, and ecology. Biodiversity is often quantified using specific indices that can range from simple to complex calculations. When selecting a biodiversity index, factors such as its ability to respond to ecological changes, focus on species richness and evenness, reliance on sample size, discriminability between samples, and frequency of use in ecological research must be considered (Fedor & Zvaríková 2018). The most used indices are species Richness, Shannon Index, Simpson Index, Inverse Simpson Index and Evenness of Simpson index (Mendes et al. 2008, Roswell et al. 2021).

In addition to traditional biodiversity indices, ecoacoustic indices, commonly used in terrestrial environments, are now being applied to marine environments, for example to evaluate the effectiveness of MPA (Bertucci et al. 2016). The biophony encodes species diversity based on the abundance and richness of different sound types (Desiderà et al. 2019, Mooney et al. 2020, Raick et al. 2023).

These indices are categorized into three forms depending on what they describe or characterized such as: acoustic intensity, complexity, and soundscape components (Sueur et al. 2014). **Intensity indices** have the inconvenience of not identifying sound source or the frequency composition, making them unsuitable for acoustic richness. **Complexity indices** are calculated within specific frequency ranges, such as bioacoustic index (BI), the amplitude index (AI) the acoustic entropy index (total entropy, H) (Harris et al. 2016). Other indices to analyse diverse soundscapes are for example acoustic richness (AR), the acoustic evenness index (AEI), the acoustic complexity index (ACI) (Pieretti et al. 2011, Depraetere et al. 2012). The third category of acoustic diversity indices, **soundscape indices**, analyse the biophony in relation to the anthropophony (Benocci et al. 2022).

The use of acoustic indices has emerged as a tool for assessing and monitoring marine environments. Acoustic indices detect changes in acoustic community composition and can help identify the presence of threats such as shipping noise and soundscape degradation over time (Bertucci et al. 2016). Acoustic methods complement traditional ecological assessment, enhancing the ability to monitor ecosystem dynamics and biodiversity patterns, offering a more holistic understanding of environmental changes (Sueur et al. 2014). This integrative approach is particularly crucial for informing policies and strategies aimed at mitigating biodiversity loss and preserving ecosystem health in the face of anthropogenic pressures and climate change (Shin et al. 2022).

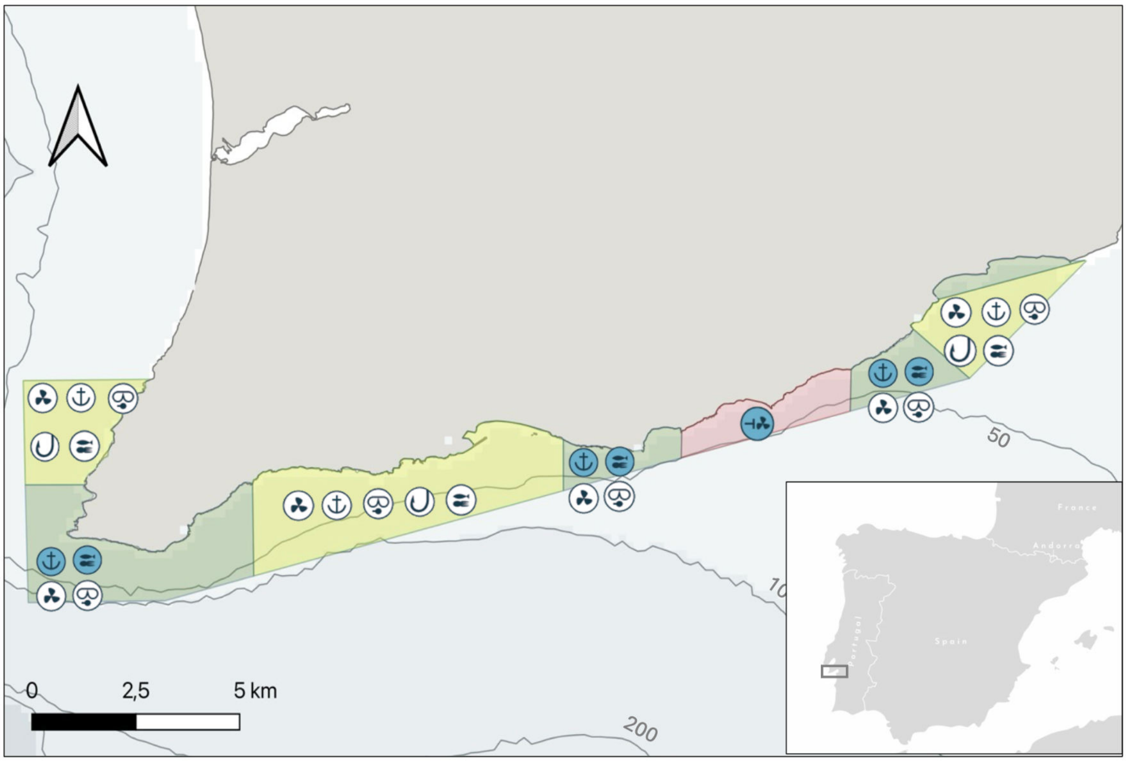
1.6. Study area: Professor Luiz Saldanha Marine Park

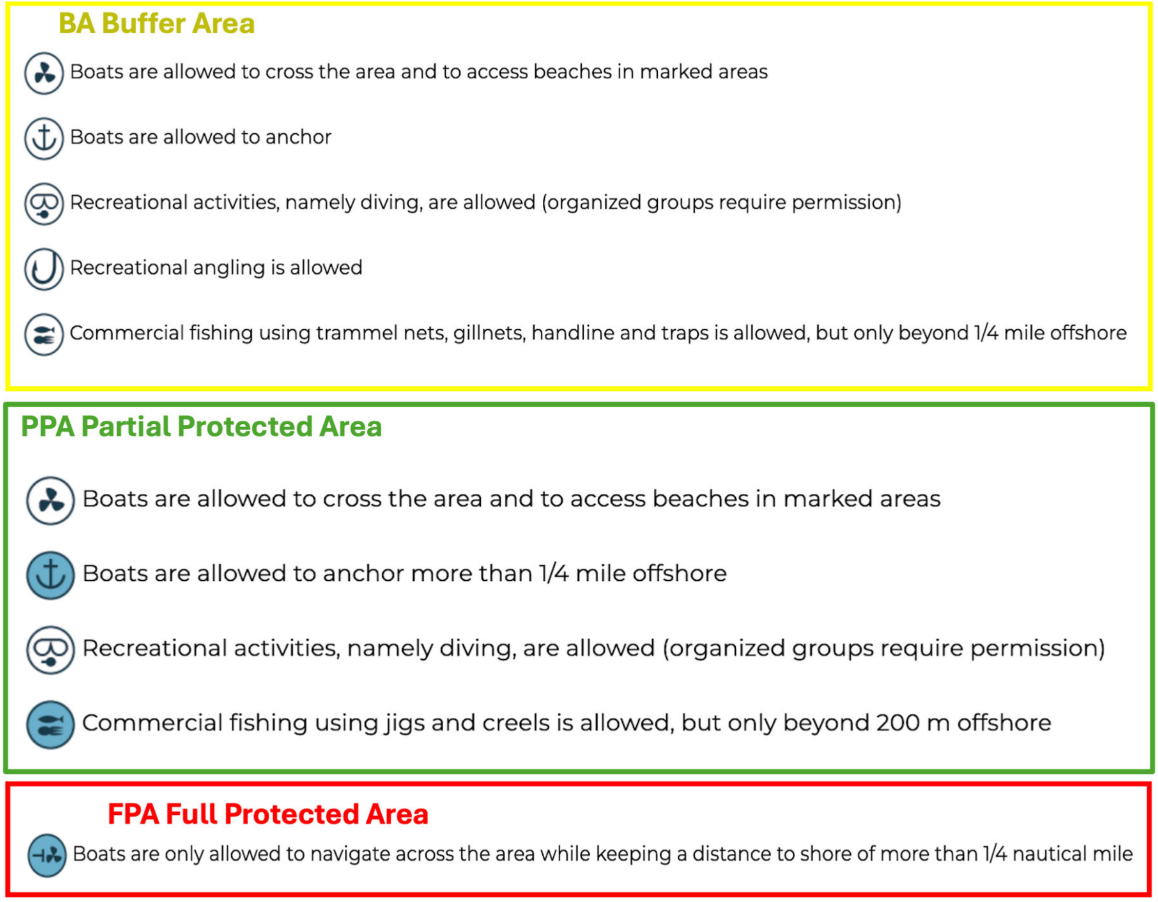
The protection of the Arrábida region began with the establishment of the Arrábida Reserve in 1971, followed by the creation of the Arrábida Natural Park in 1976. The Professor Luiz Saldanha Marine Park (PLSMP) was established in 1998 as one of the earliest marine protected areas in mainland Portugal. The management plan for the PLSMP was approved in 2005 and fully implemented in 2009 (Cunha et al. 2014).

Managed by ICNF, the park aims to preserve biodiversity, restore marine habitats, conduct research, educate the public, and promote eco-tourism (Batista et al. 2011). The park was established to conserve coastal biodiversity while supporting effective fisheries management (Gonçalves et al. 2015). Indeed, these communities rely on the park's biodiversity, as many commercially important species complete critical life stages within its boundaries (Cunha et al. 2014, Gonçalves et al. 2015).

The PLSMP comprises 38 km rocky coastline and beach shores, defined by the following geographical reference points 38º 27' 13'' N, 9º 14' 05'' W; 38º 28' 06'' N, 8º 59' 16'' W. The PLSMP is divided into eight zones with three different areas of protection, a fully protected area (FPA, 4.3 km2), four partially protected areas (PPA, total= 21 km2), and three buffer areas (BA, total = 27.7 km2) **Figure 1.4**. The FPA is a no-take/no-go zone, with expectations for monitoring, research, and educational activities. Even though this marine park contemplates one no-take area (FPA), the management and enforcement are very poor, and transgressions happen frequently (Abecasis et al. 2015). The PPAs allow non-fishing recreational activities and licenced commercial fishing with traps and jigs beyond 200 m from the shore. The BAs permit recreational activities and licenced commercial fishing with vessels smaller than 7 m. Spearfishing, commercial dive fishing, purse-seining, trawling, and dredging are prohibited in the entire park (Batista et al. 2015, Pita et al. 2020). Commercial fishing is limited to 25 species and vessels under 7 meters with specific licenses (Horta e Costa et al. 2013). The zoning scheme outlines boundaries for recreational boating, sports fishing, and maritime tourism within the park (Cabral et al. 2008, Abecasis et al. 2015).

Research, assessments and monitoring efforts of the PLSMP has been supported by several research projects, notably the BIOMARES project (2007–2015), which focused on monitoring, conservation, and public awareness within the Marine Park, utilising UVC data, habitat restoration, experimental fisheries (Cunha et al. 2014). BUFFER – Partially protected areas as buffers to increase the linked social-ecological resilience BIODIVERSA/0002/2011 (https://www.biodiversa.eu/2022/10/31/buffer/). This work was continued by the INFORBIOMARES project (2017–2020), which expanded the scope to include habitat restoration, UVC surveys, experimental fisheries, and BRUVs for monitoring the MPA (Serrão et al. 2021). Also, the project BIODIVAMP with collection of environmental DNA (2021-2022) (Coxey et al. 2022) (https://areasmarinhasprotegidasportuguesas.org/). Independent of these initiatives, this PhD project conducted acoustic monitoring at the PLSMP from 2021 to 2022.





BA Buffer Area

Boats are allowed to cross the area and to access beaches in marked areas

Boats are allowed to anchor

Recreational activities, namely diving, are allowed (organized groups require permission)

Recreational angling is allowed

Commercial fishing using trammel nets, gillnets, handline and traps is allowed, but only beyond 1/4 mile offshore

PPA Partial Protected Area

Boats are allowed to cross the area and to access beaches in marked areas

Boats are allowed to anchor more than 1/4 mile offshore

Recreational activities, namely diving, are allowed (organized groups require permission)

Commercial fishing using jigs and creels is allowed, but only beyond 200 m offshore

FPA Full Protected Area

Boats are only allowed to navigate across the area while keeping a distance to shore of more than 1/4 nautical mile

Figure 1.4 Map and protection measures of the Professor Luiz Saldanha Marine Park. Adapted from https://arrabidaparquemarinho.ualg.pt/en/areas-and-rules.

1.6.1 Habitats and fauna

The diverse habitats of PLSMP are part of the Natura 2000 list due to their unique environmental conditions. The park's location within a biogeographic transition zone between temperate and subtropical regions plays a pivotal role in fostering its high biodiversity and variety of habitats (Gonçalves et al. 2015). This remarkable diversity is further shaped by the complex bathymetry of the Lisboa-Setúbal submarine canyon system, with the Lisbon branch extending to the west and the Setúbal branch to the east, creating distinct facing slopes. The continental shelf within the marine park exhibits significant depth variation, ranging from approximately 200 m to over 1000 m, influenced by the proximity to the canyon branches and their structures (Lastras et al. 2009). Additionally, the region benefits from the ecological influence of two estuaries: the Tagus estuary, situated around 30 km north of Espichel Cape, and the Sado estuary, located to the east of the PLSMP, which further enhances its biodiversity (Oliveira 2015).

The marine park encompasses rocky and sandy substrates that extend up to 100 m deep in the west and 30 m deep in the east. It features diverse marine environments, including estuaries, intertidal zones, and shallow sublittoral areas, with sandbanks, reefs, and macroalgal beds that support rich biodiversity. To date, over 2,000 species have been identified within the park, including approximately 1,650 fauna species and 400 flora species.

Macroalgae habitats within the park present three different characteristics, first rocky reefs with seasonal occurrence of *Saccorhiza polyschides* kelp forest. Secondly, the rocky reef area hosts a diverse range of algae species, including *Asparagopsis taxiformis*, *Saccorhiza polyschides*, and *Sphaerococcus coronopifolius*. Thirdly, *Streptacantha usneoides* appears seasonally in spring (Boaventura & Re 2001). The park features soft-muddy habitats with *Atrina* sp. beds and seagrass meadows dominated by *Zostera marina*, alongside rocky areas inhabited by invertebrates such as black corals, gorgonians, and orange corals, which create essential habitats for marine life.

In a study focussing on the macroalgal environment, a total 182 different taxa across eight phyla were identified: Annelida, Arthropoda, Chordata, Echinodermata, Mollusca, Nematoda, Nemertea, and Platyhelminthes (Silva 2022). The park hosts over 150 fish species, with the most common belonging to the families Sparidae, Serranidae, Labridae, Tripterygiidae, Gobiidae, and Scorpaenidae (Serrão et al. 2021, link). From these fish observed in the park a high proportion of these species are classified as soniferous or potentially soniferous (Ríos et al. 2025).

1.7. Thesis overview

This PhD research combines Passive Acoustic Monitoring (PAM) and Baited Remote Underwater Video Systems (BRUVS) to study acoustic communities, and soundscape dynamics in benthic habitats at Professor Luiz Saldanha Marine Park (PMPLS). By using these non-invasive techniques, the study aims to provide critical insights into species-specific vocalisation patterns, habitat use and assess the behaviour patterns of near-threatened species in relation to human activities. This research focused on ecological and acoustic indicators to evaluate biodiversity, phenology, and fish behaviour using non-invasive visual and acoustic techniques. This recent methodology will significantly contribute to MPA management by improving species detection capabilities, informing conservation strategies, and advancing the development of holistic, multi-sensor monitoring frameworks for marine ecosystems.

Specifically, this thesis has the following main objectives:

i) Compile the first fish sound library for the PLSMP by integrating PAM with Baited BRUVS, aiming to identify vocal fish species and associate their sounds with specific behaviours (**Chapter II)**. This chapter aims to enhance understanding of fish acoustic communities in coastal areas in PLSMP.

ii) Analyse temporal and spatial variations in the marine soundscape produced by fish across areas with differing protection levels and habitat types, and to evaluate the influence of environmental factors on these patterns (**Chapter III)**. Compare acoustic and visual monitoring techniques and evaluate the effectiveness of acoustic indices against traditional biodiversity indices (**Chapter IV)**. These chapters will help us to understand the environmental factors affecting temporal patterns of fish acoustic communities and comparing diversity and ecoacoustic indices in the Marine Protected Area using various methods.

iii) Identify behavioural patterns in near-threatened species using non-invasive methods (BRUVS and PAM), enabling the detection of differences in foraging behaviour, social interactions, and responses to boat noise (**Chapter V)**. These chapters will help us understand the behaviour of emblematic species and the influence of human activities on these behavioural patterns.

iv) Disseminate the thesis findings through various scientific and public events, as outlined in (**Chapter VI)**. This includes publishing in peer-reviewed journals, presenting at conferences, and participating in public events to raise awareness. Additionally, it includes using digital platforms, websites and TV Journals to reach a wider audience. The aim is to engage both academia and the broader community.

CHAPTER II

Acoustic fish community
in a biogeographic transition zone
of the Northeast Atlantic

Chapter II: *Acoustic fish community in a biogeographic transition zone of the Northeast Atlantic.*

The material of this chapter is currently published in the journal ICES Journal of Marine Science

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2.1 Abstract

Fish sounds are a significant component of marine soundscapes. Recently, passive acoustic monitoring (PAM) arose as a promising tool for ecological monitoring, but a good characterization of fish acoustic communities is still needed. This study is the first to characterize the fish acoustic community at a biogeographic transition zone in the Northeast Atlantic. The research was conducted in a marine protected area (MPA) along the Portuguese mainland coast. Based on a literature review, we identified 29 (19.3%) sound-producing fish species present at this MPA, while 70 species (46.7%) were considered potentially soniferous. Using in situ acoustic recordings to detect potential fish sounds, we found 33 putative fish sounds that were categorized using a simple dichotomous classification. The temporal and spectral features of the 13 most prevalent sound types were characterized and compared among them and with available recordings to identify similarities. Finally, hydrophone recordings coupled with baited remote underwater video systems were tested as a method to identify sound sources. This study provides the first fish sound catalogue from the Portuguese mainland coast, laying the foundations to survey fish communities in coastal habitats with PAM.

Keywords: passive acoustic monitoring; marine protected areas; fish sounds; sound catalogue; acoustic communication; marine soundscape; Professor Luiz Saldanha Marine Park

2.2 Introduction

Biological sounds in the ocean are produced by a wide range of taxa, from small invertebrates to marine mammals (Ladich & Winkler 2017). Whales and other cetaceans are better known for their vocalizations, which they can use for both echolocation and communication, across a wide range of frequencies (0.02–150 kHz) (Jones et al. 2020, Miller et al. 2021). Nonetheless, other taxa also rely on sounds to communicate or to obtain relevant information from the environment: molluscs, crustaceans, fish, and their larvae have all been shown to use sound as a sensory cue (Lillis et al. 2015, 2016, Gordon et al. 2018, Ladich 2019). Invertebrate sounds are typically broadband pulses with frequencies between 2 and 12 kHz (Radford et al. 2008, Bittencourt et al. 2016), while fish, by contrast, predominantly produce lower frequency sounds (<2–3 kHz) (La Manna et al. 2021, Vieira et al. 2021, Raick et al. 2022, Puebla-Aparicio et al. 2024), both often dominating marine soundscapes. Currently, more than 980 species of fish from 133 families worldwide are known to produce sounds (Looby et al. 2022, Rice et al. 2022), with communicative functions extensively studied for some species (Amorim et al. 2015, 2023, Ladich 2019, Banse et al. 2024). Fish produce sounds through active or passive mechanisms (Fine & Parmentier 2015). Passive sounds occur inadvertently, while active (communication) sounds are deliberate, produced through specialized mechanisms, and typically associated with specific behaviours (Kasumyan 2008). Generally, active sounds are species-specific (for an exception, see Raick et al. 2022) and are usually composed of low-frequency repetitive pulses, typically below 3 kHz, but mostly below 1 kHz (Amorim 2006, Fine & Parmentier 2015). Fish sounds are commonly associated with feeding, territorial, or reproductive behaviour, and their features and production rates vary depending on the behavioural context and motivation (Amorim 2006, Parsons et al. 2022). Because fish sounds can be species- and context-specific, they can be used to monitor the abundance, behaviour, distribution, and/or diversity of species, potentially playing an important role in the monitoring and management of fish communities (Parmentier et al. 2018, Bertucci et al. 2020, Raick et al. 2022, Stratoudakis et al. 2024). Passive acoustic monitoring (PAM) is a non-invasive and cost-effective method that can be used for continuous monitoring of fish sounds, with long-term and large-scale coverage, unaffected by visibility or weather conditions (Mooney et al. 2020b). Furthermore, PAM can be integrated with other monitoring methods, such as environmental DNA or visual techniques, enhancing the overall capacity for comprehensive marine ecosystem studies (e.g. Elise et al. 2022, Souza et al. 2023). Acoustic monitoring can have valuable applications in marine protected areas (MPAs), which are recognized as crucial for safeguarding and restoring biodiversity. However, the limited understanding of underwater soundscapes, including the prevalence, diversity, along with limited awareness of PAM's utility amongst society and environmental agencies hinder its widespread adoption as a conservation tool in MPAs. This study characterizes the acoustic fish community within the Professor Luiz Saldanha Marine Park (PLSMP), situated in a biogeographic transition zone on the west coast of mainland Portugal. This MPA is a biodiversity hotspot, hosting species with north-eastern Atlantic temperate affinities but also Mediterranean and subtropical species (Henriques et al. 2007). Specifically, the objectives are to (1) list sound producing fishes in the PLSMP based on literature, (2) catalogue fish sound types recorded in situ, and (3) try to identify putative sound sources using audio-video recordings. To the best of our knowledge, this study is the first to characterize open coastal acoustic communities in mainland Portugal. It will serve as a foundation for future research and as a valuable tool for monitoring fish communities through acoustic methods.

2.3 Material and methods

2.3.1 Study site

The PLSMP extends 38 km along the west coast of mainland Portugal (**Figure 2.1**). It was established in 1998 and is composed of eight zones subject to three protection levels: a full protected area (FPA, 4.3 km2), which is a no-take no-go zone, four partial protection areas (PPA, 21 km2), and three buffer areas (BA, 27.7 km2). This MPA is an important biodiversity hotspot with more than 2000 species of flora and fauna inhabiting diverse habitats ranging from sandy bottoms with bivalve fields and seagrasses to complex nearshore rocky reefs with algae, including kelp forests and gorgonians (Henriques et al. 2007). The park's location in a biogeographic transition area between temperate and sub-tropical zones enhances its biodiversity and presents a variety of habitats (Horta e Costa et al. 2014, Gonçalves et al. 2015). Its unique geography and oceanographic conditions, which include canyons and estuaries, contribute to the region's diverse wildlife. The bathymetric features near the study area are diverse due to the Lisboa-Setúbal submarine canyon system, which allows processes such as canyon upwelling and downwelling, influencing coastal ocean circulation of nutrient-rich water, boosting food web productivity (Peliz et al. 2002). Furthermore, the coastal disposition offers varying levels of protection from the wind and water currents, with an exposed offshore area to the west and south of Cape Espichel and a sheltered area to the east. The study area is also influenced by the presence of two of the biggest estuaries in Europe: the Tagus estuary located 30 km north of the PLSMP and the Sado estuary to the east. Under north winds, the boundary of the Tagus estuary plume may extend to the south of Cape Espichel, affecting salinity values in the protected area (Oliveira et al. 2015). Similarly, the Sado estuary plume runs parallel to the coast where PLSMP is situated and can extend to Cape Espichel, potentially connecting to the Tagus plume in spring (Campuzano et al. 2018).

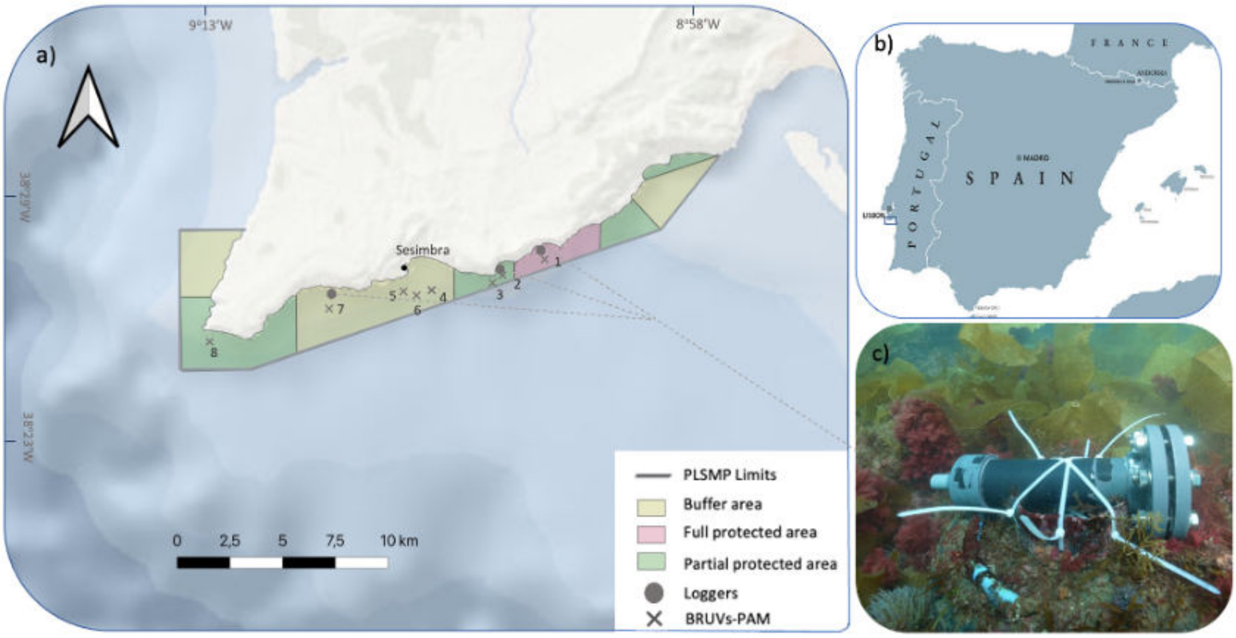


Figure 2.1. (a) Map of the PLSMP depicting the different protection areas, location of the acoustic loggers and of BRUV-PAM deployments; BRUVS-PAM deployments locations; (1) Derrocada, (2) Bahia do Armação, (3) Jardim das gorgónias, (4) Pedra do meio, (5) Batelão, (6) Maria Grecia, (7) 3 Milhas, (8) River. (b) Map of Europe with Portugal highlighted and location of the PLSMP; (c) Photograph of the acoustic logger deployed in the Partial Protected Area (2).

2.3.2 Fish community and reported soniferous species

A bibliographic review was conducted to identify listed soniferous and potentially soniferous fish species i.e. fish species that belong to families containing soniferous species, within the PLSMP. The fish database available on the marine park's geoportal https://arrabidaparquemarinho.ualg.pt/ was used to identify fish present in the area. Subsequently, a literature search was conducted for each species recorded within the PLSMP: species considered soniferous by Carriço et al. (2019) and Rice et al. (2022) or by the FishSounds database (Looby et al. 2023) were classified as soniferous. For the remaining species, a search was performed on Google Scholar with the terms 'soniferous', 'sound', or 'acoustic.' Fish species not reported as soniferous were classified as potentially soniferous if belonging to families containing soniferous species, and as potentially non-soniferous if otherwise. Further information was added to every species, such as conservation status [IUCN: VU (vulnerable), LC (least concern), NT (near threatened), CR (critically endangered), EN (endangered), DD (data deficient), NE (not evaluated)], environment (cryptobenthic, benthopelagic, pelagic, demersal, bathydemersal), depth range, climate affinity and, if known, the behaviour associated with the sound of the soniferous species (FishBase, Froese & Pauly 2024).

2.3.3 Acoustic recording

Autonomous acoustic data loggers (Audiomoth 1.2.0; Hill et al. 2019) equipped with custom-made hydrophones (Piezo tubes PZT-P5 with 24 × 20 × 20 mm, with signal pre-amplification of 50 times) were deployed in three zones with different protection levels between June 2021 and September 2022. The piezoelectric sensor has a measured response sensitivity of ca.−184.5 dB re 1 V/µPa at 1 kHz and a frequency response comparable to commercial hydrophones, namely the Brüel & Kjær 8104 (8104, Brüel & Kjær, Nærum, Denmark), and reliably captures the temporal and spectral characteristics of the various sound types (**Figure 2.S1**). Note, however, that in the deployments at PLSMP there was sometimes a visible loss of acoustic energy at frequencies around 400 Hz (see sound types #1, #10, #16, and #28 in **Figure 2.2**). This is likely because the acoustic data loggers were placed horizontally in close contact with ballast (**Figure 2.1c**, **Figure 2.S1**). The Autonomous Acoustic data loggers were deployed in: (1) the BA (buffer area) at a depth of~8.5 m in a rocky habitat; (2) the FPA (full protected area) at a depth of~6 m at an interface of a sandy and rocky habitat; and (3) the PPA (partial protected area) at a depth of~6.9 m in a rocky habitat (**Figure 2.1**). Deployments failed in December 2021 for the FPA and at the beginning of March 2022 for the PPA. The recordings were made with a continuous duty cycle at a sampling rate of 48 kHz. Due to battery autonomy and digital storage capacity, the data loggers were retrieved, reprogrammed, and deployed every ca. 60 days.

2.3.4 Detection of putative fish sounds

Acoustic recordings were analysed for the presence of putative fish sounds. Given the challenge of manually analysing large acoustic datasets, the data were subsampled. Four months were chosen to represent each of the four seasons: June for summer, September for autumn, December for winter, and March for spring. Within each month, 4 days were selected based on the moon's phases: full moon, crescent moon, waning moon, and new moon, as it may influence acoustic communities (Borie-Mojica et al. 2022). Within each selected day, 30-min recordings at four specific time periods were chosen to represent the diel period: sunrise, solar mid-day, sunset, and midnight (according to NOAA 2023).

The recordings were analysed manually through both aural and visual inspection of the spectrograms and oscillograms, using Raven Pro 1.6 (Bioacoustics Research Program, Cornell Laboratory of Ornithology; DFT size 256, contrast 80, and brightness 55; recordings down sampled to 8 kHz). Two individuals conducted this analysis, with files randomly assigned to each to prevent biases. The manual inspection of the files allowed for the identification and categorization of putative fish sounds. Putative fish sounds were determined based on their similarity to previously reported fish calls in frequency, relative duration, and temporal patterning of their pulses. Based on categorical methods from other studies (e.g. Parsons et al. 2016, Desiderà et al. 2019, Muñoz-Duque et al. 2024, Puebla-Aparicio et al. 2024), each fish sound type was characterized using a dichotomous classification system, following the steps outlined below:

(a) A preliminary study was conducted using Raven software to train observers to identify fish sounds in acoustic archives. Selected samples were later analysed.

(b) Putative fish sounds were grouped and assigned to numbers (#1, #2, #3…).

(c) Putative fish sounds were grouped according to their temporal patterns into two main categories, continuous and pulsed sound types.

(d) Then, pulsed sounds were categorized into low-frequency (peak frequency <500 Hz) pulse trains (fast and slow with pulse period under or over 50 ms) and high-frequency (peak frequency >500 Hz) pulse trains (fast and slow with pulse period under or over 50 ms). Continuous sounds included tonal sounds (frequency–non-modulated), which were further divided into low-frequency (peak frequency <500 Hz) and high-frequency (peak frequency >500 Hz), as well as noisy (wideband) sounds, which were also further divided into low (peak frequency <400 Hz, range frequency between 150 and 300 Hz) and high- frequency (peak frequency >400 Hz, range between 450 and 1000 Hz). Note that continuous sounds may be composed by pulses but are perceived as continuous through aural and visual inspection.

These classification steps are represented in **Table 2.2**.

Note that although all putative fish sound types exhibit characteristics similar to reported fish calls, it is possible that, due to the complexity of the underwater soundscapes, not all sound types originated from fish. Additionally, the loss of acoustic energy around 400 Hz frequencies in some of the recordings, as well as the distance from the source, may influence the spectral properties of the recorded sounds. However, this did not hinder the use of a dichotomous classification system or the construction of a fish sound catalogue, as most frequencies were reasonably well represented (see **Figure 2.2**).

2.3.5 Identification of putative fish sound type sources using BRUVS

Baited remote underwater video systems (BRUVS) were used to identify potential sources of the various putative fish sound types. The BRUVS design was adapted from Brooks et al. (2011). It comprised a PVC pipe frame equipped with a Go-Pro HERO 5 Black, housed in a SeaGIS (SeaGIS Pty Ltd https://www.seagis.com.au/) waterproof housing along with an additional external power supply, allowing for unattended video recording. A bait pole (~1 m long) was extended outwards from the frame into the field of view of the camera. In addition, the BRUVS were also fitted with an autonomous acoustic data logger (see above) that simultaneously recorded audio, used to identify the fish sound types. For all deployments, the BRUVS were baited with 1 kg mackerel (Scomber spp.) to attract fish into the area. The amount of bait is consistent with standard practices of 1kg of bait per sample hour (Harvey et al.2007). Deployments were made at two different periods: November 2022 and April 2023, at a depth of 2–9 m, close to the locations where the three acoustic loggers were previously deployed (see **Figure 1)**. The daily recordings lasted around 70 min each, conducted at sunrise, mid-day, sunset, and early night. For the deployments conducted at night, the BRUVS were equipped with a dive torch and left to record into the night until the storage on the camera was full (~4 h) and recovered the following day. The video was used to identify instances where sound produced by fish could be detected. This process thus aimed to test a video technique to determine the sound source or narrow down the fish species capable of producing the identified sounds. In cases where a sound source could not be determined, the fish captured in the frame were identified and included in a list of potential sound sources.

2.3.6 Multivariate analyses of putative fish sounds

To assess the similarities between the defined sound types, principal component analysis (PCA; Mardia et al. 1979) considering the 13 most frequent sound types was performed, i.e. for sounds with $n \geq 20$ occurrences. Several acoustic features were measured, namely

- sound duration (s);
- minimum and maximum frequency (the lowest and the highest frequency of each sound in the spectrogram, Hz);
- first and third quartile frequencies (Q1, which represents the frequency at 25% of the spectral frequency range, and Q3, the frequency at 75%, Hz);
- peak frequency (frequency at which the sound presents its highest energy in the power spectrum, Hz);
- number of pulses;
- pulse duration (the mean duration of a pulse, ms);
- pulse period (mean time elapsed between the peak amplitude of two consecutive pulses within a sound, ms); and
- average value of the entropy (entropy measures the disorder in a sound by analysing its energy distribution).

The values obtained for each feature were standardized to zero mean and unit variance. Some sounds with indistinct pulses may be difficult to separate and recognize due to low signal-to-noise ratio or fast pulse rates, making pulse measurement challenging (#3, #6, #11, and #16) (see **Figure 2.2**). Pulse period and pulse duration were only measured in some pulsed sound types (#1, #7, #14, and #15). Separate statistical analyses were used for sounds in which pulse period and duration could not be measured. Multivariate analyses were performed on the 13 most common sound types using 7 standardized acoustic features described above (Table S3). This led to three separate multivariate analyses: 'continuous' sound types (n = 83), 'pulsed (measured)' sound types (n = 56), and 'pulsed (not measured)' sound types (n = 65). Analyses were performed using R (R Core Team 2021) with the package 'FactoMineR' (Lê et al. 2008) and 'PCA' function (Blighe & Lun 2023). See Web **Appendix 1** for further details.

2.4 Results

2.4.1 Fish community and reported soniferous species

From the 150 fish species known to occur in the study area, 29 (19.3%) species from 17 families were identified as soniferous (**Table 2.1**), 70 (46.7%) species from 17 families as potentially soniferous, and 51 (34.0%) species from 25 families as potentially non-soniferous (**Table 2.S1**). The soniferous species belonged to various families, including Balistidae, Batrachoididae, Blenniidae, Carangidae, Gadidae, Gobiidae, Labridae, Lotidae, Molidae, Pomacentridae, Pomatomidae, Sciaenidae, Scorpaenidae, Serranidae, Syngnathidae, Triglidae, and Zeidae. According to the IUCN Red List of Threatened Species (IUCN 2023), three soniferous species in the study area have defined threatened conservation statuses: EN *Epinephelus marginatus*; VU *Mola mola*; and NT *Pomatomus saltatrix*. Four species are DD: *Balistes capriscus*, *Hippocampus hippocampus*, *Hippocampus guttulatus*, and *Zeus faber*, and two are NE: *Gaidropsarus mediterraneus* and *Cynoscion regalis*, while the other twelve were classified as LC (**Table 2.1**). The weakfish (*C. regalis*), although classified as NE for the north-eastern Atlantic, is an invasive species in Portugal originating from the East Coast of North America, where it is classified as EN. All sound-producing fish species present in the PLSMP partially or fully overlap their distribution depth range with our study sites. Most of the behaviours associated with sound production described in the literature were related to courtship, agonistic behaviour, feeding competition, and distress sounds resulting from manual stimulation (**Table 2.1**).

Table 2.1. Summary of the 29 confirmed soniferous fish species listed for the PLSMP.

Species [a]	Family	Conservation [b]	Environment [c]	Climate affinity [c]	Depth range(m)[c]	Behaviour [d]	Ref.[d]
Balistes capriscus	Balistidae	DD	Benthopelagic	Subtropical	0-100	Manual and electric stimulation	1
Halobatrachus didactylus	Batrachoididae	LC	Cryptobenthic	Subtropical	1-50	Agonistic, courtship	2, 3
Lipophrys pholis	Blenniidae	LC	Demersal	Temperate	0-8	n/a	5
Seriola dumerili	Carangidae	LC	Benthopelagic	Subtropical	1-385	n/a	1
Trachinotus ovatus	Carangidae	LC	Pelagic	Subtropical	50-200	n/a	1
Pollachius pollachius	Gadidae	LC	Benthopelagic	Temperate	40-200	Agonistic	22
Gobius paganellus	Gobiidae	LC	Cryptobenthic	Subtropical	0-15	Agonistic, courtship	18,19
Gobius cobitis	Gobiidae	NE	Demersal	Subtropical	8-35	Agonistic, courtship	18, 23
Gobius cruentatus	Gobiidae	LC	Demersal	Subtropical	15-40	Agonistic	24
Gobius niger	Gobiidae	LC	Demersal	Temperate	1-50	Agonistic, courtship	18, 23
Pomatoschistus flavescens	Gobiidae	LC	Reef associated	Temperate	0/	Agonistic, courtship	33, 34
Pomatoschistus marmoratus	Gobiidae	LC	Demersal	Subtropical	20-70	Agonistic, courtship	18,25, 26,31
Pomatoschistus pictus	Gobiidae	LC	Demersal	Temperate	0-55	Agonistic, courtship	4
Pomatoschistus minutus	Gobiidae	LC	Demersal	Temperate	4-200	Agonistic, courtship	18,27, 28, 29, 30
Symphodus melops	Labridae	LC	Reef associated	Subtropical	1-30	Agonistic	32
Gaidropsarus mediterraneus	Lotidae	NE	Demersal	Temperate	1-450	Agonistic	5
Mola mola	Molidae	VU	Pelagic	Subtropical	30 - 1515	Manual stimulation	1
Chromis chromis	Pomacentridae	LC	Benthopelagic	Subtropical	1-40	Agonistic, courtship	6
Pomatomus saltatrix	Pomatomidae	NT	Pelagic	Subtropical	0-200	Escape sounds	1
*Cynoscion regalis**	Sciaenidae	NE	Demersal	Subtropical	10-26	Agonistic, courtship	7, 8, 9, 10
Scorpaena porcus	Scorpaenidae	LC	Demersal	Temperate	?-800	n/a	11
Epinephelus marginatus	Serranidae	EN	Benthopelagic	Subtropical	8-300	Courtship	17
Hippocampus hippocampus	Syngnathidae	DD	Cryptobenthic	Subtropical	0 - 60	Agonistic, courtship	20
Hippocampus guttulatus	Syngnathidae	DD	Cryptobenthic	Temperate	0 - 30	Agonistic, courtship	21

Chelidonichthys lastoviza	Triglidae	LC	Demersal	Subtropical	10-150	Competitive feeding	12, 13, 14,15
Trigla lyra	Triglidae	LC	Bathydemersal	Temperate	150 - 400	n/a	12, 13, 20
Chelidonichthys lucerna	Triglidae	LC	Benthic	Subtropical	20-318	Agonistic	12, 13
Chelidonichthys cuculus	Triglidae	LC	Benthic	Temperate	2-100	Agonistic	12, 13
Zeus faber	Zeidae	DD	Benthopelagic	Temperate	5-400	Agonistic	16

Notes:

a Marine fish of PLSMP.

b IUCN Red List of Threatened Species (2023) Conservation status from IUCN: LC, Least Concern; VU, Vulnerable; NT, Near Threatened; EN, Endangered; CR, Critically Endangered; DD, Data Deficient.

c Environment, climate affinity and depth ranges were extracted from fishbase.org

d 1, Fish & Mowbray (1970); 2, Fine et al. (2001); 3, Jordão et al. (2019); 4, Amorim & Neves (2008); 5, Almada et al. (1996); 6, Picciulin et al. (2002); 7, Connaughton et al. (2000); 8, Gannon et al.(2007); 9, Ono & Poss (1982); 10, Amorim et al. (2023); 11, Bolgan et al.(2019); 12, Amorim & Hakins (1995); 13, Amorim (2006); 14, Amorim & Hawkins(2000); 15, Radford et al.(2018);16, Onuki & Somiya (2004); 17, Bertucci et al. (2015);18, Malavasi et al. (2008); 19, Parmentier et al. (2013); 20, Dufossé (1874); 21, Protasov (1962); 22, Wilson et al. (2014); 23, Horvatic et al. (2021); 24, Sebastianutto et al. (2008); 25, Amorim et al. (2018); 26, Lugli et al. (2008); 27, Blom et al. (2016); 28, Lindström & Lugli (2000); 29, Pedroso et al. (2013); 30, Blom et al. (2022); 31, Lugli & Torricelli (1999); 32, Bussmann et al. (2020); 33, de Jong et al.(2016), 34, de Jong et al.(2018)

* This species is considered as invasive in Portugal

2.4.2 Characterization of putative fish sounds

Recordings from a total of 16 days were analysed, producing 2542 putative fish sound detections assigned to 33 putative sound types, with a cumulative recording time of 79.5 h. Each fish sound type was characterized and included in a catalogue (**Figure 2.2**). Fish sounds were grouped into two main categories, continuous sound types (n = 20 sound types) and pulsed sound types (n = 13). **Table 2.S2** provides a qualitative characterization of the different sound types, while **Table 2.S3** provides a quantitative description of acoustic features for the 13 most common sound types. Together, these tables (**2.S2, 2.S3**) and **Figure 2.2** provide an overview of the putative fish sounds recorded at the PLSMP.

Pulsed sound types

Pulsed sound types were sub-categorized as low-frequency and high-frequency sound types (**Figure 2.2**, **Table 2.2**, and **Table 2.S2**). Within the pulsed sounds, low-frequency pulsed sounds were the most common sub-category presenting a peak frequency below ca. 500 Hz. Within this subcategory, four sound types were classified as fast pulse trains (pulse period under 50 ms #3, #6, #11, and #19) and five as slow pulse trains (pulse period over 50 ms #14, #7, #15, #33, #42). Notably, within the low-frequency fast train sub-category, sound types #3 (n = 382) and #6 (n = 143) were the most abundant sounds. Within the low-frequency slow train sub-category, sound types #14 (n = 172) and #15 (n = 654) were the most abundant. Within the pulse train high-frequency sub-category, four sound types were included (#1, #10, #16, #28), all with fast trains. Sound types #1 (n = 94) and #16 (n = 92) presented the most detections in this sub-category (see description of the sound types in **Table 2.S2**).

Continuous sound types

Continuous sound types were divided into tonal sounds and noisy sounds (**Figure 2.2**, **Table 2.2**, and **Table S2**). Within the tonal sub-category, three high-frequency sounds were included (#9, #29, #43). Sound type #9 (n = 35) was the most common, usually composed of a sequence of 3–11 short tonal sounds with a peak frequency of 500 Hz. Ten sound types were included in the tonal low-frequency sub-category (#2, #4, #5, #8, #17, #18, #26, #34, #38, and #40). This was the sub-category with the many sound types, the prevalent being #2, #4, and #8. Because sound type #8 often occurred after #4, they are probably part of a sound sequence produced by the same individual. Sound type #4 was a broadband sound presented in sequences of 3–4 sounds with an average peak frequency of 250 Hz. Sound type #8 was a low-frequency sound with a longer duration of around 500 ms and a peak frequency within 250-500 Hz. Another Sub-category included four wideband noisy sound types (#25, #31, #36, #37) and three low-frequency sound types (#20, #24, #39). From these noisy sounds, sound type #20 was the most common with 61 detections.

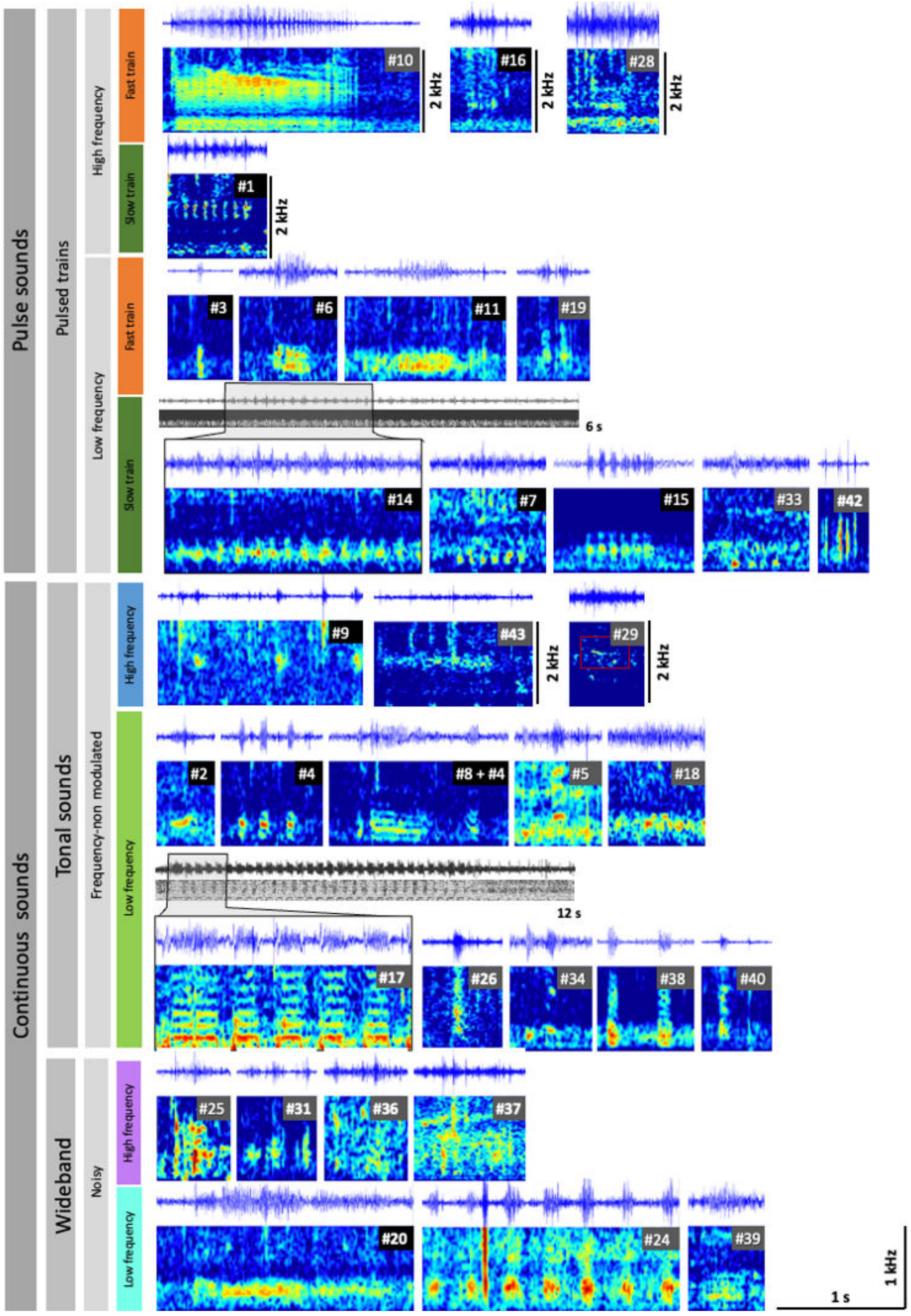


Figure 2.2. Catalogue of putative fish sound types registered at Professor Luiz Saldanha Marine Park. Each sound type was named numerically. The 13 most common sound types (≥ 20 detections) are in black. Spectrograms were produced with FFT=256, frequency range from 0 up to 1 kHz (except when indicated).

Table 2.2. Dichotomy of the main sound categories found on the analysed recordings.

Categories	Sub-category		Frequency	Definition
Pulsed sounds	Pulse trains	Low frequency	Fast train	Fast pulsed sounds, with an average duration of 400–600 ms and a peak frequency below 500 Hz. Pulse period under 50 ms.
			Slow train	Serial of short slow train pulses (duration between 30 and 3500 ms) with peak frequency below 500 Hz. Pulse period over 50 ms.
		High frequency	Fast train	Series of high-frequency pulse trains with a peak frequency above 500 Hz (in this dataset it ranged 600–800 Hz). Pulse period under 50 ms.
Continuous sounds	Tonal sounds	Frequency-non modulated	Low frequency	Low-frequency tonal sound with a duration between 600 and 2000 ms and a peak frequency below 500 Hz.
			High frequency	Tonal sound that has a peak frequency above 500 Hz.
	Wideband	Noisy	Low frequency	Continuous noisy sound with a duration of 1000 and 2000 ms and a peak frequency below 400 Hz.
			High frequency	Broadband, continuous sound with a duration of <1000 ms and a peak frequency above 400 Hz (in this dataset ranged between 450 and 1000 Hz).

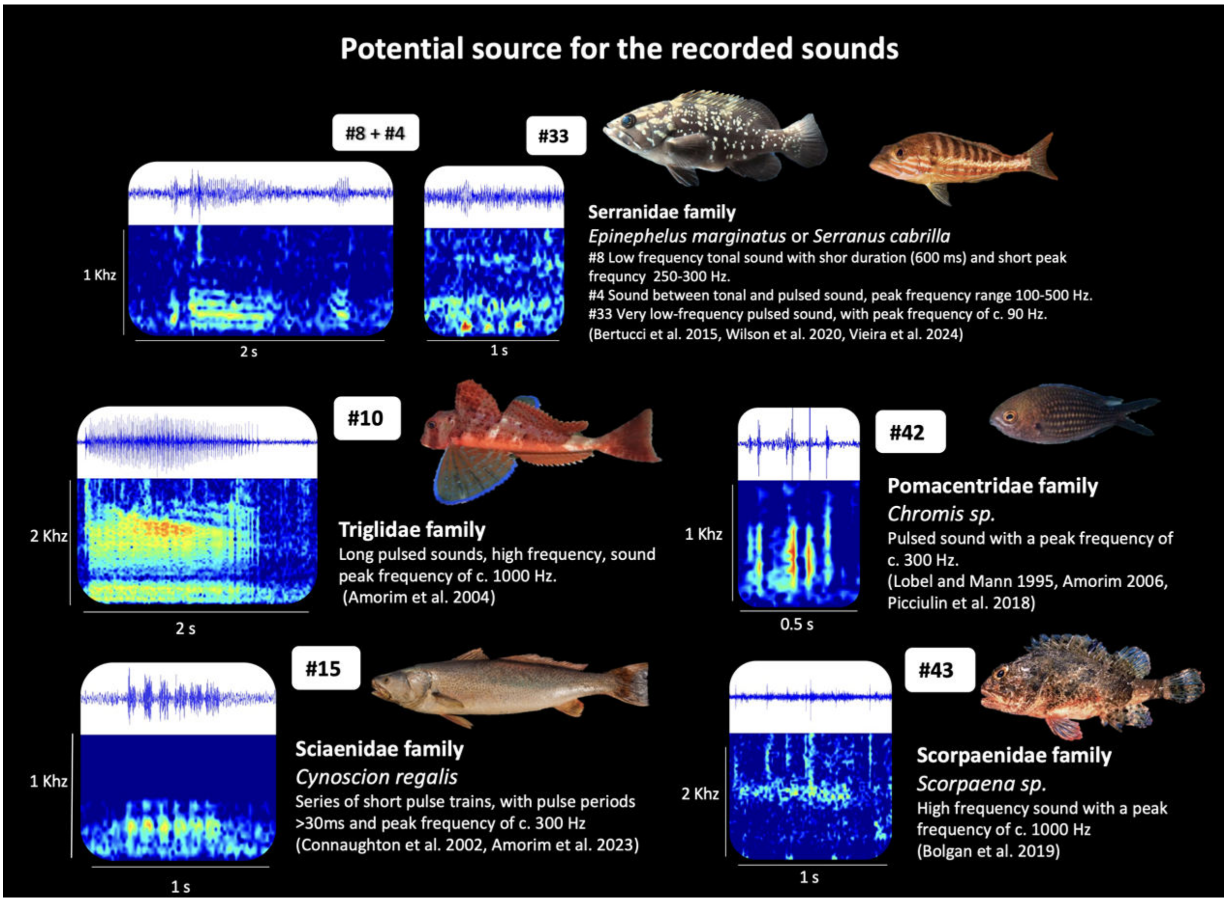
2.4.3 Sound similarities

Three PCAs were conducted on the 13 most frequent sound types using 7 standardized acoustic features shown in **Table 2.S3**. The PCA revealed distinct patterns and separations among the sound types. For pulsed (measured) sounds, PC1 and PC2 explained 85.8% of the variance, with peak frequency, entropy, and Q3 being the most influential variables, while sound type #1 was most distinct (see **Figure 2.S2a**). Pulsed sounds (with pulse period and duration not measured) had 77.9% variance explained by PC1 and PC2, with duration and Q1 being the most influential variables and sound type #16 showing the clearest differentiation (see **Figure 2.S2b**). For continuous sounds, PC1 and PC2 explained 79.4% of data variability, with Q1, Q3, and peak frequency driving the first component and duration influencing the second. Sound type #9 stood out among continuous sounds, while #2, #4, and #8 were more similar (see **Figure 2.S2c**). See **Appendix 1** for a detailed description of the results obtained in these analyses.

2.4.4 Identification of putative fish sound type sources

From the 52 BRUVS deployments, 12 were discarded due to deployment conditions (e.g. unstable rig, blocked vision, strong currents). Of the 40 analysed videos, 13 included putative fish sounds. These belonged to the sound types #3, #6, #9, #16, #20, and #38 (**Table 2.S4**). Several species that are known to be soniferous were also detected in these videos, namely *B.capriscus*, *Coris julis*, *Ctenolabrus rupestris*, *Diplodus cervinus*, *Diplodus sargus*, *Diplodus vulgaris*, *Serranus cabrill*a, *Muraena helena*, *Labrus mixtus*, and Trachurus spp. Sound types #6, #16, #20, and #38 only appeared in one video. The most common sound was sound type

#3, which was found on six videos with eight potentially soniferous species: *D.cervinus*, *D.sargus*, *D.vulgaris*, *C.julis*, *C.rupestris*, *M.helena*, *S. cabrilla*, and Trachurus spp. (**Table 2.S4**). As none of the species showed any obvious behaviour associated with the occurrence of sounds, source identification was not possible. To further aid in identifying the sound sources, we also compared them with sounds reported in the literature. **Figure 2.3** displays potential sound types linked to specific fish families/species. Sounds #4 and #8 are similar to sounds associated with the Serranidae family, identified by Bertucci et al. (2015) and Wilson et al. (2020). Sound #33 is also similar to sounds described by Bertucci et al. (2015), Wilson et al. (2020), and Vieira et al. (2024) and can be associated with the Serranidae family. Sound #10 is characteristic of the Triglidae family as described in Amorim et al. (2004). Sound #15 resembles sounds produced by the Sciaenidae family, specifically the sounds produced by the *Cynoscion regalis*, as reported in Comenaughton et al. (2002) and Amorim et al. (2023). Sound #42 is similar to sounds from the Pomacentridae family, identified by Amorim et al. (2006) and Picciulin et al. (2018). Sound #43 matches sound from the Scorpaenidae family, namely the sounds of Scorpaena sp. reported in Bolgan et al. (2019).



Serranidae family
Epinephelus marginatus or *Serranus cabrilla*
#8 Low frequency tonal sound with shor duration (600 ms) and short peak frequncy 250-300 Hz.
#4 Sound between tonal and pulsed sound, peak frequency range 100-500 Hz.
#33 Very low-frequency pulsed sound, with peak frequency of c. 90 Hz.
(Bertucci et al. 2015, Wilson et al. 2020, Vieira et al. 2024)

Triglidae family
Long pulsed sounds, high frequency, sound peak frequency of c. 1000 Hz.
(Amorim et al. 2004)

Pomacentridae family
Chromis sp.
Pulsed sound with a peak frequency of c. 300 Hz.
(Lobel and Mann 1995, Amorim 2006, Picciulin et al. 2018)

Sciaenidae family
Cynoscion regalis
Series of short pulse trains, with pulse periods >30ms and peak frequency of c. 300 Hz
(Connaughton et al. 2002, Amorim et al. 2023)

Scorpaenidae family
Scorpaena sp.
High frequency sound with a peak frequency of c. 1000 Hz
(Bolgan et al. 2019)

Figure 2.3. Potential sources (family/species of soniferous fish) of sounds recorded at PLSMP were identified by cross-referencing results from the bibliography review with field and BRUVS observations. References to articles describing the sounds produced by fish species or families are provided. Images from free sources: *E. marginatus*—Silvia Tavares iNaturalist (2019), *S.cabrilla*—Tim Camerom iNaturalist (2018), Triglidae—family Silvia Tavares iNaturalist (2020), *C.chromis*—Silvia Tavares iNaturalist (2020), *C.regalis*—Museum of Comparative Zoology, Harvard University, iNaturalist, Scorpaenidae family—Fricke (2018).

2.5 Discussion

This study highlights a wide biodiversity of putative fish sounds recorded at the PLSMP while evidencing the existing gap of information on sound sources. Moreover, it provides the first fish sound catalogue for mainland Portugal and the Atlantic Iberian coast, contributing to increasing the knowledge of fish acoustic communities, providing an initial framework for the use of PAM as a tool to monitor MPAs and coastal areas. Fish community and reported soniferous species. We reviewed the literature as an approach to estimate the soniferous fish species occurring in a specific study site. Considering that only a small percentage of soniferous fish species have been reported thus far, potentially coniferous species (i.e. those closely related to known soniferous species) were also listed. This approach contributes to filling in the gaps in the current understanding of fish acoustic communities (Parmentier et al. 2021, Looby et al. 2022, Parson et al. 2022). Out of the 150 fish species listed for PLSMP, only 29 species were confirmed as soniferous, while 70 species were considered potentially soniferous, lacking documented acoustic activity studies. Similar to other regions worldwide (Parmentier et al. 2021), the number of vocal fish species identified along the Portuguese coast will likely increase, as many species have not yet been acoustically studied. The methodology of listing potentially soniferous fish species, describing their sound, and cross-referencing with recorded putative fish sound types has proven to be adaptable and applicable in various marine environments. For example, Carriço et al. (2019) listed potential soniferous species from Azores seamounts, while Puebla-Aparicio et al. (2024) applied the same methodology to the Mozambique Island coral reefs. Another example is the study conducted by Parmentier et al. (2021), which identified soniferous species on coral reefs in French Polynesia. As in our research, these studies also noted a low percentage of reported soniferous fish species.

2.5.1 Characterization of putative fish sounds

Sounds produced by fish are predominantly low frequency, formed by pulses and usually with short duration (Amorim 2006, Parsons et al. 2016). We detected, identified, and described 33 sound types and classified them into two main categories, pulsed and continuous sounds, and then further into different sub-categories. From all the detected sound types, only 13 were abundant enough to be characterized for different acoustic parameters and to be evaluated using multivariate analyses. Multivariate analyses indicated that some sounds were clearly differentiated within their category but highlighted similarities between some of the sound types. For example, pulsed sound types such as #1 and #16 and the continuous sound type #9 were clearly differentiated within their category. In contrast, within the pulse sounds, #7 and #15 as well as #6 and #11 were not clearly separated by the PCA analyses. The same stands for sound #2 and #4 within the continuous sound category. The lack of distinctiveness in these sound types suggests they may be produced by the same species, e.g. as variations of a sound type, or by different species generating similar calls. Note that the ability to use PAM to detect and properly identify each sound depends on several factors that influence the SNR. For example, quiet sounds such as the ones produced by several goby species should be detected only rarely (Parmentier et al. 2013, Amorim et al. 2018).

2.5.2 Diversity of putative fish sounds

A considerable diversity of putative fish sounds contributing to the marine soundscape of PLSMP was found during the analysis of acoustic recordings. We detected, identified, and described 33 sound types. Compared to other fish acoustic communities, we found that the fish sound richness (the diversity of putative fish sounds) falls well within the range observed in other locations. For example, in the region of Macaronesia, Muñoz-Duque et al. (2024) described 43 fish sounds from a total of 188 listed fish species, including 35 soniferous and 102 potentially soniferous species. In the Mediterranean Sea, specifically at Tavolara-Punta Coda Cavallo MPA, Desiderà et al. (2019) identified 12 fish sound types from a total of 53 fish species identified through underwater visual census. Bertucci et al. (2020) identified 22 fish sounds in a coral reef from Polynesia, while a second study by Raick et al. (2023), encompassing a wider range of Polynesian islands and water depths, detected 45 fish sound types. Also in coral reefs, but, respectively, in the Mozambique Island and Hawaii, 47 and 85 sound types were identified (Tricas & Boyle 2014, Puebla-Aparicio et al. 2024). Lastly, Wang et al. (2017) found 66 fish sound types in the Pearl River estuary, a location within a global hotspot of fish biodiversity with 834 reported species. The above studies highlight that the PLSMP presents high fish acoustic diversity, comparable to other biodiversity hotspots. This is consistent with its position as a biogeographic and oceanographic transition zone between warm and cold temperate waters in the north-eastern Atlantic region (Briggs & Bowen 2012), harbouring a high diversity of fish species, from subtropical to species with more northern-temperate affinities (Henriques et al. 2007).

2.5.3 Identification of putative fish sound type sources

Cross-reference with fish sounds from the bibliography

In our study, we observed some similarities between putative fish sounds and previously reported sound types (**Figure 3**). Sound types #4 and #8 are produced consecutively, thus likely

by the same species. When compared to the list of potentially soniferous species in the PLSMP, several Serranidae species were noted as possible candidates, namely *Serranus atricauda*, *S.hepatus*, *S.cabrilla*, *E.marginatus*, and *Anthias anthias*. Although *S. atricauda* is present in the marine park, it is not a frequent species and is mostly observed during summer (Gonçalves et al. 2002). Both sound types #4 and #8 are fairly common sound types present throughout the whole year and widely distributed, decreasing the likelihood that they are produced by this species. *Anthias anthias* is also an improbable source for these sound types, as the habitat range of these species is at depths greater than 40 m (Schneider 2012), and the hydrophones for this study were placed in shallower water (<10m). Another possible candidate is the Dusky grouper (*E.marginatus*). These sounds are similar to what is reported for groupers (Epinephelus spp.) described by Bertucci et al. (2015) and Wilson et al. (2020), supporting the suggestion that they could be associated with the Serranidae family. The calls from the Nassau grouper (*E.striatus*) described by Wilson et al. (2020) show similarities with two distinct types of calls. Sound type #4 resembles the alarm call (N1) characterized by low-frequency pulses, while sound type #8 is similar to the courtship call (N2). Similar sounds have also been documented in other locations within the Madeira and Azores archipelagos where the Dusky grouper is found (Vieira et al. 2024). However, due to deeper water preferences (8–300 m) and low abundance of this species in the PLSMP (only two individuals reported by divers in a wreck ship diving spot), it is an unlikely sound source for sound types #4 and #8. Consistently, a telemetry study that released 30 *E.marginatus* individuals on the Portuguese coast found that three of them travelled within PLSMP between May and June 2019, highlighting their migratory behaviour and low rate of occupation in PLSMP (Silva et al. 2022). Nevertheless, we cannot exclude the possibility of *S. cabrilla* and *S. hepatus* (less frequent in shallow waters and rocky habitats) being responsible for these two sound types, as they are a common Serranidae species present year-round and in Madeira and Azores archipelagos (Gonçalves et al. 2002, Vieira et al. 2024). Furthermore, sound type #33 was similar to the agonistic sound of *E.striatus* (Wilson et al. 2020) and the boom series described for *E. marginatus* (Bertucci et al. 2015, Vieira et al. 2024). Another sound comparable to bibliography reports is the sound type #10 that has similarities to the grunt produced by the grey gurnard, *Eutrigla gurnardus*, during competitive feeding (Amorim et al. 2004). Sound type #15 was one of the most common sound types, detected as part of a chorus with over 600 detections in one recording of 30 min, whose most likely source is the invasive species weakfish (*C. regalis*). In addition to the similarity in acoustic characteristics between sound type #15 and weak fish sounds (Amorim et al. 2023), the sound type #15 chorus was recorded in June (summer) during sunset, consistent with the reported patterns of sciaenid spawning choruses, including weakfish (Connaughton et al. 2002, Vieira et al. 2022). The chorus was also observed closer to the Sado Estuary, where weakfish are known to occur since 2014 (Morais & Teodósio 2016). The sound type #42 bears a close resemblance to the characteristics of previous confirmed reports of the genus Chromis (Lobel & Mann 1995, Amorim 2006, Picciulin et al. 2018) presented in the list of potentially soniferous species in our study area (**Table S2**). Sound type #43 was reported at other locations within the Madeira and Azores archipelagos (Vieira et al. 2024) and is possibly produced by a species of the genus Scorpaena. It resembles the /kwa/ recorded in the *Posidonia oceanica* meadows of the Mediterranean Sea, which is produced by species of the genus Scorpaena, as described by Bolgan et al. (2019). Both the /kwa/ and #43 showed peak frequencies above 600 Hz and sounded alike to the human ear. Notice that this sound type was rarely detected and at a low signal-to-noise ratio. From this family, the most common species in the PLSMP are *Scorpaena scrofa* and *S.notata* with *S.maderensis* being less frequent but still occurring in the park.

Although the suggestions regarding the potential sources of the different sound types in our study are based on similarities with sounds of other confirmed soniferous species, it is important to note that our hypotheses remain untested. Indeed, increasing use of PAM has led to the detection of numerous unidentified fish sounds. However, the documentation of known sounds has not kept pace with these detections. Nevertheless, these unidentified sounds are valuable for assessing biodiversity and habitat health (Mooney et al. 2020a, Parsons et al. 2022).

Challenges in the identification of fish sound sources

As mentioned above, one important part of the effectiveness of PAM as a monitoring tool relies on the identification of sound-producing species (Mouy et al. 2018). Hence, cataloguing fish sounds using combined audio and video recordings in situ has been used to increase our knowledge and understanding of soniferous fish species (Mouy et al.2018, Carriço et al. 2020b, Puebla-Aparicio et al. 2024). Other studies have carried out laboratory recordings to catalogue fish sounds (Bolgan et al. 2019). Although captivity studies are valuable, there is clearly a need to characterize and identify the sound sources in the natural habitat. The usage of BRUVS in this study was intended to serve this purpose. Analysis of the videos identified six distinct sound types linked to various fish sources, but determining the exact source was challenging. In this study, thirteen potential fish sounds were detected using BRUVS, belonging to the following sound types: #3, #6, #9, #16, #20, and #38. Species such as *C.julis* and *D.sargus*, which are potentially soniferous species (see **Table S2**), were the most abundant in the videos. However, no sounds were directly linked to a fish in the videos. It is possible that sounds could have been made by other fishes not visible on screen, as fish sounds can travel different distances, typically up to 20 meters, depending on environmental factors and source level (Carriço et al. 2020a). Challenges in identifying sound sources included the presence of a bait pole and box, which added noise as fish interacted with them. Also, the detected fish sounds could not be linked to a specific species due to the presence of multiple fish species in the frame, making it difficult to associate their behaviour with the sounds. It is also likely that fish are less prone to making sounds during competitive feeding in comparison with other contexts such as agonistic interactions or reproduction (Ladich et al. 2004). To address these challenges, potential solutions include removing the bait and employing remote underwater video, as in Puebla-Aparicio et al. (2024), which can mitigate some of the additional noise. Another option could be an array of hydrophones combined with a camera to determine the position of the sound source and thus attribute sounds to individuals. This combination allows for sound localization using the time difference of arrival of the sound to the different hydrophones, complemented with video analysis to identify the soniferous fish (Mouy et al. 2018). Directional hydrophones, which are designed to detect sounds from specific directions (Mouy et al. 2023), could help to restrict the considered sound sources. Other proposed systems use 360◦ cameras with acoustic recorders that have been applied in etho-acoustical studies of bottlenose dolphins (Maralunda et al. 2017). Combining audio recordings with visual data allows researchers to correlate sounds with specific species and behaviours, providing a more comprehensive understanding of the context in which sounds occur. Nevertheless, complementing field with captivity studies could improve our knowledge on fish acoustic communication. While captivity studies allow controlled experiments, field studies capture the complexities of natural habitats where fish can express their complete acoustic repertoires. Different studies, such as Bolgan et al. 2019, Pereira et al. (2020), and Mouy et al. (2023), highlight the importance of utilizing both field and captivity approaches in understanding fish sounds. Species that breed in known spawning sites or that use nests to breed or as shelters could ease field studies, as acoustic recordings (or videos) could target these locations. For example, the acoustic repertoire of the Lusitanian toadfish was determined by placing hydrophones near nesting males (Amorim et al. 2008).

2.5.4 Importance of fish sounds for research and public awareness

The field of fish bioacoustics has faced historical constraints due to the absence of an easily accessible and comprehensive inventory of known soniferous fishes, a resource readily available for other taxa such as cetaceans and birds. This limitation has hindered researchers' ability to systematically study and understand the acoustic behaviours and communication patterns of fish species (Mouy et al. 2018, Looby et al. 2022). Bioacoustics researchers have recognized the need to identify soniferous fishes and to create a comprehensive database of fish species and their sounds (Lindseth & Lobel 2018, Rountree et al. 2019), which is crucial for several reasons. These datasets serve as valuable resources for researchers in the identification of soniferous species, preventing unnecessary duplication of research efforts, and revealing general trends in fish sound production (Looby et al. 2022, Parsons et al. 2022). Moreover, the inclusion of negative results helps researchers to avoid potential biases in their analyses and may contribute to a more nuanced understanding of the conditions and contexts under which fish engage in sound production. In addition, the development of fish sound catalogues serves as reference databases for identifying vocalizations at the species or family level (Parmentier et al. 2005, Rountree et al. 2020), facilitating regional comparisons (Vieira et al. 2024), and supporting analyses across different geographical regions (Parmentier et al. 2005). Fish sounds, including those from unknown sources, can serve as ecological indicators if they occur across broad geographic areas and persist throughout the year (Di Iorio et al. 2018, Vieira et al. 2024). Unidentified sounds may also contribute to acoustic metrics such as sound richness and diversity, offering valuable insights into habitat conditions, ecosystem health, and marine biodiversity (Staaterman et al. 2017). Future research should focus on expanding these catalogues and developing tools to automate sound recognition to enhance the efficiency of PAM for fish ecology studies (Bas et al.2017). Furthermore, informing stakeholders about the significance of sound in the underwater environment can promote conservation efforts. Popular media often highlights soniferous marine mammals (https://www.nmmf.org/). However, over 980 fish species can produce sounds, compared to 130 marine mammals (Spriel et al. 2023). To counter this common misconception, outreach activities such as informative videos, podcasts, social media posts, and websites can help increase ocean literacy. Sharing research findings is vital for raising awareness of fish sounds among a broad audience. Resources such as the links listed below also contribute to increasing ocean literacy on fish sounds and already include data/results from the present study: https://www.fishbioacoustics.pt,https: //www.wo-pam.com/, and https://www.glubs.org/. Integrating these catalogues into citizen science programs and interactive exhibits allows visitors to engage with underwater soundscapes, fostering marine conservation awareness and promoting environmental stewardship (Parsons et al. 2022). Indeed, organizations such as UNESCO emphasize that ocean literacy is crucial for raising awareness about the conservation, restoration, and sustainable use of our oceans.

2.6 Conclusions

This study provides a baseline for research on acoustic fish communities in PLSMP and other regions. A high diversity of fish sounds was found for this MPA, revealing the importance of the fish acoustic communities and paving the way for future monitoring programs. The potential of PAM for wildlife monitoring, habitat assessment, and acoustic pollution analysis depends on the existence of comprehensive and reliable databases. Creating a fish acoustic catalogue and database is also crucial for improving machine learning applications in marine ecology, aiding species identification, biodiversity monitoring, and impact assessment. Machine learning is already used to detect vocalizations, identify species, and analyse acoustic data, supporting conservation and management efforts (Bermant et al. 2019, Stratoudakis et al. 2024). Furthermore, changes in fish sound type diversity can be used to monitor shifts in fish communities (Desiderà et al. 2019). This new catalogue of putative fish sounds thus constitutes an important first step in that direction and should be a part of a global effort in marine bioacoustics. Further, PAM is a low-cost, minimal-impact, and relatively low-time-consuming method (if automation methods are applied) and allows for continuous monitoring even at depths and in areas difficult to reach. These are important characteristics, especially since, in general, MPAs are reported to be understaffed and underbudgeted leading to poor law enforcement and management actions (Álvarez-Fernández et al. 2017).

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Appendix 1 - Multivariate analysis of putative fish sounds

Principal Component Analysis (PCA) was conducted on the 13 most frequent sound types to explore similarities between sound types. Separate analyses were performed for pulsed measured in which pulse period and duration could be measured (pulsed measured), pulsed in which pulse period and duration could not be measured (pulsed not measured), and continuous sound types as explained in section 2.6. In general, PCA assumes correlation and linear relationship between features that were tested through a linearity test. The first two principal components scores (PC1 and PC2) were plotted to determine the visual separation of sound types. Additionally, principal components analysis shows which of the acoustic features are more influential in explaining the relationship between sounds (Fan et al. 2018). Only Principal Components (PC) with Eigenvalues > 1 were included. These three analyses were based on the 10 standardised acoustic features shown in **Table 2.S3.** However, minimum frequency, maximum frequency and number of pulses were excluded for the multivariate analysis. Minimum frequency and maximum frequency were excluded because these metrics may be dependent on SNR and are redundant with Q1 and Q3. For this analysis the number of pulses was also redundant with sound duration.

The pulsed (measured) sound types were #1, #7, #14, and #15. The first two principal components for the pulsed (measured) sound analysis cumulatively accounted for 85.8 % of the variance (53 % for the first PC and 32.8 % for the second PC; see **Figure 2.S2a**). The variables entropy, peak frequency and Q3 were positively correlated amongst themselves (Pearson correlation coefficient between Q3-peak frequency = 0.98, Q3-entropy = 0.88, peak frequency-entropy = 0.92) consistent with their high loadings on the first component of the PCA (Q3 = 0.93, peak frequency = 0.95, entropy = 0.86). These three variables were negatively correlated to pulse duration. The variables duration and Q1 were also correlated (duration-Q1 = 1) and loaded in the PC2 with a loading coefficient of 0.93. Sound type #1 was the most distinct pulsed sound, mostly due to the peak frequency, entropy and Q3. Sound type #15 formed a cloud of points with lower variability and overlapped with sound types #7 and #14. Sound types #7 and #14 were more dispersed (**Figure 2.S2a**). Sound types #7 and #15 had similar pulse periods and pulse duration but #7 presented higher variation in sound duration and peak frequency.

The pulsed (not measured) sound types were #3, #6, #11, and #16. The first two principal components accounted for 77.9 % of the variance, the first principal component explaining 54.8 % and the second 23 % (**Figure 2.S2b**). The variables duration and Q1 were correlated (Pearson correlation coefficient between duration-Q1 = 0.21) and with loadings in the second dimension of the PCA of (duration = 0.48) and (Q1 = 0.57). Sound type #16 was clearly the most differentiated sound associated with the variables' peak frequency, entropy and Q3 that were positively correlated between them (Q3-peak frequency = 0.95 Q3-entropy = 0.73, entropy-peak frequency = 0.80) and consistent with their high loadings on the first component of the PCA (Q3 = 0.86, peak frequency = 0.95, entropy = 0.76). Sound types #6 and #11 exhibited a strong overlap and a high dispersion related to duration and Q1. Aurally, these two sound types #6 and #11 were quite similar, with differences mostly on duration suggesting that they could have the same provenance and therefore hinting at potential subtypes.

The most frequent continuous sound types were #2, #4, #8, #9, and #20. The first two principal components for this analysis accounted for 79.4 % of data variability for this sound class. First dimension explained 60.6 % and the second dimension explained 18.8 %. The first dimension is explained by the variables Q1, Q3 and peak frequency that were positively correlated and presented loadings values over 0.85. Duration was represented in the second PCA component with a loading value of 0.57. Sound type #9 was separated from the other continuous sounds by the frequency variables (peak frequency, Q1 and Q3) that were positively correlated between them (Pearson correlation coefficient between Q1-Q3 = 0.94, Q1-peak frequency = 0.78, Q3-peak frequency = 0.87), presenting also high loading values (Q1= 0.85, Q3= 0.95, peak frequency = 0.85) in the first component of the PCA (**Figure 2.S2c**). Sound types #2, #4 and #8 were similar in terms of acoustic features. Some sounds of sound type #20 were more detached due to a higher duration, but also present overlap with sound types #2, #4 and mostly #8. Sound types #8 and #20 were longer, low-frequency sound types; however sound type #8 had a spectral pattern clearly different of sound type #20 with visible harmonics (see spectrograms in **Figure 2.2**).

2.8 Supplementary Material

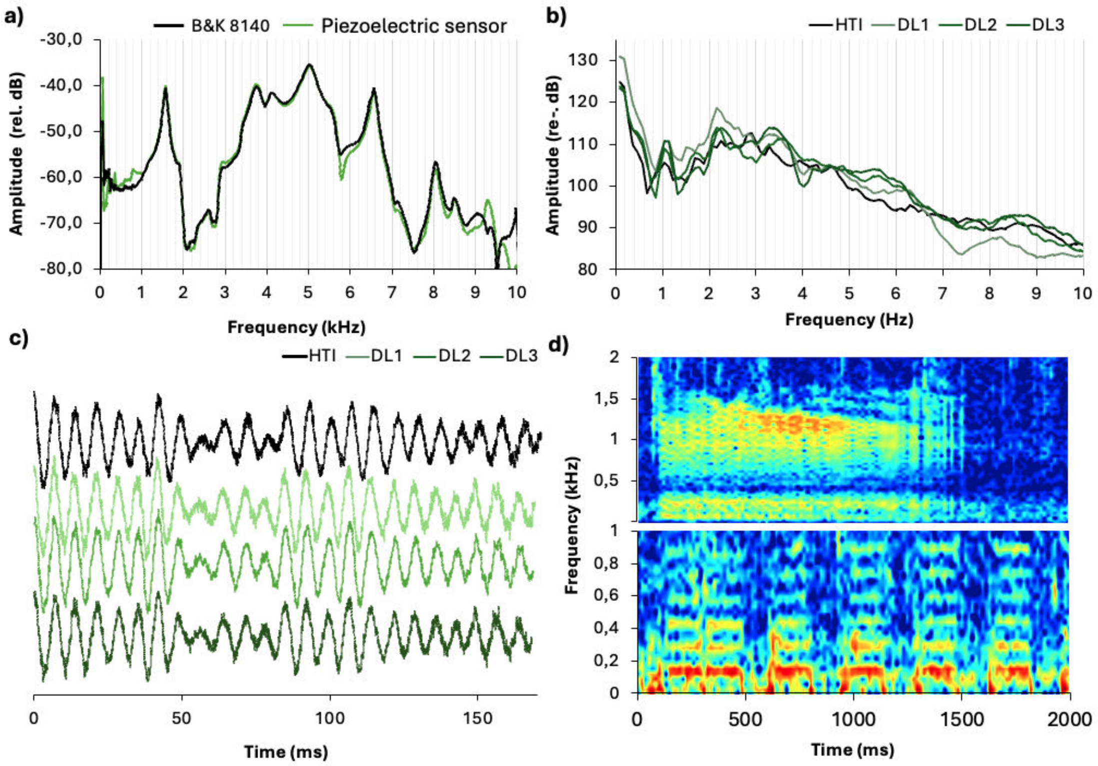

Figure 2.S1. Characterization of the custom-made acoustic loggers used in the present study. (a) Comparison between the piezoelectric sensor used in the custom-made acoustic loggers and the reference hydrophone Brüel & Kjær 8104 (8104, Brüel & Kjær, Nærum, Denmark; sensitivity −205 dB re. 1 V/µPa; frequency response from 0.1 Hz to 180 kHz). The sensor reliably captures the temporal and spectral features of the sounds, although its sensitivity decreases below approximately 150 Hz. (b, c) Comparison between simultaneous field boat noise recordings using custom-made acoustic loggers and the commercial hydrophone HTI (High Tech Inc., Gulfport, MS, USA; sensitivity of −165 dB re. 1 V/µPa, frequency response up to 6 kHz within ±1 dB): (b) shows the averaged spectra (Hamming window; FFT 512 points) and (c) details of the waveforms from each recording. These recordings were made at the pier of Sesimbra port, with the acoustic loggers vertically positioned at the bottom, and the HTI positioned nearby. Note that the four recordings are very similar, without an observable decrease around 400 Hz. (d) Shows two examples of detected putative fish sounds (ST #10 and #17). In sound #10, a clear decrease around 400 Hz is observed, while in sound #17, no obvious alteration is noted. This suggests that the horizontal deployment, as shown in Fig. 1, might be responsible for a significant loss of energy around 400 Hz.

Table 2.S1. Summary of ichthyofauna found on the Professor Luiz Saldanha Marine Park presenting the likelihood of being soniferous (soniferous – S; potentially soniferous - PS; or potentially non-soniferous - PNS). Conservation status (CS), environment and depth range are also presented. Fish family is ordered alphabetically.

Species	Family	Soniferous	CS[a]	Environment[b]	Depth range[b]
Hyperoplus lanceolatus	Ammodytidae	PNS	LC	Demersal	6-30
Argentina sphyraena	Argentinidae	PNS	NE	Bathydemersal	50-700
Atherina boyeri	Atherinidae	PNS	LC	Demersal	1-/
Atherina presbyter	Atherinidae	PNS	LC	Pelagic	1-64
Balistes capriscus	Balistidae	**S**	DD	Demersal	0-100
Halobatrachus didactylus	Batrachoididae	**S**	LC	Criptobenthic	10-50
Belone belone	Belonidae	PNS	LC	Pelagic	0-/
Coryphoblennius galerita	Blenniidae	**PS**	LC	Demersal	1-10
Lipophrys pholis	Blenniidae	**S**	LC	Demersal	0-8
Lipophrys trigloides	Blenniidae	**PS**	LC	Demersal	0-1
Microlipophrys canevae	Blenniidae	PNS	LC	Demersal	0-2
Parablennius gattorugine	Blenniidae	PNS	LC	Demersal	3-32
Parablennius incognitus	Blenniidae	**PS**	LC	Demersal	0-2
Parablennius pilicornis	Blenniidae	**PS**	LC	Demersal	0-25
Parablennius rouxi	Blenniidae	**PS**	LC	Demersal	0-42
Parablennius sanguinolentus	Blenniidae	**PS**	LC	Demersal	0-1
Arnoglossus imperialis	Bothidae	PNS	LC	Demersal	20-350
Arnoglossus laterna	Bothidae	PNS	LC	Demersal	10-200
Arnoglossus thori	Bothidae	PNS	DD	Demersal	15-300
Bothus podas	Bothidae	PNS	LC	Demersal	15-400
Callionymus lyra	Callionymidae	PNS	LC	Demersal	1-440
Callionymus reticulatus	Callionymidae	PNS	LC	Demersal	0-110
Capros aper	Caproidae	PNS	LC	Demersal	40-700
Decapterus macarellus	Carangidae	**PS**	LC	Pelagic	0-400

Naucrates ductor	Carangidae	**PS**	LC	Reef associated	0-300
Seriola dumerili	Carangidae	**S**	LC	Reef associated	1-385
Seriola rivoliana	Carangidae	**PS**	LC	Reef associated	5-245
Trachinotus ovatus	Carangidae	**S**	LC	Pelagic	50-200
Trachurus mediterraneus	Carangidae	**PS**	LC	Pelagic	0-500
Trachurus picturatus	Carangidae	**PS**	LC	Benthopelagic	305-370
Trachurus trachurus	Carangidae	**PS**	LC	Pelagic	0-1050
Spicara maena	Centracanthidae	PNS	LC	Pelagic	30-130
Macroramphosus scolopax	Centriscidae	PNS	LC	Demersal	25-600
Centrolophus niger	Centrolophidae	PNS	LC	Bathypelagic	40-1050
Citharus linguatula	Citharidae	PNS	LC	Demersal	/-300
Alosa alosa	Clupeidae	**PS**	LC	Pelagic	0-300
Sardina pilchardus	Clupeidae	**PS**	LC	Pelagic	10-100
Conger conger	Congridae	PNS	LC	Demersal	0-1171
Taurulus bubalis	Cottidae	**PS**	LC	Demersal	0-200
Engraulis encrasicolus	Engraulidae	PNS	LC	Neritic	0-400
Pollachius pollachius	Gadidae	**S**	LC	Benthopelagic	40-200
Trisopterus luscus	Gadidae	**PS**	NE	Benthopelagic	30-100
Trisopterus minutus	Gadidae	**PS**	NE	Benthopelagic	1-440
Apletodon dentatus	Gobiesocidae	PNS	NE	Demersal	1-15
Diplecogaster bimaculata	Gobiesocidae	PNS	LC	Benthopelagic	0-20
Lepadogaster candolii	Gobiesocidae	PNS	NE	Demersal	3-7
Lepadogaster lepadogaster	Gobiesocidae	PNS	LC	Demersal	3-7
Lepadogaster purpurea	Gobiesocidae	PNS	LC	Demersal	/-/
Gobius bucchichi	Gobiidae	**PS**	LC	Demersal	1-3
Gobius cobitis	Gobiidae	**S**	NE	Demersal	10-35

Gobius cruentatus	Gobiidae	**S**	LC	Demersal	15-40
Gobius gasteveni	Gobiidae	**PS**	LC	Demersal	35-270
Gobius niger	Gobiidae	**S**	LC	Demersal	1-50
Gobius paganellus	Gobiidae	**S**	LC	Demersal	0-15
Gobius xanthocephalus	Gobiidae	**PS**	LC	Demersal	3-36
Pomatoschistus flavescens	Gobiidae	**S**	LC	Reef associated	0-/
Pomatoschistus marmoratus	Gobiidae	**S**	LC	Demersal	20-70
Pomatoschistus minutus	Gobiidae	**S**	LC	Demersal	4-200
Pomatoschistus pictus	Gobiidae	**S**	LC	Demersal	1-55
Thorogobius ephippiatus	Gobiidae	**PS**	LC	Demersal	6-40
Acantholabrus palloni	Labridae	**PS**	LC	Reef associated	30-500
Centrolabrus exoletus	Labridae	**PS**	LC	demersal	0-/
Coris julis	Labridae	**PS**	LC	Demersal	0-120
Ctenolabrus rupestris	Labridae	**PS**	LC	Reef associated	0-50
Labrus bergylta	Labridae	**PS**	LC	Reef associated	1-50
Labrus mixtus	Labridae	**PS**	LC	Reef associated	2-200
Labrus viridis	Labridae	**PS**	VU	Reef associated	1-/
Symphodus bailloni	Labridae	**PS**	LC	Reef associated	1-50
Symphodus cinereus	Labridae	**PS**	LC	Demersal	1-/
Symphodus mediterraneus	Labridae	**PS**	LC	Demersal	1-50
Symphodus melops	Labridae	**S**	LC	Reef associated	1-30
Symphodus ocellatus	Labridae	**PS**	LC	Reef associated	1-30
Symphodus roissali	Labridae	**PS**	LC	Reef associated	1-30
Symphodus rostratus	Labridae	**PS**	LC	Reef associated	1-50
Ciliata mustela	Lotidae	PNS	LC	Demersal	17-22
Gaidropsarus mediterraneus	Lotidae	**S**	NE	Demersal	1-450

Gaidropsarus vulgaris	Lotidae	**PS**	LC	Demersal	20-120
Merluccius merluccius	Merlucciidae	**PS**	LC	Demersal	30-1075
Mola mola	Molidae	**S**	VU	Pelagic	30-1515
Dicentrarchus labrax	Moronidae	PNS	LC	Demersal	10-100
Chelon auratus	Mugilidae	PNS	LC	Neritic	10-20
Chelon labrosus	Mugilidae	PNS	LC	Demersal	0-120
Chelon ramada	Mugilidae	PNS	LC	Neritic	10-20
Mugil cephalus	Mugilidae	PNS	LC	Benthopelagic	0-120
Mullus surmuletus	Mullidae	**PS**	LC	Demersal	5-409
Muraena helena	Muraenidae	PNS	LC	Reef associated	1-801
Phycis phycis	Phycidae	**PS**	LC	Benthopelagic	13-614
Chromis chromis	Pomacentridae	**S**	LC	Reef associated	2-40
Pomatomus saltatrix	Pomatomidae	**S**	NT	Pelagic	0-200
*Cynoscion regalis**	Sciaenidae	**S**	NE	Demersal	10-26
Sarda sarda	Scombridae	PNS	LC	Pelagic	80-200
Scomber colias	Scombridae	PNS	LC	Pelagic	/-/
Scomber japonicus	Scombridae	PNS	LC	Neritic	0-300
Scomber scombrus	Scombridae	PNS	LC	Pelagic	0-1000
Thunnus albacares	Scombridae	PNS	LC	Pelagic	1-1602
Thunnus obesus	Scombridae	PNS	VU	Pelagic	0-1500
Thunnus thynnus	Scombridae	PNS	LC	Pelagic	0-985
Scophthalmus rhombus	Scophthalmidae	PNS	LC	Demersal	5-50
Zeugopterus punctatus	Scophthalmidae	PNS	LC	Demersal	1-40
Zeugopterus regius	Scophthalmidae	PNS	LC	Demersal	10-180
Helicolenus dactylopterus	Scorpaenidae	**PS**	LC	Bathydemersal	50-1100
Scorpaena notata	Scorpaenidae	**PS**	LC	Demersal	10-700
Scorpaena porcus	Scorpaenidae	**S**	LC	Demersal	/-800

Scorpaena scrofa	Scorpaenidae	**PS**	LC	Demersal	20-500
Scorpaena maderensis	Scorpaenidae	**PS**	LC	Demersal	10-500
Anthias anthias	Serranidae	**PS**	LC	Reef associated	30-300
Epinephelus marginatus	Serranidae	**S**	EN	Reef associated	8-300
Serranus atricauda	Serranidae	**PS**	DD	Demersal	3-150
Serranus cabrilla	Serranidae	**PS**	LC	Demersal	5-500
Serranus hepatus	Serranidae	**PS**	LC	Demersal	5-100
Buglossidium luteum	Soleidae	PNS	LC	Demersal	5-450
Dagetichthys lusitanicus	Soleidae	PNS	DD	Demersal	0-125
Monochirus hispidus	Soleidae	PNS	LC	Demersal	10-100
Solea senegalensis	Soleidae	PNS	DD	Demersal	12-100
Solea solea	Soleidae	PNS	DD	Demersal	0-150
Boops boops	Sparidae	**PS**	LC	Demersal	0-350
Diplodus annularis	Sparidae	**PS**	LC	Benthopelagic	0-90
Diplodus bellottii	Sparidae	**PS**	LC	Benthopelagic	0-100
Diplodus cervinus	Sparidae	**PS**	LC	reef associated	30-300
Diplodus puntazzo	Sparidae	**PS**	LC	Benthopelagic	0-150
Diplodus sargus	Sparidae	**PS**	LC	Demersal	0-50
Diplodus vulgaris	Sparidae	**PS**	LC	Benthopelagic	0-160
Lithognathus mormyrus	Sparidae	PNS	LC	Demersal	1-150
Oblada melanura	Sparidae	**PS**	LC	Benthopelagic	/-30
Pagellus acarne	Sparidae	**PS**	LC	Benthopelagic	/-500
Pagellus bogaraveo	Sparidae	**PS**	NT	Benthopelagic	150-700
Pagellus erythrinus	Sparidae	**PS**	LC	Benthopelagic	/-300
Pagrus auriga	Sparidae	**PS**	LC	Benthopelagic	/-170
Pagrus pagrus	Sparidae	**PS**	LC	Benthopelagic	0-250
Sarpa salpa	Sparidae	**PS**	LC	Benthopelagic	5-70
Sparus aurata	Sparidae	**PS**	LC	Demersal	1-150
Spondyliosoma cantharus	Sparidae	**PS**	LC	Benthopelagic	5-300

Entelurus aequoreus	Syngnathidae	**PS**	LC	Demersal	5-50
Hippocampus guttulatus	Syngnathidae	**S**	DD	Demersal	0-30
Hippocampus hippocampus	Syngnathidae	**S**	DD	Demersal	0-60
Nerophis lumbriciformis	Syngnathidae	**PS**	LC	Demersal	0-30
Syngnathus abaster	Syngnathidae	**PS**	LC	Demersal	0-5
Syngnathus acus	Syngnathidae	**PS**	LC	Demersal	0-110
Syngnathus typhle	Syngnathidae	**PS**	LC	Demersal	1-20
Sphoeroides marmoratus	Tetraodontidae	**PS**	LC	Demersal	1-100
Echiichthys vipera	Trachinidae	PNS	NE	Demersal	2-150
Trachinus draco	Trachinidae	PNS	LC	Demersal	1-30
Chelidonichthys cuculus	Triglidae	**S**	LC	Demersal	15-400
Chelidonichthys lastoviza	Triglidae	**S**	LC	Demersal	10-140
Chelidonichthys lucerna	Triglidae	**S**	LC	Benthic	20-318
Chelidonichthys obscurus	Triglidae	**PS**	LC	Demersal	20-170
Lepidotrigla cavillone	Triglidae	**PS**	NE	Demersal	30-450
Trigla lyra	Triglidae	**S**	LC	Bathydemersal	/-700
Tripterygion delaisi	Tripterygiidae	PNS	LC	Demersal	0-40
Zeus faber	Zeidae	**S**	DD	Benthopelagic	5-400

a IUCN Red List of Threatened Species (2023) Conservation status from IUCN: LC, Least Concern; VU, Vulnerable; NT, Near Threatened; EN, Endangered; NE Not Evaluated, CR, Critically Endangered; DD, Data deficient.

b Environment and depth ranges were extracted from fishbase.org

*This species is considered an invasive species in Portugal.

Table 2.S2. Description of sound types. Highlighted sounds indicate the thirteen sound types with ≥ 20 occurrences that were used for the similarity analyses. Note that pulsed sounds are marked by distinct, individual pulses that can be detected both audibly and visually. On the other hand, tonal sounds are generally continuous, with their energy concentrated in a specific frequency range. While tonal sounds may include pulses, they were perceived as continuous during aural and visual inspection.

Sound type	Categories	Sub-categories	No. Detections	Description
#1	Pulsed	Train-high frequency	94	Series of high-frequency pulse trains with a peak frequency of about 900 Hz. Sounds like the creaking of an old door.
#2	Continuous	Low frequency	22	Continuous frequency-modulated sound, with a peak frequency of approximately 400 Hz
#3	Pulsed	Fast train	382	Short single or fast short double pulsed low frequency sound with a peak frequency of about 245Hz.
#4	Continuous	Low frequency	568	Sound between a tonal and pulsed sound. It is a broadband sound with a frequency range of 250 Hz. Can occur isolated but is usually in a sequence. Potentially Serranidae family *Epinephelus marginatus* or *Serranus cabrilla* (Bertucci et al. 2015, Wilson et al. 2020).
#5	Continuous	Low frequency	1	A low-frequency pulsed sound slightly modulated. Sounds like the buzzing of a fly.
#6	Pulsed	Train-low frequency	143	Low-frequency fast pulsed sound with a duration of 500 ms and a peak frequency of approximately 290 Hz. Sounds similar to a snore
#7	Pulsed	Train-low frequency	20	A series of low-frequency pulses with an average duration of 1000 ms and a peak frequency of 300 Hz.
#8	Continuous	Low frequency	147	Low-frequency tonal sound with a short duration (around 600 ms) and a peak frequency range of 250-500 Hz. Potentially Serranidae family, *Epinephelus marginatus* or *Serranus cabrilla* (Bertucci et al. 2015, Wilson et al. 2020).
#9	Continuous	High frequency	35	Tonal sounds that last approximately 2500 ms and have a peak frequency of 500 Hz. Sounds like a barking dog. Usually in a sequence.
#10	Pulsed	Train-high frequency	1	A long-pulsed, high-frequency with peak frequency approximately of 1000 Hz sound that sounds like a long croak.
#11	Pulsed	Train-low frequency	101	A low-frequency fast pulse sound similar to a grunt with an approximate peak frequency of 250 Hz

#14	Pulsed	Train-low frequency	172	A pulse train with a slow pulse rate, lasting for about 3500 ms with an average peak frequency of 250 Hz. Sounds like a helicopter.
#15	Pulsed	Train-low frequency	651	Serial of short slow train pulses (< 630 ms), with pulse period around 90 ms, and peak frequency of about 300 Hz. Potentially Sciaenidae family *Cynoscion regalis* (Connaughton et al. 2022, Vieira et al. 2022)
#16*	Pulsed	Train-high frequency	92	Pulsed high frequency sound with a peak frequency of 800 Hz.
#17	Continuous	Low frequency	1	Continuous sound with frequency not modulated. Peak frequency at 125 Hz. Long sound series.
#18	Continuous	Low frequency	7	Continuous sound with frequency modulation. Peak frequency at 300 Hz.
#19	Pulsed	Fast train	16	Fast two pulsed sounds, with an average duration of 400 ms and a peak frequency of 250 Hz.
#20	Continuous	Low frequency	61	Continuous sound with a peak frequency of 300 Hz
#24	Continuous	Train-low frequency	3	Continuous sound with a frequency range of 150-300 Hz. Sounds like biting.
#25	Continuous	Wideband	2	Continuous tonal noise. Sounds like a duck.
#26	Continuous	Low frequency	1	Continuous broadband sound showed a peak frequency of 500 Hz
#28	Pulsed	High frequency	2	Pulse sound high-frequency with a peak frequency of 600 Hz
#29	Continuous	High frequency	3	High-frequency sound has a peak frequency of approximately 1000 Hz. Sounds like the neighing of a donkey.
#31	Continuous	Wideband	1	Pulsed sound with a peak frequency of approximately 450 Hz.
#33	Pulsed	Train-low frequency	2	Very low-frequency pulsed sound, with peak frequency of 90 Hz.
#34	Continuous	Low frequency	1	Continuous Frequency Modulated sound with peak frequency of approximately 200 Hz.
#36	Continuous	Wideband	1	Broadband, continuous sound with a peak frequency of 850 Hz.
#37	Continuous	Wideband	1	Broadband continuous sound with a peak frequency of 1000 Hz.
#38	Continuous	Low frequency	1	Tonal non- modulated sound showing a peak of approximately 200 Hz

#39	Continuous	Low frequency	1	Tonal sound with a peak frequency of 300 Hz. Sounds like the neighing of a horse.
#40	Continuous	High frequency	1	Continuous, tonal, frequency modulated sound, with a peak frequency of 625 Hz
#42	Pulsed	Train-low frequency	1	Pulsed sound with a peak frequency of approximately 300 Hz. Potentially Pomacentridae family, *Chromis sp*. (Lobel & Mann 1995, Amorim 2006, Picculin et al. 2018)
#43	Continuous	High frequency	8	High frequency sound perceived as tonal with a peak frequency of approximately 1000 Hz. Similar to the /Kwa/ belonging to the Scorpaena (Bolgan et al. 2018).

Table 2.S3. Average ± Standard deviation (and range: maximum - minimum) values of measured acoustic variables for the 13 types of sound that had at least n ≥ 9 non-overlapped occurrences with a sufficiently good signal to noise ratio for the measurement of acoustic parameters. Units are expressed as Hertz (Hz) and seconds (s). *Pulsed no measured sounds. Note that the loss of acoustic energy around 400 Hz frequencies in the recordings and the distance from the source may influence the spectral characterization of sounds, including average entropy.

Sound	N	Sound type	Sound duration (s)	Min-freq. (Hz)	Max-freq. (Hz)	Q1 or 25 %freq. (Hz)	Q3 or 75%freq. (Hz)	Peak freq. (Hz)	Average entropy	No. pulses	Pulse duration (ms)	Pulse period (ms)
#1	12	pulsed	1.38±0.32 (0.52-0.032)	473.08±17.58 (376.07-376.07)	1338.88±44.51 (1145.3-1145.3)	1.38±0.32 (0.52-0.52)	744.14±46.49 (640.63-640.63)	1113.28±50.85 (921.87-921.87)	4.09±0.057 (3.81-3.81)	11±11 (23-12)	30±30 (40-30)	90±90 (170-80)
#2	11	tonal	0.15±0.02 (0.29-0.03)	134.5±26.1 (326.36-25.64)	579.82±57.99 (991.16-392.84)	0.15±0.02 (0.29-0.03)	271.31±22.32 (484.38-218.75)	400.57±43.19 (750-281.25)	2.76±0.14 (3.56-1.98)			
#3*	20	single pulsed	0.12±0.01 (0.21-0.07)	48.45±9.71 (169.22-0)	401.2±13.62 (529.92-282.93)	0.12±0.01 (0.21-0.07)	178.91±8.96 (250-125)	263.28±7.37 (328.13-187.5)	2.73±0.05 (3.03-2.25)			
#4	20	tonal	0.1±0 (0.15-0.05)	40.4±5.38 (72.73-0)	482.46±34.16 (827.83-294.12)	198.44±13.47 (281.25-93.75)	317.19±17.6 (468.75-187.5)	256.25±10.29 (343.75-187.5)	2.94±0.09 (3.8-2.31)			
#6*	14	single pulsed	0.33±0.04 (0.59-0.17)	42.74±6.84 (73.17-0)	468.07±26.2 (721.95-370.73)	40.43±22.04 (250-0.17)	222.1±13.92 (312.5-140.63)	289.06±14.54 (421.88-187.5)	2.9±0.08 (3.7-2.3)			
#7	10	pulsed	0.77±0.15 (1.71-0.39)	45.55±9.94 (110.38-6.9)	358.29±25.81 (507.32-258.54)	0.77±0.15 (1.71-0.39)	156.25±13.02 (234.38-125)	248.26±21.23 (390.63-187.5)	2.77±0.17 (4.11-2.13)	5±1 (12-2)	60±10 (90-40)	130±100 (170-60)
#8	12	tonal	0.53±0.07 (1.11-0.35)	22.54±4.07 (48.78-0)	467.13±36.34 (809.76-358.73)	0.53±0.07 (1.11-0.35)	175.78±11.07 (265.63-109.38)	303.39±26.05 (562.5-203.13)	2.98±0.09 (3.83-2.57)			
#9	20	tonal	0.09±0.01 (0.12-0.06)	513.05±14.71 (574.36-418.8)	736.17±11.88(788.99-658.12)	609.38±11.48(656.25-531.2)	651.042±8.17 (687.5-593.75)	630.21±6.87(656.25-593.75)	2.133±0.042 (2.39-1.84)			
#11*	16	pulsed	0.68±0.07 (1.25-0.43)	36.43±6.38 (103.48-0)	418.1±18.23 (531.19-289.74)	49.33±19.26 (218.75-0.43)	214.84±18.18 (343.75-109.38)	266.6±18.4 (468.75-140.63)	2.68±0.08 (3.3-2.14)			
#14	16	pulsed	6.94±3.2 (54.39-0.76)	60.48±33.73 (549.97-351.83)	390.7±27.4 (470.09-2.67)	15.25±11.49 (187.5-0.76)	164.06±16.14 (375-93.75)	285.16±4.4 (312.5-250)	2.98±0.05 (3.4-2.61)	23 ± 3 (41-4)	60±0 (70-40)	150±100 (230-120)
#15	16	pulsed	0.63±0.04 (0.93-0.31)	41.02±4.07 (75.55-6.04)	392.91±14.73 (492.56-329.38)	0.63±0.04 (0.93-0.31)	232.03±4.95 (250-171.88)	291.41±1.91 (296.88-281.25)	2.56±0.03 (2.75-2.31)	3 ± 0.03 (3 -2)	60±0 (130-40)	90±0 (100-80)
#16*	15	pulsed	0.23±0.04 (0.61-0.07)	408.99±31.58 (551.28-151.09)	1081.57±45.41 (1358.97-670.94)	0.23±0.04 (0.61-0.07)	654.17±24.58 (875-437.5)	827.08±46.11 (1171.88-531.25)	3.5±0.11 (4.17-2.72)			
#20	20	tonal	0.86±0.18 (4.04-0.39)	114.07±7.61 (163.27-34.19)	387.12±8.64 (538.46-357.49)	0.86±0.18 (4.04-0.39)	203.13±2.78 (234.38-187.5)	289.84±4.74 (343.75-250)	2.49±0.06 (3.32-2.04)			

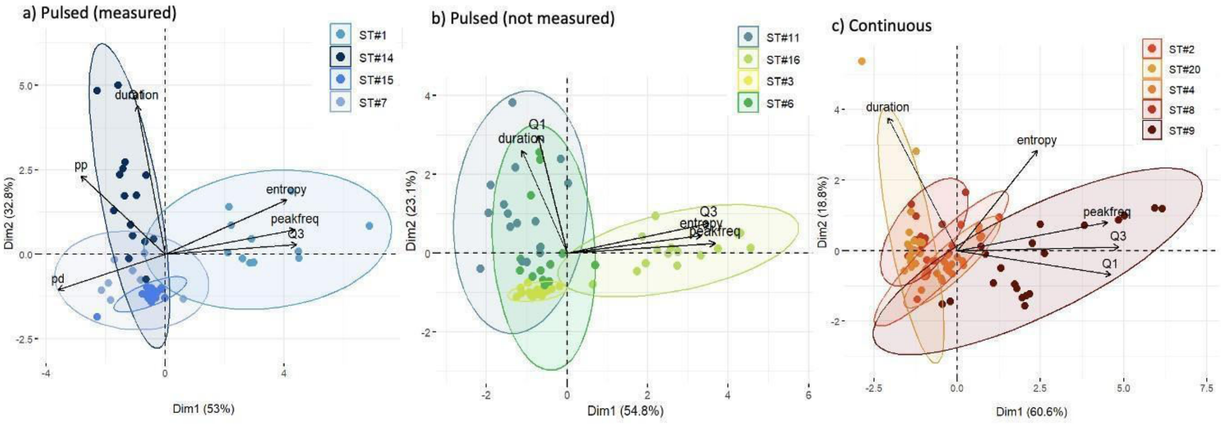

Figure 2.S2. Principal Component Analysis PCA biplots of the 13 most common putative fish sound types analysed by categories: a) Pulsed (measured) sounds; b) Pulsed sounds in which pulse period and duration could not be measured; c) Continuous sounds. The following acoustic features were considered and represented as vectors: sound duration, frequency percentile 25 % (Q1), frequency percentile 75 % (Q3), peak frequency, pulse duration, and pulse period. Pulse duration and pulse period were only used in a).

Table 2.S4: Summary of putative fish sound type sources based on Baited Remote Underwater Video system recordings (1 hour). Sound types identified on the videos and vocal and potentially vocal species that appear on the frame. Occurrence (number of individuals of every species). Location of video deployment with the number of identifications on the map Figure 1. Depth of the deployment in meters, season and area of protection.

Sound type/ Species	Occurrence	Location	Depth (m)	Season	Area
#16					
Balistes capriscus	1	4. Pedra do meio	12	April	PPA
Coris julis	1				
Ctenolabrus rupestris	1				
#20					
Diplodus cervinus	1	8. River Gurara Popa	23,4	April	PPA
Coris julis	1				
Diplodus sargus	1				
Serranus cabrilla	1				
#3					
Diplodus cervinus	1	2. Baía da Armação, 7. 3milhas	7	November	PPA
Trachurus sp.	1		8.7	April	BA
Coris julis	4	1. Derrocada, 2. Baía da Armação, 7. 3milhas	6, 7, 8.7	November	FPA, PPA,BA
Ctenolabrus rupestris	3				
Diplodus sargus	2				
Diplodus vulgaris	1	2. Baía da Armação	7	November	PPA
Murena helena	1				
Serranus cabrilla	1				
#38					
Coris julis	2	7. 3milhas	7	April	BA
Ctenolabrus rupestris	1				
Diplodus vulgaris	1				
Diplodus sargus	1				
#4					
Coris julis	1	7. 3milhas	7	November	BA

Diplodus sargus	1				
#6					
Coris julis	2	1. Derrocada	6	November	FPA
Ctenolabrus rupestris	2				
#9					
Coris julis	1	4. Pedra do meio	12	April	PPA
Ctenolabrus rupestris	1				
Coris julis	1	2. Baía da Armação	8	April	PPA
Labrus mixtus	1				

CHAPTER III

Assessing temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area

Chapter III: *Assessing temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area*

Noelia Ríos, Jodanne Pereira, Paulo J. Fonseca, Gonçalo Silva, Miguel Pessanha Pais, Manuel Vieira and Maria Clara P. Amorim

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3.1 Abstract

Passive Acoustic Monitoring (PAM) is a cost-effective, non-invasive technique with potential applications for long-term monitoring of biodiversity, species behaviour, habitat health and noise pollution at wide temporal and spatial scales. This research aimed to study temporal patterns in the acoustic fish community in a Marine Protected Area, the Professor Luiz Saldanha Marine Park (PLSMP), in Portugal. Three acoustic recorders were deployed between June 2021 and September 2022 in three sites with different rock/algae coverage within the marine park. Acoustic recordings were analysed for putative unidentified fish sounds and analysed for seasonal and diel patterns. We compared fish sounds abundance and acoustic diversity indices (sound type Richness, Shannon, Simpson and Evenness) at the different spatiotemporal scales. Results show an increase in fish sound production and higher diversity indices at sunset and night, with no distinct seasonal patterns. We further explore the patterns in the occurrence of fish sounds relative to the type of habitat, and the influence of environmental factors like temperature, type of substrate or proximity to an estuary. We found that total abundance and diversity of sounds were higher in Zone 1 (rocky habitat with kelp forest *Saccorhiza polyschides*) compared to Zone 3 (interface rock/sand habitat with *Treptacantha usneoides*). Other factors, such as the distance to the nearest estuary, also seemed to influence fish sound occurrence. In conclusion, our results highlight the patterns of occurrence of fish sounds in a marine protected area, supporting the use of PAM as a supporting tool for monitoring and conservation efforts in the marine environment.

Keywords: Acoustic communication, Marine soundscape, Fish sounds, Acoustic biodiversity indices, Professor Luiz Saldanha Marine Park

3.2 Introduction

The marine environment is rich in a variety of sounds, with biological (animals' passively and actively produced sounds), geological (e.g. wind, waves, rain sounds), and anthropogenic origins (e.g. shipping, drilling, dredging) that collectively create a soundscape (Miksis-Olds et al. 2018, Duarte et al. 2021). Biological sounds are produced by a diverse range of species, from marine mammals (Miller et al. 2021) to sea turtles (Melo et al. 2023) and invertebrates (Coquereau et al. 2016). Notably, fish sounds are significant contributors to marine soundscapes on a wide range of environments, including coral reefs (Bertucci et al. 2020, Puebla-Aparicio et al. 2024), rocky reefs (Desiderà et al. 2019, Muñoz-Duque et al. 2024), coastal areas (Bolgan et al. 2019), seamounts (Carriço et al. 2020), and estuaries (Vieira et al. 2021). Fish sounds are usually composed of a series of short-duration pulses, although some species produce tonal sounds resulting from a continuous sequence of muscle contractions (Fine & Parmentier 2015). These sounds typically have frequencies below 2 kHz but can reach up to 5 kHz (Mooney et al. 2020). They can contain valuable information about their function (Amorim 2023) and are produced in various contexts, including fights, mating, spawning, competition for food, group cohesion, and schooling (Amorim 2006, Larsson 2009, Van Oosterom et al. 2016). Currently, it is known that families containing soniferous species make up nearly two-thirds of all actinopterygian species (Rice et al. 2022).

Passive acoustic monitoring (PAM) involves recording the soundscape, including fish sounds, allowing for non-intrusive ecological insights over large areas and time spans (Duarte et al. 2021). Studying marine soundscapes can thus help to assess marine species phenology by looking into vocalisation patterns (Sueur et al. 2019) and identifying daily, moon and annual cycles (Ruppé et al. 2015). Researchers have found that fish species use various strategies to communicate, concentrating their calls at specific temporal, environmental or biological conditions (Desiderà et al. 2019, Bertucci et al. 2020, Vieira et al. 2022). For example, Sciaenidae, Gadiidae and some Batrachoididae often show vocal patterns with increased activity at dusk and/or night and during the breeding season (Connaughton et al. 2002, Nordeide et al. 2008, Parsons et al. 2009, Feng & Bass 2013, Vieira et al. 2019, 2022), while moon phases can affect spawning in Serranidae, reducing activity during full moon periods (Mann et al. 2009). Research in an Atlantic seamount at deeper waters (ca. 190 m deep) found that fish acoustic activity was most common at dusk and night throughout the year, with no clear seasonal pattern (Carriço et al. 2020). These studies suggest increased fish activity and likely spawning behaviour during night-time, possibly as a strategy to reduce the risks of predation (Hobson & Chess 1978, Lobel 1978, Ferraro 1980, Holt et al. 1985). Furthermore, competition for acoustic space can drive species within a community to call at specific times of day, reducing overlap, as proposed by the Acoustic Niche Hypothesis (Krause 1993). Evidence of acoustic partitioning in fish supports the existence of these niches (Bertucci et al. 2020, Muñoz-Duque et al. 2024). For example, a study in the Mediterranean Sea found that three fish species displayed different use of acoustic resources across various locations, utilising a wider range of both temporal and spectral resources in areas with higher acoustic richness (Bolgan et al. 2022), suggesting they were avoiding acoustic competition. In the Madeira archipelago, the acoustic fish community exhibited noticeable differences of activity between day and night. Also, fish sounds exhibited less spectral overlap at night, suggesting temporal differences in acoustic niche use (Muñoz-Duque et al. 2024). Understanding the temporal patterns of fish vocalisation is thus useful for interpreting acoustic data and for monitoring acoustic communities (La Manna et al. 2024). This knowledge can provide valuable insights into the ecosystem's status, dynamics, and overall health, aiding in the development of effective management plans (Pijanowski et al. 2011, Di Iorio et al. 2018) namely in Marine Protected Areas (MPAs). Moreover, PAM-based baseline metrics could be invaluable for assessing the effectiveness of MPAs in restoring biodiversity (Rossiter & Levine 2014, Soga & Gaston 2018).

The present study aims to analyse for the first time the temporal patterns of fish sound production in the MPA Professor Luiz Saldanha Marine Park (PLSMP) using PAM and investigate whether fish sound production is influenced by temporal and environmental factors. By using both sound abundance and biodiversity indices based on putative unidentified fish sounds, the study will assess the existence of acoustic temporal patterns, namely the existence of diel and seasonal variations. Moreover, it will study the variability in the occurrence of fish sounds relative to the type of habitat, and the influence of environmental factors like temperature, type of substrate or proximity to an estuary. The study provides valuable insights into fish acoustic communities within the PLSMP, which could contribute to monitoring habitat health, fish community dynamics, and documenting important biological processes within this MPA.

3.3 Methods

3.3.1 Study site

The PLSMP encompasses a total area of 53 km^2 along 38 km of the western coast of Portugal. This MPA is divided into eight zones with three different levels of protection: four partially protected areas (PPA) allow non-fishing recreational activities and licensed commercial fishing beyond 200 m from the shore, three buffer areas (BA) permit recreational activities and licensed commercial fishing with smaller vessels, and one fully protected area (FPA) which is a no-take zone with permission only for monitoring, research, and education activities.

The PLSMP is a highly biodiverse area with over 1300 species of fauna, including 150 fish species (https://arrabidaparquemarinho.ualg.pt/) that inhabit various habitats like sandy bottoms, seagrasses, rocky reefs, and kelp forests. The region's high biodiversity is driven by its distinctive geography and oceanographic conditions. For instance, the Sado estuary's proximity, along with the Setúbal and Lisbon submarine canyon system, influences coastal ocean circulation, promoting upwelling currents and enhancing primary productivity (Peliz et al. 2002). The Sado estuary also plays a crucial role in shaping the physical and chemical conditions of the area (Reid & Wood 1976), providing habitat for various species to complete their life cycles (Potter et al. 2015). Moreover, its orientation and surrounding orography provide varying levels of exposure to dominant wind and water currents, exhibiting an exposed offshore area to the west and south of Cape Espichel and a sheltered area to the east (Oliveira et al. 2015).

3.3.2 Acoustic recording

To record marine soundscapes, custom-made autonomous acoustic recorders (Audiomoth 1.2.0, Hill et al. 2019) equipped with custom-made hydrophones (Piezo tubes PTZ-P5 with 24 × 20 × 20 mm, with a 50 x signal pre-amplification) were deployed within the PLSMP to record the soundscape between June 2021 and September 2022. The sensitivity of these custom-made hydrophones was ca. -184.5 dB re. 1V/ µPa at 1 kHz. These devices were deployed at three locations, one in each different protection area to capture the variability within this MPA: Zone 1 (BA, 38.4292N, -9.1652W), 8.5 m deep and characterised by a rocky habitat with an extensive rocky reef region and a *Saccorhiza polyschides* kelp forest; Zone 2 (PPA, 38.4357N, -9.0636W) approximately 6.9 m deep and composed by a rocky reef area with a variety of algae species, including *Asparagopsis taxiformis*, *Saccorhiza polyschides*, and *Sphaerococcus coronopifolius*; and Zone 3 (FPA, 38.4465N, -9.0406W) approximately 6 m deep exhibiting a small rocky reef area with seasonal occurrences of *Treptacantha usneoides* in spring, along with a vast expanse of sand (**Figure 3.1**) (Boaventura & Ré, 2001). The continuous acoustic recordings were made at a sampling rate of 48 kHz. Due to the constraints imposed by battery life and storage capacity, data loggers were retrieved, reprogrammed, and redeployed every ca. 58 days. These acoustic recorders have been used in previous studies (e.g. Muñoz-Duque et al. 2024) and their characterization is detailed in Ríos et al. (2025).

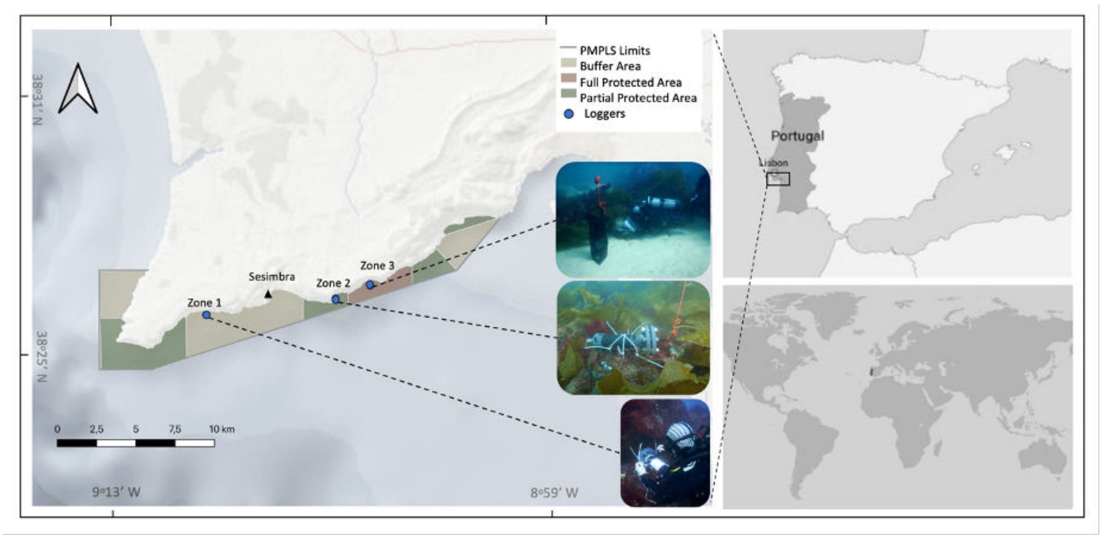

Figure 3.1. Map of the autonomous acoustic recorder locations in the Professor Luiz Saldanha Marine Park.

3.3.3 Detection of putative fish sounds

The acoustic recordings were manually analysed to annotate putative fish sounds. To streamline this highly time-consuming process, specific days were chosen for analysis in each recording location (Zone 1, Zone 2, Zone 3). Four months were chosen to represent the four seasons, June (summer), September (autumn), December (winter) and March (spring). Within each of these months, four days were chosen based on the moon phase - full moon, crescent moon, waning moon, and new moon - to capture the broadest variability of fish sounds, as moon phase can influence vocal activity in some fishes (e.g. Parsons et al., 2016). For each selected day, four distinct periods of 30-minute recordings were extracted to represent the diel cycle: sunrise, solar mid-day, sunset, and night (as per NOAA, 2023). These datasets were composed of 30-minute sample files with the above selections (3 zones, 4 months/seasons, 4 days covering the different moon phases, and four periods for the diel cycle). From the 192 samples, only 157 sample files were analysed, due to some files being incomplete or damaged. The analysis was divided equally between two observers. Since fish sounds usually have energy below 2 kHz, these 30-minutes files were down-sampled to 8 kHz to improve computer processing time. To prevent biases, files were randomly assigned to each observer. Each of these audio files was manually analysed both aurally and visually (spectrogram and oscillogram) using Raven Pro 1.6 (Yang 2024) (DFT size = 256, contrast = 80 and brightness = 55). The manual inspection of the files allowed for the identification, categorisation, and quantification of the various sound types. Sound types were defined for both observers, and any sounds that were not identified as potentially produced by fish were excluded and not categorised. Fish sounds were identified based on their similarity to previously reported fish calls, in frequency, duration and timing. Characteristics of fish sounds can be indicative of species or behavioural context; hence, similar sounds were placed into categories based on spectral and temporal features (Parsons et al. 2016, Desiderà et al. 2019, Puebla-Aparicio et al. 2024). The sounds were categorised into different types based on a dichotomous classification of the main fish sound categories and included in a comprehensive catalogue (Ríos et al. 2025). In short, sounds were classified into pulsed and continuous sound types (Ríos et al. 2025). Pulsed sounds were categorized into low and high frequency pulse trains (each divided into slow and fast pulse trains). Continuous sounds were divided into tonal frequency non-modulated sounds, and noisy wideband sounds, each further divided into low or high frequency sounds (**Figure 3.2).** Sounds were then quantified and analysed for temporal (seasonal and daily) and spatial patterns. It is important to note that although each putative fish sound exhibits characteristics similar to reported fish calls, this is not a guarantee that all these sounds originated from fish.

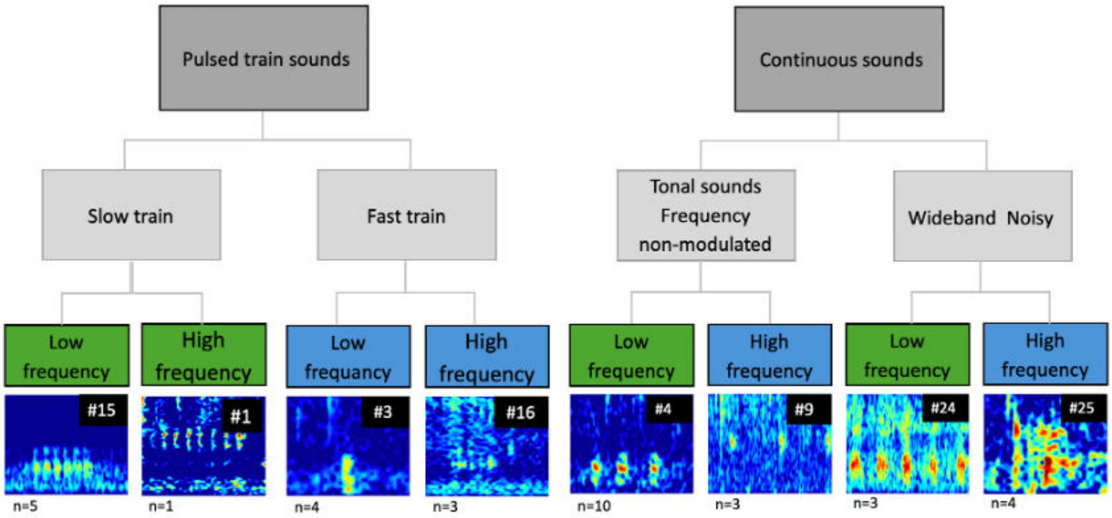

Figure 3.2. Dichotomic classification framework used for categorising putative fish sounds based on their temporal and spectral characteristics. Sounds were classified into two main

categories: (1) *Pulsed train sounds*, which include high-frequency and low-frequency signals further divided into *fast* and *slow* pulse trains, and (2) *Continuous sounds*, which encompass *tonal sounds* (with non-modulated frequency) and *wideband noisy* signals. Each classification is represented with a spectrogram, illustrating the acoustic properties of each sound type. Sound examples from each category are available for reference. The number of different sounds within each category is denoted by N. For further methodological details, see Ríos et al. (2025).

3.3.4 Analysis of temporal and spatial patterns

The occurrence of the different sound types was quantified in the different sampled 30-minute files. For the evaluation of acoustic diversity, traditional ecological biodiversity indices were adapted, using sound types instead of species. The Sound Type Richness (R), Shannon's Diversity Index (H), Simpson Index (D), Inverse Simpson Index (1/D) and Simpson's Evenness (1/DR) were calculated and compared between seasons, diel periods, and zones. Sound Type Richness, similar to Species Richness which represents the number of species or attributes present in a community, is the simplest metric used to describe diversity in an acoustic community. A higher number indicates a greater level of diversity (Gotelli & Colwell 2001). Shannon's index is a comprehensive metric that combines both measures of richness and abundance (Morris et al. 2014). However, due to the consolidation of these variables into a single index, it can be sensitive to small sample sizes and may not accurately reflect the presence of rare classes (sound types), potentially leading to an underestimation of diversity (Sandoval et al. 2018). In traditional ecology, the Simpson Index can be understood as the likelihood that two randomly selected individuals are from different species. Lower values suggest a higher chance that the individuals are from the same species, indicating lower diversity (Morris et al. 2014). Inverse Simpson's index, as the name suggests, is the inverse of Simpson's index. This transformation serves to make the index intuitively increase as the diversity increases. In this study, we use Inverse Simpson's Index. Finally, Simpson's Evenness represents the extent to which the calls (or individuals) are distributed across the defined sound types (or species in traditional ecology). Lower values indicate the dominance of a few sound types, and high values indicate a relatively even number of calls belonging to each sound type (Morris et al. 2014).

To visually explore fish sound patterns sound occurrence matrices (using percentage values of occurrence of each sound type) were made. One-way ANOVAs were used to compare the differences in the abundance and diversity of the sound types between seasons and time of day. The factor season had 4 levels - June, September, December, March, each with 12 replicates - 3 zones × 4 days per month; the number of sounds in a day corresponded to the average of occurrences in the 4 × 30-minute periods. The factor time of day also had 4 levels - Sunrise, Mid-day, Sunset, Night, each also with 16 replicates - 3 zones × 4 months. However, due to equipment failures or continuous presence of high levels of anthropogenic noise, some recordings were missing or discarded, resulting in a reduced sample size for certain comparisons. For nonparametric data a Kruskal-Wallis test was used instead (Kruskal & Wallis 1952). Post-hoc tests, Tukey HSD test or a Dunn's test, depending on whether the data was considered parametric or not, were used to test for pairwise differences. The normality and the homogeneity of the data were respectively tested with the Shapiro-Wilk and Levene tests. Analyses were conducted on the total abundance of fish sounds as well as on individual sound types with over 100 detections (#3, #4, #6, #8, #11, #14 and #15; see Ríos et al. 2025 for the description of specific sound types), and to acoustic diversity indices described above.

To further explore the environmental and spatio-temporal factors affecting the occurrence of putative fish sounds, a Principal Coordinates Analysis (PCoA) was carried out using both categorical and quantitative variables The considered categorical predictor variables were month (June, September, December, March), time of day (simplified as day (sunrise, mid-day) and night (sunset, night)), and zone (zone 1, zone 2, zone 3). The quantitative variables were the percentage of rock, water temperature, wave fetch and distance to the Sado estuary. The percentage of rock habitat was calculated using habitat data layers from https://eunis.eea.europa.eu/ in QGIS and considering a 20 m radius from the hydrophone (see map in Supplementary Material **Figure 3.S1**). Sea Surface Temperature (SST) was obtained from the west side of the park (38.4166N, -9.1333W; depth: 0.5 m; E.U. Copernicus Marine Service Information, https://doi.org/10.48670/moi-00021). Wave fetch was considered as the distance over water that the wind travels in different directions before reaching the shore. The total wave fetch is calculated as the sum in km of 16 different directions and the average of the tree polygons close to the coordinates where every acoustic logger was placed (Burrows et al. 2012).

The PCoA is a statistical technique that reduces the dimensionality of data used to study the similarity or dissimilarity of sample composition (Borcard et al. 2018). The analysis was based on a zero-adjusted Bray-Curtis dissimilarity matrix (Clarke et al. 2006) between samples, calculated from the abundance of all fish sounds except for the rare sounds (i.e. with less than 10 detections), namely #1, #2, #3, #4, #6, #7, #8, #9, #11, #14, #15, #16, #19 and #20. Fish sound abundances were square root transformed to reduce the impact of outliers and dominant sounds, revealing any potential underlying patterns. The function w*cmdscale* (Vegan package) was used to perform the weighted PCoA on the Bray-Curtis dissimilarity matrix (Oksanen et al. 2019). The function envfit (Vegan package) was used to assess the correlation between environmental factors and ordination axes, with significance tested via permutation (n = 999). To identify and remove redundant variables prior to this analysis, a Pearson correlation matrix was created using the corrplot package (Wei 2021) to examine the relationships between variables. The analysis indicated that some variables such as the percentage of rock (i.e. rock cover), zone (i.e. protection status), distance to the estuary and wave fetch were highly correlated (Supplementary Material **Figure 3.S2**). Distance to Sado estuary and percentage of rock were used as proxy of zone and wave fetch. The temporal variables—month and diel cycle—were encoded into binary (0/1) dummy variables to incorporate this categorical data in the analysis. Statistical analyses were performed in the R environment version 4.2.2 (R Core Team 2021).

3.4 Results

A total of 157 sample files were analysed corresponding to a cumulative recording time of 79.5 hours encompassing 16 days. We found 2542 putative fish sounds spread over 33 sound types.

3.4.1 Seasonal patterns

There was no clear seasonal pattern in the occurrence of fish sounds (**Figure 3.3, Table 3.1**). In terms of individual sound types, several sounds had a higher prevalence in June, but others showed higher abundance in other sampled months (see matrix in **Figure 3**). Of the seven sound types with more than 100 detections, only sound types #3 showed a significant difference in the number of detections made during the different months (Kruskal-Wallis H=14.185, N=381, P=0.007, **Figure 3.3).** Sound #3 had higher abundance in June than in any other month (Dunn test p=0.04). Sound #15 was detected as a chorus in one of the days of June, but detections were almost non-existent in other days. However, we did not find significant differences among months for this sound type. Diversity indices showed no clear pattern in terms of seasonal occurrence (**Figure 3.3, Table 3.1).**

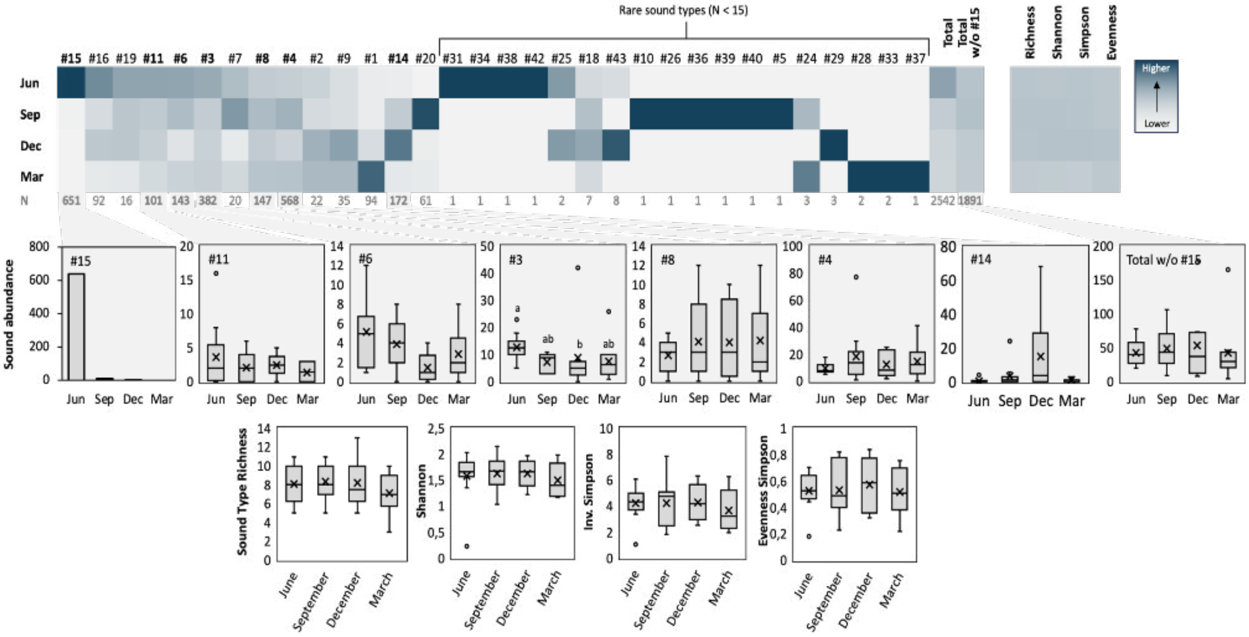

Figure 3.3. Seasonal patterns of unidentified fish sounds sampled in the Professor Luiz Saldanha Marine Park. The matrix shows the seasonal patterns of the different sound types, with darker blue colours representing a higher occurrence of each sound. The matrix is arranged according to the prevalence of sounds in June (summer). The box plots show the seasonal patterns in the abundance of the different sound types with over 100 detections. Box plots of the total abundance and sound diversity indices are also presented. The plot with the total abundance does not include detections from sound type #15, as these detections were mainly concentrated in one sound file that contained choruses. Different letters atop the box

plots indicate significant pairwise differences given by post-hoc tests (Tukey HSD test or Dunn's test) with a p-level of 0.05.

Table 3.1. Seasonal variations of acoustic based biodiversity indices and abundance of the seven sound types with more that 100 detections. Differences between seasons were evaluated by applying parametric (ANOVA; F) or non-parametric (Krustal-Wallis; H) tests depending on whether data followed parametric test assumptions. Average values measured on each acoustic variable are also presented. Significant values are in bold.

	Index	**June**	**September**	**December**	**March**	**Tests value**	**df/N**	**p-value**
Biodiversity indices	**Shannon Index**	5.21	5.44	5.58	4.49	1.026 (F)	3	0.47
	Simpson Index	4.23	4.33	4.47	3.55	0.336 (F)	3	0.79
	Evenness	0.52	0.53	0.57	0.51	0.020 (F)	3	0.92
	Sound Type Richness	8.08	8.36	8.25	7.1	4.86 (H)	3	0.59
Sound types abundances	**#15**	53.25	0.90	0.25	0	7.56 (H)	651	0.11
	#14	0.5	3.45	14.75	1	7.62 (H)	172	0.11
	#3	12.75	7.36	9.0	7.6	14.185 (H)	381	**0.007**
	#6	5.16	3.90	1.5	2.88	4.19 (H)	143	0.24
	#8	2.66	4.09	4.0	2.22	2.10 (H)	147	0.71
	#11	3.66	2.09	2.5	1.4	3.66 (H)	101	0.45
	#4	9.66	18.54	12.37	14.9	18.421 (H)	568	0.61
	Total without #15	49.91	52.72	60	46	2.1993 (H)	1852	0.53

Water temperature (SST) showed some seasonal variation. The minimum temperature was 13.8°C in December, ranging from 14-15ºC between December and April, and the maximum was 20.7°C in September, with an average temperature of 16.1 ± 1.7°C across the four seasons. Boxplots showing the variability of calling activity and water temperature can be found in Supplementary Material **Figure 3.S3**.

3.4.2 Daily patterns

A significantly higher abundance of sounds was observed at sunset and night during the study year (Kruskal-Wallis H=20.29, N=2503 p<0.0001, **Figure 3.4).** Pairwise post-hoc tests showed a significant difference between night and sunrise (Dunn-test p=0.008), night and mid-day (Dunn-test p=0.001), sunset and sunrise (Dunn test p=0.039), and sunset and mid-day (Dunn test p=0.008). There were significant differences among the different times of day in sound type Richness (ANOVA- F=7.97, df=3 p<0.0001, **Figure 3.4**).The sunset and night periods presented significantly higher Richness, with mean values of 7.60 and 7.75, respectively, when compared to sunrise and mid-day with mean values of 5.46 and 4.81, respectively (Tukey HSD test: sunset-sunrise p=0.03, sunset-mid-day p=0.005, night-day p=0.01, night-mid-day p=0.002). A comparable trend was observed for Evenness Index, (ANOVA- F=11.97, p<0.001, **Figure 3.4**) with significantly lower evenness during sunset and night periods. The mean Evenness values for sunset and night were 0.55 and 0.48, respectively, and 0.73 and 0.81 for sunrise and mid-day (Tukey HSD test: sunset-sunrise p=0.031, sunset-mid-day p=0.0006, night-sunrise p=0.001, night-mid-day p=0.00001) (**Table 3.2**). Shannon and InvSimpson did not show differences between seasons. In terms of individual sound types with more than 100 total detections, only #14 presented a significant difference (Kruskal-Wallis test: H=13.25, N=20 p=0.0041, **Figure 3.4**), with a higher number of these sounds occurring at night relative to sunrise and mid-day (Dunn test: night-sunrise p=0.004, night-mid-day p=0.024). The high number of vocalisations of #15 during sunset is attributed to a single chorus event found in only one of the files, precluding the detection of significant diel effects.

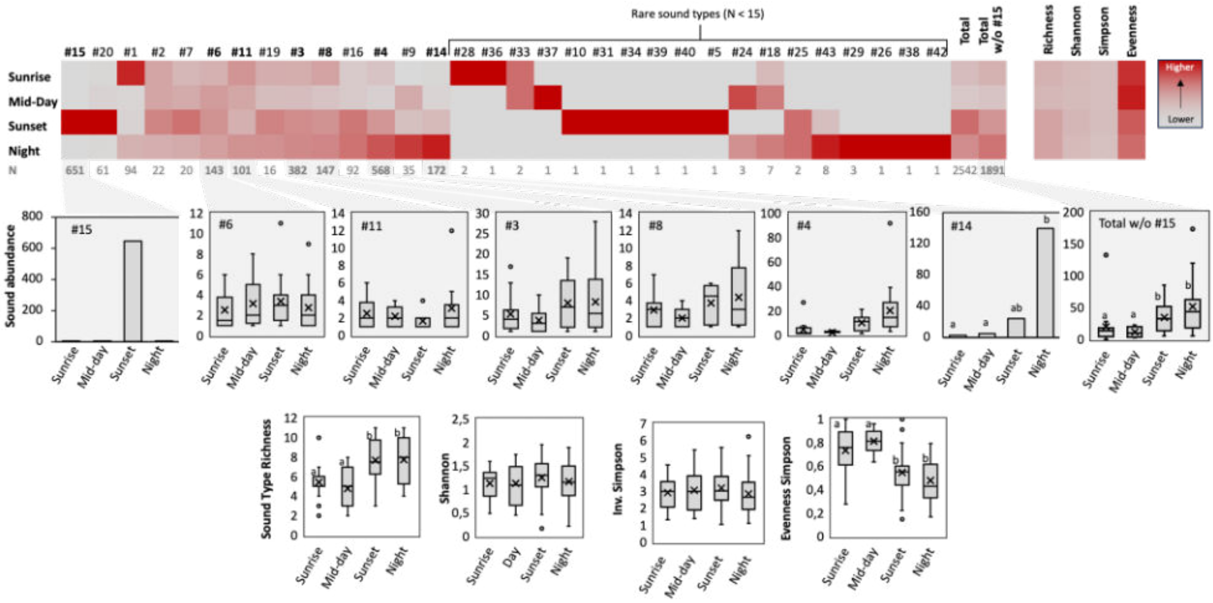

Figure 3.4. Time of day patterns in unidentified fish sounds sampled in the Professor Luiz Saldanha Marine Park. The matrix represents the diel patterns of the different sound types, with the darker red colour representing a higher occurrence of sounds. The matrix is organised to emphasise the prevalence of sound during the night period. The boxplots represent the daily patterns of the abundance of the different sound types that had above 100 sound detections. The plot with the total abundance does not include the detections from #15 as these were mostly concentrated in one session containing choruses. The last row of boxplots illustrates the diversity indices for the different periods of the day. Different letters atop the box plots indicate significant pairwise differences given by a post-hoc test with a p-level of 0.05.

Table 3.2. Daily variations of acoustic based biodiversity indices and abundance of the seven sound types with more that 100 detections. Differences between time of day were evaluated by applying parametric (ANOVA; F) or non-parametric (Krustal-Wallis; H) tests depending on whether data followed parametric test assumptions. Average values measured on each acoustic variable are also presented. Significant values are in bold.

	Diel period	Sunrise	Mid-day	Sunset	Night	Tests value (F/H)	df/N	p-value
Biodiversity indices	**Sound Type Richness**	5.46	4.81	7.6	7.75	7.97 (F)	3	**<0.0001**
	Shannon Index	4.28	4.21	4.88	4.55	0.856 (F)	3	0.47
	Simpson Index	0.71	0.69	0.70	0.66	0.646 (F)	3	0.59
	Evenness	0.73	0.81	0.55	0.48	11.97 (F)	3	**<0.001**
Sound types abundances	**#15**	1	1	107.33	1.33	22.438 (H)	651	0.52
	#14	1.50	1.25	4	12.72	13.25 (H)	172	**<0.0001**
	#3	5.28	3.69	8	8.25	48.293 (H)	381	0.18
	#6	2.50	3.16	3.38	2.78	15.251 (H)	143	0.68
	#8	2.91	2	3.75	4.42	35.373 (H)	147	0.33
	#11	2.50	2.20	1.63	3.10	21.485 (H)	101	0.54
	#4	4.66	2.40	10.25	20.25	22.438 (H)	568	0.52
	Total without #15	19.93	10	34.81	51.82	20.29 (H)	1852	**0.0001**

3.4.3 Spatial patterns

The total abundance of sounds was higher in Zone 3 than in the other zones mainly because of sound type #15, which was part of a chorus and was therefore detected in high abundances in one sampling day of June (**Figures 3.5, 3.6**). However, when removing sound type #15, Zone 1 presented more fish sounds than the remaining, with Zone 2 showing the lowest abundance. Sound types #4 and #3 were dominant in Zone 1 and Zone 2. Sound type richness increased from Zone 3 to Zone 1. Similar patterns were observed for the Simpson's and Shannon Indices, with higher values in Zone 1 compared to Zone 3, and intermediate values for Zone 2. In contrast, the Simpson's Evenness was similar among zones (**Figure 3.6**). In terms of individual sound types, most of the detections occurred in Zone 1, the most notable exception being sound type #15 in Zone 3. However, none of the individual sound types with more than 100 detections showed marked differences in occurrence among zones.

Note that results regarding differences in zones should be regarded with care as there was only one acoustic recorder per zone. Hence no statistical comparisons were performed.

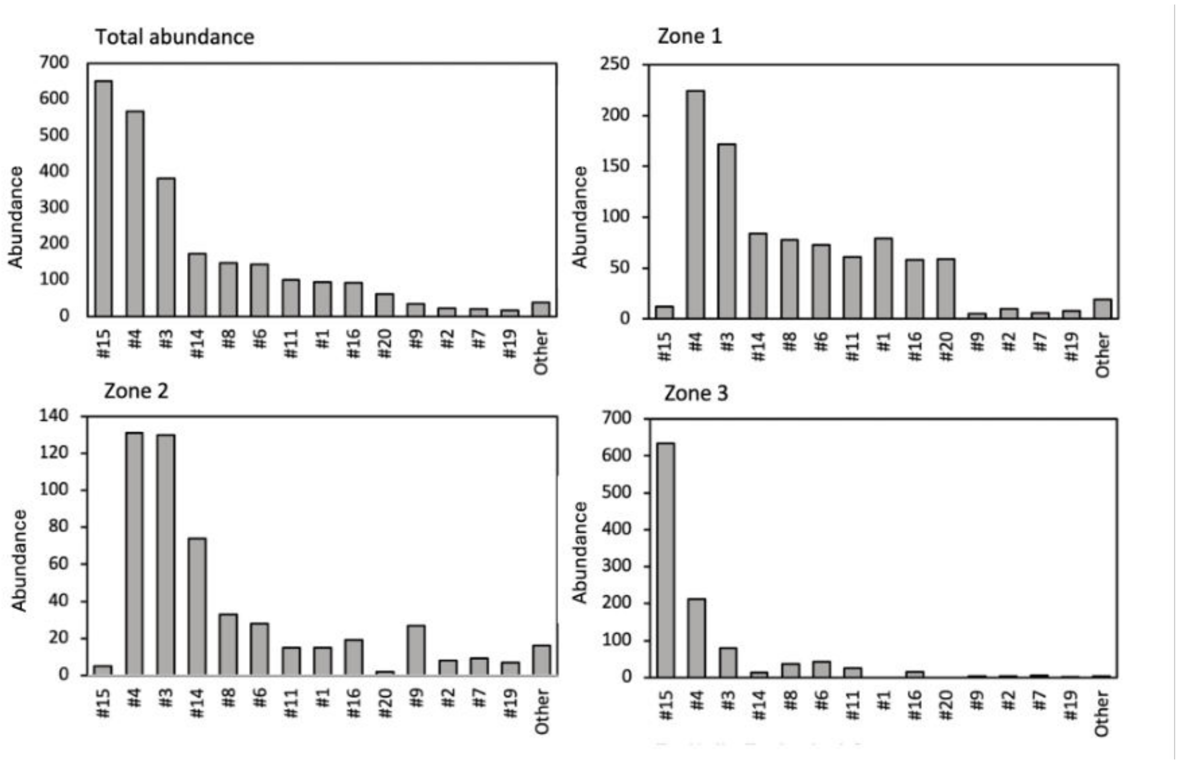

Figure 3.5. The abundance of fish calls for the different protection areas and the overall abundance. "Other" in the graphs represents sound types with less than 15 detections.

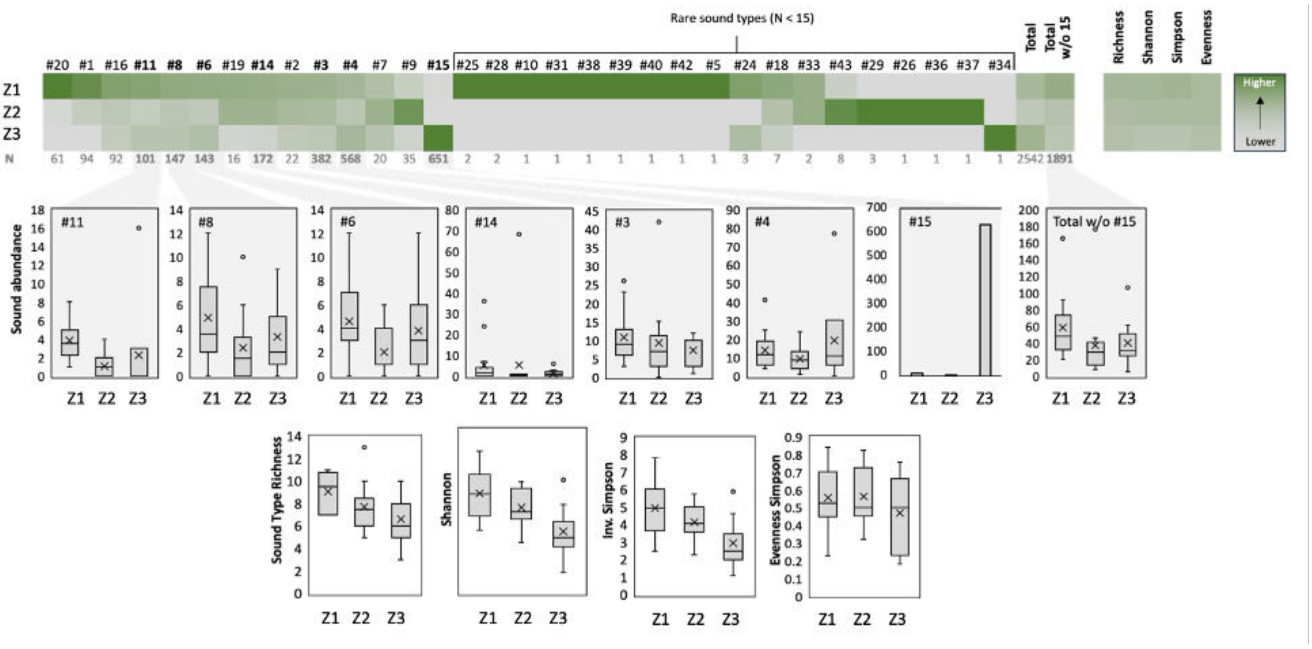

Figure 3.6. Spatial patterns in fish sounds sampled in the Professor Luiz Saldanha Marine Park. The matrix represents the spatial patterns of the different sound types, with the dark green colour representing a higher occurrence of sounds. The matrix is organised to emphasise the prevalence of sound in Zone 1 (Z1). Boxplots displaying the abundance of sound types with over 100 detections are shown. A separate plot shows the total abundance, excluding detections from #15 due to their concentration in one sound. The final row of boxplots shows the sound diversity indices. Zones were simplified Z1, Z2 and Z3 to correspond to Zones 1, 2 and 3.

3.4.4 Multivariate analysis of fish sound patterns

The indirect gradient analysis of the PCoA plot (**Figure 3.7**) using envfit revealed that the main gradient (horizontal axis) was significantly correlated with the time of day ($R^2 = 0.4282$, $p = 0.001$), confirming a strong influence of diel patterns on community composition. The second gradient (vertical axis) was primarily correlated with Season June ($R^2 = 0.1047$, $p = 0.022$) and temperature ($R^2 = 0.0839$, $p = 0.026$), suggesting a secondary influence of seasonal and environmental factors. The multivariate analysis showed that sounds #3 ($R^2 = 0.3441$, $p = 0.001$), #4 ($R^2 = 0.5139$, $p = 0.001$), #6 ($R^2 = 0.0946$, $p = 0.019$), #8 ($R^2 = 0.3464$, $p = 0.001$), #11 ($R^2 = 0.0762$, $p = 0.036$), #14 ($R^2 = 0.1268$, $p = 0.003$), and #16 ($R^2 = 0.1362$, $p = 0.003$) were the most important in differentiating community composition along the temporal gradient. In summary, the analysis highlights the time of day as the primary driver of variation in community composition, with additional but less pronounced contributions from seasonal and environmental variables.

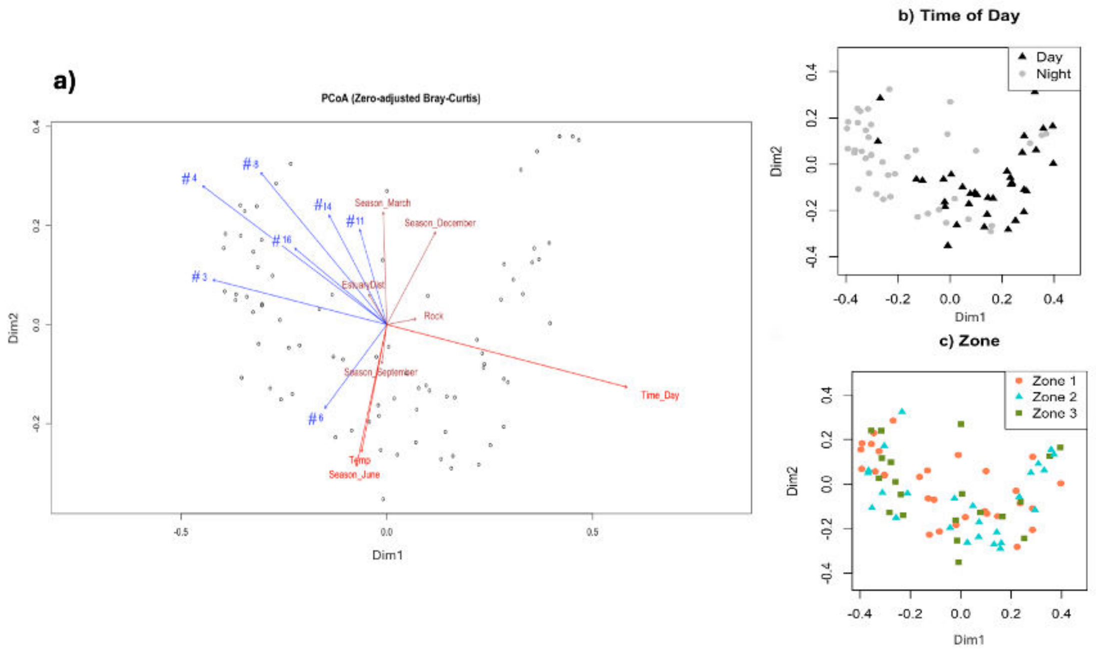

Figure 3.7. a) Two-dimensional Principal Coordinate Analysis (PCoA) based on zero-adjusted Bray-Curtis dissimilarity matrix. The length of superimposed vectors represents the correlation with the axes. All predictor variables are represented, but the significantly correlated ones are signalled with a red colour and larger font size. Significantly correlated sound types are shown as blue vectors. **b)** PCoA plot represented in a), but with identified day and night samples night. **c)** PCoA plot represented in a), but with identified zones.

3.5. Discussion

Here, we present the first study examining the temporal patterns of fish vocalisations in the Professor Luiz Saldanha Marine Park. The observed acoustic patterns reveal variations in daily activity. while also indicating possible variations on the spatial distribution within the marine park. Specifically, fish acoustic behaviour appears to be influenced by several factors, including diel cycles and environmental conditions like temperature. Overall, our study supports that acoustic monitoring can be a valuable tool for studying fish behaviour, biodiversity, and ecosystem dynamics, providing insights into the factors that influence fish sound occurrences. This information is essential for developing more effective conservation strategies and improving management practices.

3.5.1 Temporal patterns

We did not find a seasonal pattern in sound type occurrence. However, several studies have described seasonal patterns of fish sounds, often with increased detections during summer months, attributed to fish choruses (McCauley & Cato 2000, Buscaino et al. 2016, Rice et al. 2016, 2017, Sánchez-Gendriz & Padovese 2017, Vieira et al. 2021). In this study, only one chorusing event (#15) was recorded, occurring during summer. The lack of a connection between the seasons and fish vocalisations may be partially explained by the absence of additional chorusing events, due to a low number of chorusing species in the area. For example, field recordings conducted in various species of Sciaenids, showed a consistent positive relation with the reproductive season and with water temperature (Rice et al. 2016, Monczak et al. 2017, Vieira et al. 2022).

Considering the sound types that are not produced in chorus (the majority), increased vocalisation behaviour was still expected in spring/summer months, associated with higher temperature and food availability. Indeed, sound type #3 occurred more frequently in the summer (June). Factors such as seasonal migrations, water depth, temperature, food availability, and fish behaviour, including reproduction, are known to influence sound production in fish (Rice et al. 2017, Vieira et al. 2021). Seasonal influences on specific sound types reveal distinct patterns, suggesting that certain sounds are associated with particular behaviours at different times of the year. For example, depending on species, reproduction can occur at different seasons and may occur over extended periods. Also, moderated temperatures (14ºC to 20ºC) influenced by upwelling events in the study area, could be shaping seasonal specificities. Indeed, conflicting results exist in the literature, with previous studies showing a relationship between acoustic activity and seasonal variation of water temperature, while others have not, suggesting that the effect of temperature on the rate of sound production is species-specific (Ladich 2018). Seasonal factors related to mating and spawning behaviour may intertwine with the effects of temperature on calling activity. Further studies could examine the relationship between temperature and other variables influencing seasonal patterns on fish acoustic communities.

Time of day was the main structuring factor in our study, with a higher number of vocalisations observed during the night and sunset, consistent with numerous other studies (McCauley & Cato 2000, Parsons et al. 2016, Rice et al. 2017), although in some communities the inverse can also be observed (e.g. Muñoz-Duque et al. 2024). Increased sound type Richness and lower Evenness were also observed at night and during sunset, indicating a higher number of detected sound types, but with specific sound types dominating during these periods. Our findings are consistent with a study on an Atlantic seamount, where the highest abundances of sounds were observed at dusk and night (Carriço et al. 2020) and with studies in coral reefs where night presented more abundance of fish sounds (Bertucci et al. 2020), despite the differences between locations such as depth, temperature, and habitat structure. Sound production appears to follow fish diel patterns of activity, associated with specific behaviours such as feeding, agonistic interactions and reproductive activity (Amorim 2006, McCauley 2012), with fish vocalisations showing nocturnal patterns in several species (McCauley & Cato 2000, Parsons et al. 2016, Rice et al. 2017). Numerous fish species exhibit most of their reproductive activity at dusk and night, greatly influencing their vocal behaviour. Examples are Sciaenids, Groupers, and some Toadfishes (Mann et al. 2009, McIver et al. 2014, Vieira et al. 2019). The increased production of sounds in low light may be a way to avoid predation risks (Ladich & Friedrich 2019). Indeed, the only detected chorus (sound #15) also occurred at dusk, and is likely from the non-native sciaenid, the weakfish, *Cynoscion regalis* (Ríos et al. 2025, Amorim et al. 2023).

3.5.2 Spatial patterns

The three sampled zones in our study represent a spatial habitat, with zone 3 being the closest and Zone 1 the farthest to the Sado estuary (**Figure 3.1**). Zones farther from the estuary presented a higher proportion of rocky habitats and a greater richness and diversity of fish sound occurrences (see **Figure 3.6**).

The Sado estuary features a longitudinal intertidal sandbank spanning around 10 km2 (Rodrigues & Quintino 1993). Sand areas result in less biodiversity, while rocky shores support a wide variety of species (Tuya et al. 2019). Estuaries are demanding environments that host a variety of fish species, including resident, migratory, and straggler species from marine and freshwater habitats (Reid & Wood 1976, Elliott et al. 2007). These communities are typically dominated by a small number of key species (Magurran & Henderson 2003), resulting in lower species richness compared to adjacent marine environments (Martino & Able 2003). Also, most of fish acoustic behaviours associated with estuaries are often related to spawning (Lowerre-Barbieri et al. 2013, Vieira et al. 2022), consistent with the detected chorus (sound #15) at zone 3, near the estuary.

Most of the sounds were recorded in Zone 1 (Buffer Area), which also showed the highest values of Sound Type Richness, Shannon Index, and Simpson Index. Substrate composition likely plays an important role in shaping fish communities, contributing to the patterns observed in our study area. The PLSMP has two distinct habitats: a sandy bottom habitat on the eastern side with large *Atrina pectinata* beds and a rocky region on the western side dominated by *Saccorhiza polyschides* algae (Cunha et al. 2014). The acoustic recorder in Zone 1, was placed in a rocky habitat, while in Zone 3 it was near a large sandy area with 30% of rock and with the seasonal presence of *Treptacantha usneoides*. Rocky shores are known to support diverse species assemblages, potentially contributing to the higher diversity levels observed there. Indeed, structural substrate complexity has been shown to influence fish communities' composition by increasing species richness (Sánchez-Caballero et al. 2017, Castaño et al. 2021).

A higher abundance and diversity of sound types in Zone 1 relative to Zone 3 could be surprising given that Zone 3 is designated as a no-take no-go zone. Nevertheless, it represents a small area where non-compliance with regulations can be observed as evidenced by the presence of boats and reported illegal fishing in the area (Cunha et al. 2011, Álvarez-Fernández et al. 2017). Furthermore, data from previous studies conducted in the area using Underwater Visual Census (UVC) and Baited Remote Underwater Video Survey (BRUVS) from 2011-2020 (Cunha et al. 2014, Serrão et al. 2021) did not reveal significant differences in richness and diversity among protection zones, likely due to the visual techniques focusing on demersal species with highly dispersal patterns. These results are in concordance with acoustic results from this study and suggest that biodiversity and abundance are influenced by factors beyond protection status, such as depth and habitat composition. Note, however, that differences between zones should be interpreted with caution due to the lack of replicates per zone. Future studies should record at multiple locations within each zone to confirm the observed fish sound patterns.

3.5. Conclusions

This study examines fish acoustic communities in PLSMP, focusing on temporal patterns while exploring other sources of variability. Diel patterns were the main temporal factor affecting fish acoustic communities, with higher occurrence of fish sounds during sunset and night. Additionally, environmental factors such as temperature and habitat type (rock percentage and proximity to the estuary) also seem to influence fish sound occurrence, though more replicates are needed per zone to ascertain this suggestion. Fish sound production can be influenced by a diversity of factors and changes in sound patterns can indicate shifts in population dynamics or environmental quality (Jarriel et al. 2024). This makes understanding fish acoustic communities vital for conservation efforts (Bolgan et al. 2023). Fish sounds could be used to detect changes in the environment, allowing specific measures to be taken to achieve targeted conservation strategies (Stratoudakis et al. 2024) or to identify critical areas for key fish species' biological cycles (Amorim et al. 2023). In sum, this first study on the spatiotemporal patterns of the fish acoustic community in PLSMP highlights the potential of PAM to extract ecologically relevant information from recordings in this MPA and lays the foundation for future acoustic research along the Portuguese continental coast.

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3.7 Supplementary Material

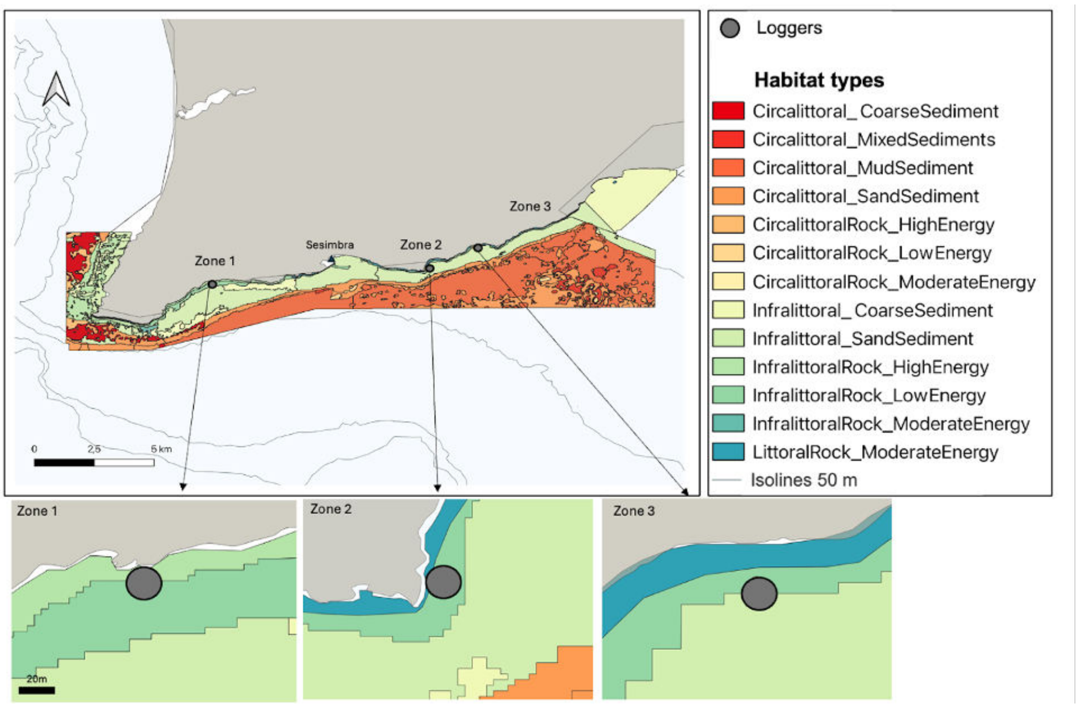


Figure 3.S1. Map showing detailed descriptions of habitat types in the Professor Luiz Saldanha Marine Park. Hydrophone deployment was conducted in three different zones. The scope of the acoustic recording area was calculated based on the distance to the coast and to assess habitat coverage. Circalittoral is a rocky subzone dominated by animals, below the algae-dominated infralittoral. Infralittoral is the shallow water region closest to the shore, excluding the intertidal zone. High energy habitats are extremely wave-exposed with strong tidal streams, while low energy habitats are wave-sheltered with weak tidal streams.

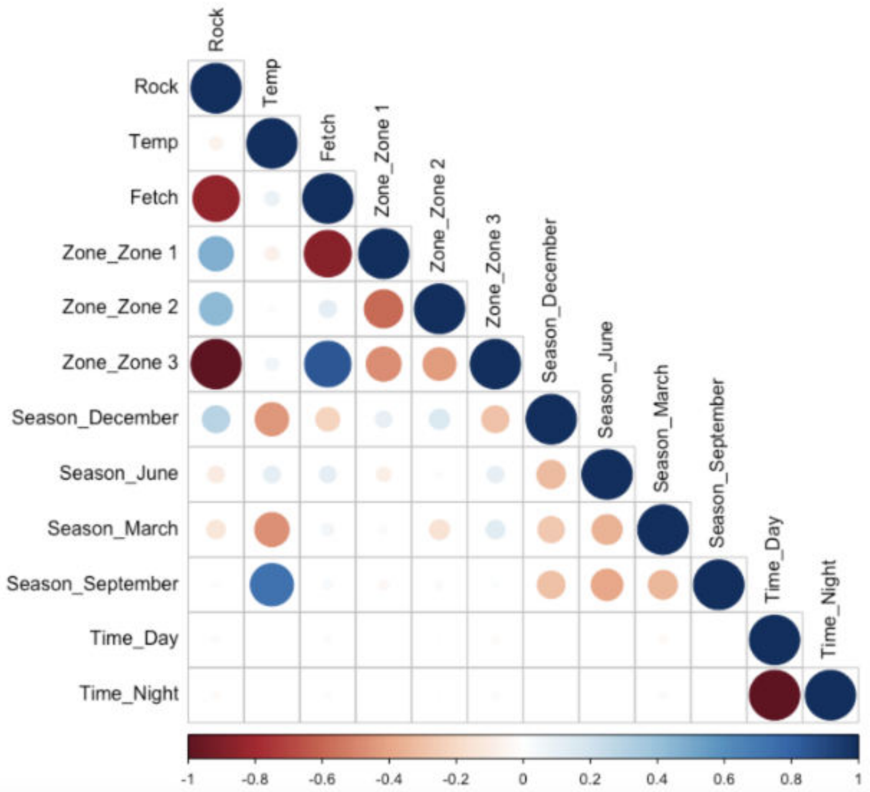


Figure 3.S2. Correlation matrix showing which variables included in the model were correlated. The matrix presents the environmental variables (rock percentage, temperature, wave fetch and estuary distance), and spatial-temporal variables (zone, season and time of day). The qualitative variables were converted to dummies (0-1) for the correlation. Blue gradient represented positive correlation between variables, and red-brown gradient represented negative correlation.

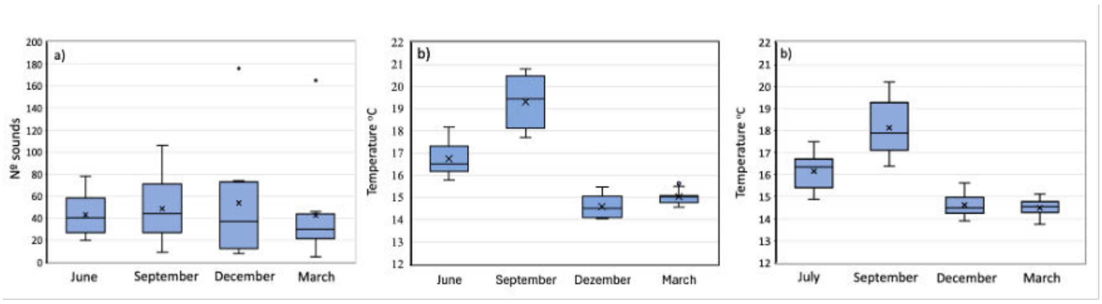


Figure 3.S3. a) Boxplots showing the abundance of sound types analysed for four representative months, each corresponding to a season: June (summer), September (autumn), December (winter), and March (spring). b) Boxplots of Sea Surface Temperature (SST) data obtained from the Copernicus Marine Service (2021) for all days within the analysed months. c) For reference, additional in situ temperature measurements recorded using HOBO TidbiT MX Temperature 400' logger, deployed on the mooring line of buoy PNA1 (38.4357°N, 9.0536°W) at a depth of 3 m (IPMA). Note: In situ temperature recordings began after June 2021; therefore, data for July is shown instead.

CHAPTER IV

Monitoring coastal fish communities: Visual vs Acoustic Methods

Chapter IV: *Monitoring Coastal fish communities: Visual vs. Acoustic Methods*

Noelia Ríos, Mariana Coxey, María Jimenez, Gustavo Franco, Miguel Pessanha Pais, Emanuel J. Gonçalves, Manuel Vieira, Maria Clara P. Amorim, Gonçalo Silva

The material of this chapter has now been corrected by the co-authors.

4.1Abstract

Effective biodiversity monitoring is crucial for evaluating the state of marine ecosystems. Under the European Marine Strategy Framework Directive (MFSD), coastal fish populations are typically surveyed using visual techniques such as Underwater Visual Census (UVC) or Baited Remote Underwater Visual Systems (BRUVS). However, UVC may not detect all fish species, especially those that are hidden or elusive, while BRUVS can lead to an overestimation of carnivorous species and underestimation of cryptic ones. Both visual techniques face limitations related to visibility, depth, weather conditions and the feasibility of long-time monitoring. To address these gaps, Passive Acoustic Monitoring (PAM) offers a promising complementary approach for long-term, non-invasive and cost-effective biodiversity assessment. However, there is still a lack of cross-validation between these different methods of assessing diversity. This study compares abundance, and biodiversity indices obtained from UVC, BRUVS, and PAM to assess fish communities through species and sound diversity in the Professor Luiz Saldanha Marine Park (PLSMP). The comparison of monitoring methods showed clear differences in their ability to detect patterns in fish community metrics. Visual techniques revealed higher abundance and species richness, whereas PAM detected comparable patterns of diversity to the visual methods. Specifically, UVC recorded higher species richness and diversity in the Full Protected Areas (FPA), whereas in the Buffer (BA) and Partial Protected Areas (PPA), BRUVS and PAM also detected greater species richness and diversity. Additionally, the ecoacoustic indices Acoustic Complexity Index (ACI) and Acoustic Entropy (H) showed strong correlations with traditional diversity indices, suggesting their potential as a quick but still reliable proxy for marine ecological assessment. These results highlight the importance of combining monitoring methods, as such an approach offers a more comprehensive understanding of fish communities and better captures the complexity and ecological variability of marine ecosystems.

Key words: Baited Remote Underwater Video Systems (BRUVS), Underwater Visual Census (UVC), Passive Acoustic Monitoring (PAM), Biodiversity indices, Ecoacoustic indices

4.2. Introduction

Marine Protected Areas (MPAs) have demonstrated significant effectiveness for preserving species, populations and habitats (Edgar et al. 2014). Studies show that MPAs, particularly no-take marine reserves, can lead to substantial increases in fish biomass and density (Molloy et al. 2009). Fish are often the primary targets of fishing and thus show the most immediate benefits from protection, making them key indicators requiring regular monitoring (Marshall et al. 2019). Effective MPAs rely on strong planning, governance, and adaptive management. In the management phase, long-term, non-invasive monitoring is crucial to achieve conservation goals (Dunham et al. 2020, Grorud-Colvert et al. 2021). Reaching environmental targets also depends on using appropriate ecological indicators (Cardoso-Andrade et al. 2022). The EU Marine Strategy Framework Directive (MSFD) promotes an ecosystem-based approach by defining 11 qualitative descriptors—ranging from biodiversity and non-indigenous species to commercial fish stocks, food webs, contaminants, marine litter, and underwater noise— to assess and achieve Good Environmental Status in marine waters (European Commission, 2008). These descriptors are only effective if translated into indicators that are regularly calculated using methods that reflect communities and support timely responsive management (Gaines et al. 2010, Pendred et al. 2016).

For example, multi-method approaches have been effectively applied to assess fish assemblages or target species, such as rare and highly mobile elasmobranchs, within marine protected areas (Honeyman et al. 2023, Batista et al. 2024). Underwater Visual Census (UVC) and Baited Remote Underwater Video Systems (BRUVS) are among the most commonly used methods for monitoring reef fish assemblages (Henriquez et al. 2013, Cheal et al. 2021). Both are cost-effective methods for quickly collecting data on marine fauna (Lowry et al. 2011, Ghazilou et al. 2019). However, both methods are known to yield different results in terms of species richness and abundance - UVC records more herbivores and cryptic species, while BRUVS are usually more effective for sampling mobile predators and carnivorous species (Colton & Swearer 2010, Lowry et al. 2011). Despite their utility, the efficiency of visual techniques is affected by factors such as poor visibility, depth and weather conditions, which restricts when and where surveys can be performed (Colton & Swearer 2010, Wraith et al. 2013). Adding to this, even subtle differences in BRUVS methodology, such as bait type and quantity, deployment times and water currents (Heagney et al. 2007, Dorman et al. 2012), or in UVC, such as swim speed and transect dimensions (Pais & Cabral, 2018), can lead to compatibility constraints due to biases that are difficult to account. Additionally, they often require highly trained scientist, making them costly and logistically challenging (Langlois et al. 2020). These factors may limit their feasibility for long-term, continuous monitoring, especially over large spatial scales (Cheal et al. 2021).

Recent advances in acoustic instrumentation and the application of Artificial Intelligence (AI) for data analysis have enabled Passive Acoustic Monitoring (PAM) to be employed for continuous monitoring of marine ecosystems (Dziak et al. 2023). By recording underwater soundscapes, PAM offers insights into species presence, environmental sounds, and the influence of human activities (Baumgartner et al. 2018). The biological component of a habitat's acoustic environment, known as the biophony, encodes species diversity that may be quantified through the abundance and richness of different sound types (Desiderà et al. 2019, Mooney et al. 2020, Raick et al. 2023). Furthermore, various acoustic features — known as ecoacoustic indices — can be extracted from recordings to assess the diversity and abundance of biological sounds, providing valuable tools for quantitatively analysing soundscapes (Pieretti & Danovaro 2020). Ecoacoustic indices have been used in different environments such as seagrass meadows (Ceraulo et al. 2018), deep seamounts (Carriço et al. 2020), temperate reefs (Harris et al. 2016), and coral reefs (Bertucci et al. 2016, Minier et al. 2023). Acoustic Complexity Index (ACI) was the first acoustic index to assess sounds in inshore marine habitats (McWilliam & Hawkins 2013). Sound Pressure Level (SPL) normally reflects environmental sound energy and can indirectly estimate vocal fish diversity (Gage et al. 2001) and abundance (Rowell et al. 2017) and entropy index (H) was identified as a proxy for biodiversity on temperate reefs (Harris et al. 2015).

However, PAM is not without limitations. It is naturally biased towards soniferous species, while background noise from both natural and anthropogenic sources can easily mask relevant acoustic signals. Accurately identifying sound sources remains challenging, as many species' vocalisations are still unclassified and most soundscapes remain poorly characterized. Additionally, variables such as source level, water depth, and substrate type influence sound propagation and detection range, which in turn complicates efforts to detect and interpret acoustic signals (Ross et al. 2023).

While both visual and acoustic methods have their strengths and weaknesses, a combination of both may offer a promising solution for long-term biodiversity monitoring in coastal ecosystems (Buscaino et al. 2025), with visual surveys providing detailed information on species composition, and hydrophones providing continuous, non-invasive detection of soundscape diversity, enhancing efficiency and coverage (Mooney et al. 2020, Dziak et al. 2023, Hildebrand et al. 2024). Taking advantage of methodological complementarity can be an important asset for efficient monitoring, but it can also lead to issues regarding comparability and representativity. This study compares three different survey methods: UVC, BRUVS and PAM, in terms of their ability to capture fish assemblage composition and diversity indices and assesses whether they are comparable across space and time. The research took place at the Professor Luiz Saldanha Marine Park (PLSMP), a marine protected area (MPA) in Portugal, established in 1998, although the current conservation measures were only in force since 2009. The aims of this study were to i) assess and compare the sampling effort needed to estimate species richness across the three methods, ii) Assess diversity indices from the three survey methods across protection levels and seasons to compare their ability to reveal spatial and temporal patterns in fish communities and iii) contrast traditional diversity indices with ecoacoustic indices obtained through acoustic monitoring.

4.3. Material and methods

4.3.1. Study area

The current study was carried out in the MPA Professor Luiz Saldanha Marine Park (PLSMP) (**Figure 4.1**). The park covers 53 km^2 along the western and southern coasts of the Setúbal Peninsula and reaches depths of 100 m (Henriques et al. 2007, Gonçalves et al. 2015). The PLSMP is divided in eight areas with three different levels of protection: one Full Protected Area (FPA), a no take zone; four Partial Protection Areas (PPAs) where non-extractive anthropogenic activities are allowed, specifically recreational activities; and three Buffer Areas (BA) where only artisanal fisheries and recreational fishing with licence and restrictions are allowed.

The PLSMP is a biodiversity hotspot listed in Natura 2000, with over 2000 species identified, including 150 fish species in various habitats such as sandy bottoms, seagrasses, rocky reefs, and kelp forests (Cunha et al. 2014). The FPA, is characterized by small rocky reefs with seasonal occurrences of the algae *Treptacantha usneoides* in spring, surrounded by a large sandy area. The PPA area selected for this study is characterized by rocky reefs with diverse dominant algae species, including *Asparagopsis taxiformis*, *Saccorhiza polyschides*, and *Sphaerococcus coronopifolius*. The BA area selected is characterized by rocky habitats with a large rocky reef and a kelp forest dominated by *Saccorhiza polyschides* (Boaventura & Ré 2001).

The region's biodiversity and dynamics are influenced by its geography and oceanographic conditions, with the Tagus and Sado estuary and submarine canyons system nearby promoting upwelling currents and primary productivity (Peliz et al. 2002).

4.3.2 Monitoring techniques

Data from UVC and BRUVS were collected in two seasons, spring (April-June) and autumn (September-October) in 2019 as part of the INFORBIOMARES project (Serrão et al. 2021). Data from PAM was collected between June 2021 and September 2022 (**Figure 4. 1**).

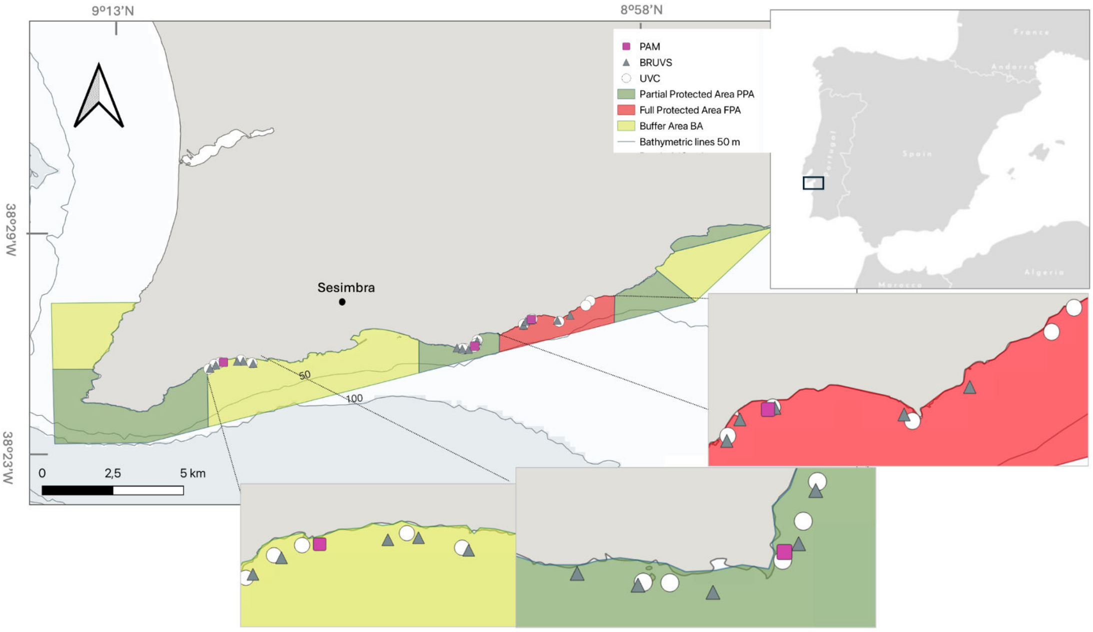


Figure 4.1. Parque Marinho Professor Luiz Saldanha (PMPLS) showing its limits and the different areas with their protection levels. Sampling locations are pinpointed, with Underwater Visual Census (UVC) points as white circles, Baited Remote Underwater Systems (BRUVS) deployment points as dark grey triangles, and Passive Acoustic Monitoring (PAM) hydrophones as purple squares. Bathymetric lines are represented for 50, 100 and 200 m.

a) Underwater Visual Census (UVC)

In 2019, reef fish communities were monitored using UVC in spring (May-June) and autumn (September-October) on the three different protection areas (**Figure 4.1**). The sampling design consisted of 15 sampling points per season, with 5 points allocated to each protection area: Buffer Area (BA), Partial Protected Area (PPA), and Full Protected Areas (FPA) (**Figure 4.1**). Three transects (25 x 4 m) in each of the 5 points were conducted to record demersal/benthic species on the initial pass, followed by three return pass transects (25 x 2m) focusing only on crypto benthic species. The fish survey covered fish on the rocky nearshore reef and in the water column above. Fish species were identified to the lowest taxonomic level possible (family, genus, and species) and counted. Abundances from both passes per transect were standardised by area and pooled together. Each sampling point was surveyed with a time effort of approximately 30 min per site.

b) Baited Remote Underwater Video System (BRUVS)

In 2019, BRUVS were also used to monitor reef fish assemblages at 15 sampling points per season, with 5 points assigned to each protection level: BA, PPA, and FPA. Sampling was carried out in spring (May–June) at all 15 points, and in autumn (October–November) at 10 points across two zones (PPA and FPA, **Figure 4.1**). Due to adverse weather and deployment issues, no autumn samples were collected in the BA. The setup of BRUVS was adapted from Letessier et al. (2013) and consisted of a stainless-steel frame trapeze (designed for stability on rock habitat monitoring), with two GoPro HERO 5 cameras (**Figure 4.S1)**. The two cameras were mounted approximately 80 cm apart with an inward convergence angle of 7º to allow for an optimal field of view, and were encased in SeaGIS waterproof housings (SeaGIS Pty Ltd https://www.seagis.com.au/). Stereo-BRUVs were calibrated using SeaGIS CAL software and a 3D calibration cube, following methods described by Boutros et al. (2015). Video was recorded at 1920x1080 pixels, 60 frames per second, in the "medium" field of view setting. A bait canister was placed 100 cm at the cross-section in front of the cameras. We used around 700 g chopped mackerel (*Scomber* sp.) as bait in every deployment, according to the standard rule-of-thumb of 1 kg of bait per 60 min of deployment (Harvey et al. 2013). BRUVS were deployed during the day, at least one hour after sunrise and recovered no later than one hour before sunset (Axenrot et al. 2004). BRUVS deployments lasted for 30 min, excluding the first 5 min to ensure that cameras reached the bottom and any deployment disturbance was minimised.

Videos were processed and analysed with the EventMeasure software v.5.22 (SeaGIS Pty Ltd). For each video analysed, total recording time and sampling location were documented. Fish species were identified to the lowest taxonomic level possible (with classification at the family level when more specific identification was not possible) and the maximum number of individuals per species per frame (MaxN) was recorded (Harvey et al. 2001).

c) Passive Acoustic Monitoring (PAM)

To capture marine soundscapes, three custom-made autonomous acoustic recorders were used. These acoustic recorders (Audiomoth 1.2.0, Hill et al. 2017) were equipped with custom-made hydrophones (Piezo tubes PTZ-P5 with dimensions of 24 × 20 × 20 mm, with a 50x signal pre-amplification and a measured sensitivity of about −184.5 dB re 1 V/µPa at

1 kHz), with a frequency response similar to commercial hydrophones (Brüel & Kjær 8104). These sensors were positioned in each type of protection area, at the locations: BA 38.4292N, 9.1652W - at a depth of 8.5 m; PPA 38.4357N, 9.0636W - approximately at 6.9 m deep; and FPA 38.4465N, 9.0406W - around 6 m deep (**Figure 4.1**). The sensors record both temporal and spectral features of different sound types that were used from June 2021 to September 2022 (see Ríos et al. 2025 for more details). The continuous acoustic recordings were conducted at a sampling rate of 48 kHz. To manage battery life and storage limitations, the data loggers were retrieved, reprogrammed, and redeployed every 58 days.

Acoustic data were collected over a 15-month period. To align with other monitoring methods, recordings were analysed for fish sounds by subsampling data from two representative months—March (spring) and September (autumn)—each corresponding to one season. Four days in each month were selected based on moon phases (full moon, crescent moon, waning moon, new moon) that may influence acoustic communities (Mann et al. 2009). Within each day, four periods of 30-min (sunrise, solar mid-day, sunset, midnight) were chosen to represent the diel period (according to NOAA, 2023). The daily periods were combined to create a unified sampling dataset. By sampling across different moon phases and times of day, we aimed to maximise the detection of fish sound diversity.

A total of 12 sampling points per season (4 moon days x 3 sites), each of 2 h (i.e. grouping the 4 daily 30 min periods), were used for the analyses.

The recordings were manually analysed by visually and aurally inspecting the spectrograms and oscillograms (DFT size 256, contrast 80, and brightness 55; recordings down-sampled to 8 kHz) using Raven Pro 1.6 (Yang 2024, Bioacoustics Research Program, Cornell Laboratory of Ornithology). The manual inspection of the files enabled the identification and categorization of potential fish sounds, following Rios et al. (2025). These potential fish sounds were identified based on their similarity to previously documented fish calls in terms of frequency, relative duration, and temporal patterning of pulses. Following methods used in previous studies (Parsons et al. 2016, Desiderà et al. 2019, Puebla-Aparicio et al. 2024, Muñoz-Duque et al. 2024), we classified each fish sound type based in a dichotomous key described by Ríos et al. (2025). Diversity indices (see below) were calculated using sound occurrences, with sound types replacing species.

4.3.3. Data analyses

a) Diversity indices

Abundance (total species counts, or sound types) and diversity indices were calculated and compared across sampling methods (UVC, BRUVS, PAM) for each season and protected area. Diversity was assessed using Richness (S), Shannon Index (H'), Simpson's Index (1-D) and Simpson's Eveness (E) (Magurran 2013).

Abundance is the total number of individuals counted in the sample (pi=∑ni/N, pi = proportion de la specie i (ni: number of individuals per specie i divided N total number of individuals). Richness (S) represents the number of different species or sound types in a community.

A high Shannon Index indicates a diverse community where species are present in similar proportions (high evenness), while a low value can either mean low richness or high dominance (ni = number of individuals of species *i,* N= total number of individuals, S = species richness).

$$H = -\sum_{i=1}^{n} \quad p_i p_i$$

The Simpson Diversity Index reflects the probability of two individuals belonging to different species or sound types, while evenness indicates the distribution of calls across species or sound types (where pi is abundance, ni number of individuals of species i, divided N total number of individuals).

$$1 - D = 1 - \sum_{i=1}^{S} \quad p_i^2$$

Evenness was only used for the comparison with acoustic indices where H′=Shannon index, S=species richness).

$$E = H' \, ln \, ln \, (S)$$

b) Ecoacoustic indices

Using the PAM datasets, the following ecoacoustic indices were calculated using R software (R Core Team, 2021): Acoustic Complexity Index (ACI), Acoustic Entropy (H) and Sound Pressure Level (SPL). ACI was derived from the "Seewave" library following the method described by Sueur et al. (2008). This index quantifies the complexity of an acoustic recording by computing the mean absolute amplitude difference between consecutive windows within each frequency bin, summing the results across all bins (Pieretti et al. 2011). ACI values decrease when the acoustic signal is more monotonous (t = amplitude or intensity of the sound signal at frequency bin, f and time step t, F = total number of frequency bins, T = total number of time steps).

$$ACI = \sum f = 1^{F} \frac{\sum_{t=1}^{T-1} \left| I_{f,t+1} - I_{f,t} \right|}{\sum_{t=1}^{T} I_{f,t}}$$

H was calculated based on the Shannon evenness index. This index combines temporal entropy, derived from the acoustic envelope, and spectral entropy, calculated from the frequency spectrum (Sueur 2018). The algorithm provided in the “Seewave” R package (Sueur et al. 2008) was used. Higher values of H indicate a more monotonous signal (Bohnenstiehl et al. 2018).

$$H = Hs \times Ht$$

Hs = spectral entropy (measures the frequency distribution entropy)

$$Hs = -\sum FP(f)\log_2 P(f)$$

Ht = temporal entropy (measures the variability over time)

$$Ht = -\sum P(t)\log_2 P(t)$$

Full bandwidth SPL was determined by adapting the script published by Merchant et al. (2015). SPL is widely employed to assess temporal variations in soundscapes, either across the full frequency spectrum or within specific frequency bands (Pieretti & Danovaro 2020). All indices were computed using a Fast Fourier Transform (FFT) size of 1024, a Hann window function, and no overlap using the full bandwidth on recordings down-sampled to 8000 Hz. The values were averaged per minute (p = root-mean-square sound pressure of the signal in pascals and compared to the reference sound pressure in water - p0 = r 1×10^{-6} Pa).

$$SPL = 20\left(\frac{p}{p_0}\right)$$

c) Statistical analysis

To assess the sampling sufficiency for estimating richness for each method, we constructed cumulative richness curves using the *specaccum* function from the vegan R package (Oksanen et al. 2025). To harmonize sampling effort across methods, a random subset of UVC points was excluded. Outlier fish groups were also removed to enhance data consistency and comparability

Data on species observations or sound detections were compiled into an abundance matrix for each method and 100 permutations with random sampling were used to account for sample order variability. We also estimated the total species richness (species pool) using the *specpool* function from vegan, based on presence–absence data. This function extrapolates the number of species (or sound types) in the community, going beyond the observed richness. We used the Chao estimator, which is the default method in *specpool* and provides a conservative (lower-bound) estimate of richness. We applied the small sample correction, as recommended by Chiu et al. (2014). All samples within each method were pooled to estimate total richness, allowing method comparisons under consistent assumptions, while acknowledging potential differences due to sample type and size.

The statistical analysis was divided into two parts: 1) The comparison of methods across season or protection level for abundance, Richness (S), Shannon (H'), Simpson (1-D), and Evenness (E); 2) The comparison between traditional diversity indices and ecoacoustic indices for acoustic data. All analyses were conducted using R software version 4.4.0 (R Core Team, 2024) with a significance level set at α=0.05.

1) A General Linear Mixed Model (GLMM) was constructed to simultaneously evaluate the effects of method, season, and protection areas, including all two- and three-way interactions, with a nested random structure (season:protection) to account for spatio-temporal dependency. This full model, also fitted using *glmmTMB* (Brooks et al. 2017), allowed for a comprehensive comparison of patterns in ecological indices across all methods under different seasonal and protection contexts. EMMs were extracted with *emmeans* (Lenth et al. 2025) and plotted using *ggplot2*, with one plot per index, showing the interaction between Season and Protection for each Method. All data wrangling was performed with *dplyr* (Wickham et al. 2025), and multiple plots were arranged using the *patchwork* (Pedersen et al. 2025) package.

2) To investigate the relationships between traditional indices and ecoacoustic indices, a correlational analysis was conducted (N=6, average of indices was considered for the 3 protection areas for 2 seasons). The three ecoacoustic indices were compared against abundance and four diversity indices (Richness, Shannon, Simpson and Evenness) calculated from fish sound types, in a pairwise Spearman correlation matrix. Model performance (R, p-values) was summarised, and multicollinearity among indices was assessed using Variance Inflation Factors (VIF) (Miles 2014), with the Shannon-Wiener model used as an example. To evaluate this, Variance Inflation Factors (VIFs) were calculated for all indices. All VIF values were below the commonly used threshold of 5, suggesting that multicollinearity was not a significant issue.

4.4. Results

4.4.1 Sampling methods overview

A total of 25 visual censuses identified 2123 individuals from 43 species and 17 families. The UVC results indicate that Labridae (39%), Sparidae (27%), Gobiidae (12%), Tripterygiidae (9%), Blenniidae (8%), and Serranidae (5%) were the most common observed families. A total of 25 BRUVS deployments identified 632 individuals from 39 species and 18 families. The six more common families in BRUVS were Sparidae (49%), Labridae (27%), Mugilidae (7%), Serranidae (7%), Carangidae (6%), and Gobiidae (4%). Out of the 150 fish species in the PLSPM database (Serrão et al. 2021, link), 28 (18.6%) were identified as soniferous, 71 (47.3%) were considered as potentially soniferous, and 51 (34.0%) as potentially non-soniferous **Figure 4.2**. The analysis of 24 acoustic samples identified 971 potential fish sounds from 25 different sound types in the considered seasons, with the most common sounds been #4, #3, #8, #1, #6, #20, #14 (see Ríos et al. 2025 for a description of the fish sound types). Of the identified species, 31 were recorded by both UVC (72%) and BRUVS (79%), while only four species were detected by all three monitoring methods—acknowledging that only five sound types were associated with their sound sources (Ríos et al. 2025). It should be noted that these sound sources were inferred by comparison with sounds reported in the literature, rather than being directly identified. List of species detected on each method is presented in **Table 4.S1.** Abundance of species/sounds are described in **Figure 4.S2.**

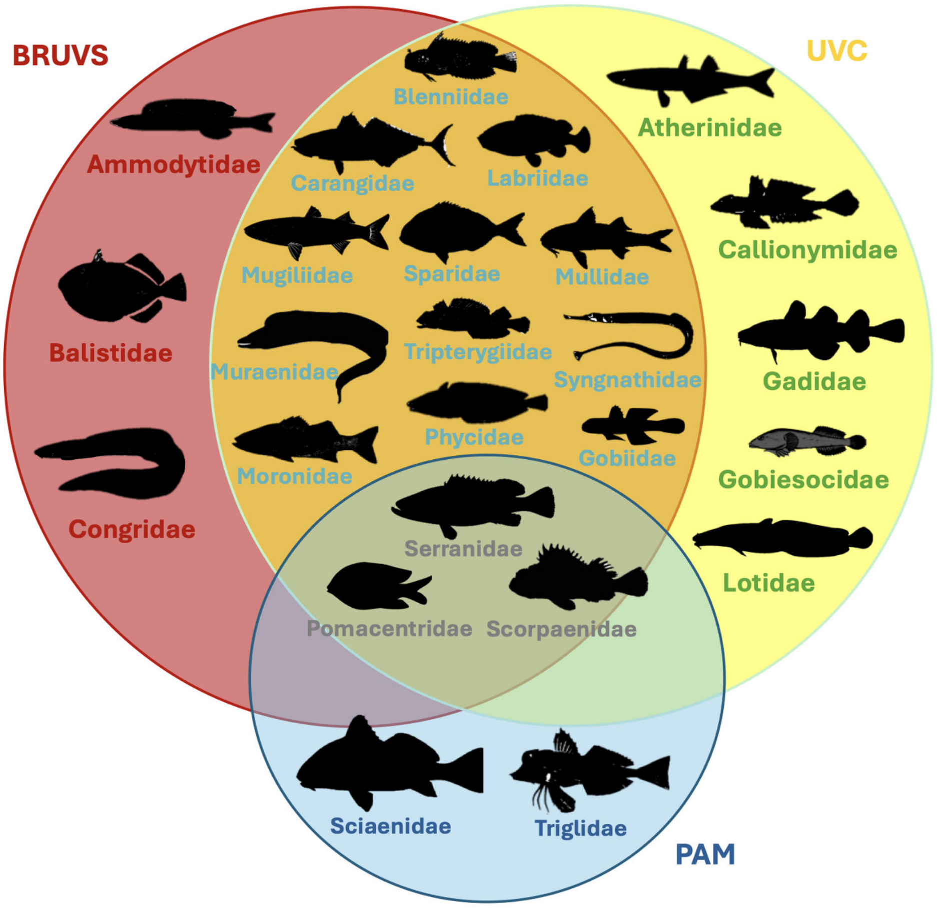


Figure 4.2. Conceptual diagram illustrating the complementarity of BRUVS, UVC, and PAM in the assessment of reef fish assemblages in PLSMP. The red sector (BRUVS) represents families more effectively detected using video-based approaches. The yellow sector (UVC) includes families that are predominantly recorded through diver-based visual censuses. The blue sector (PAM) highlights soniferous families identifiable through passive acoustic recordings identified by bibliographic comparison. Families located in the overlapping areas, orange sector, may be detected by more than one method, reflecting methodological complementarity. In contrast, families outside these intersections are restricted to a single sampling technique, indicating taxon-specific limitations of each method.

4.4.2 Sampling methods comparison

a) Species/sounds accumulation curves

The species accumulation curves (**Figure 4.3**) highlight clear differences in detection capacity among the three survey methods. UVC recorded a higher number of species compared to BRUVS (37 species *vs.* 33 species ~10 samples) with few replicates, suggesting greater detection efficiency for UVC with few samples. At 20 samples, both methods detected a similar number of species, but the UVC curve stabilised around 25 samples, whereas BRUVS curve did not reach a plateau, indicating higher efficiency and stronger detection power for UVC compared to BRUVS. In contrast, PAM showed a substantially lower asymptote, reflecting the detection of fewer sound types than species, with the curve plateauing after approximately 15 samples. This suggests intermediate efficiency in capturing (acoustic) diversity. The wider confidence intervals at low sample sizes across all methods reflect higher variability at early stages of sampling, which narrows as the number of samples increases. Notably, none of the curves fully flattened, suggesting that rare species or sounds remained undetected. It is important to note that methods like *specpool* (Chao variant) estimate minimum richness, not maximum, so actual total richness may be higher

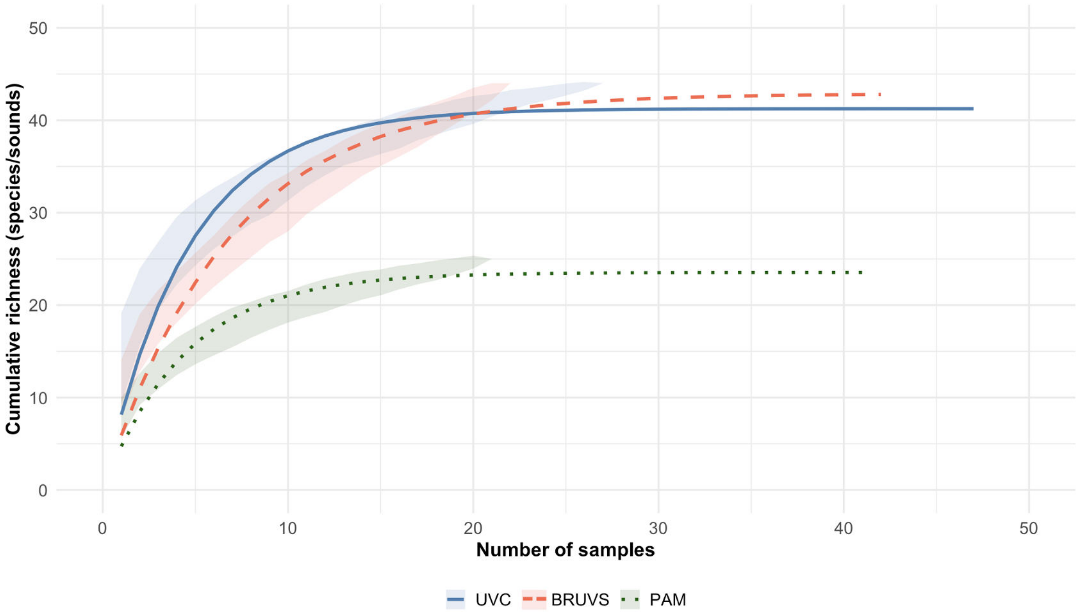


Figure 4.3. Cumulative species/sound richness curves for three different monitoring methods Underwater Visual Census (UVC), Baited Remote Underwater Visual System (BRUVS), Passive Acoustic Monitoring (PAM) across seasons and protection areas. Lines indicate the mean cumulative richness, while shaded areas represent the 95% confidence intervals obtained through resampling.

b) Abundance and diversity indices

Fish assemblages showed distinct spatial and seasonal patterns in abundance, species richness (S), Shannon diversity (H'), and Simpson Index (1-D) and Evenness (E) depending on the survey method (**Table 4.1**). **UVC** recorded the highest abundance in the FPA during spring (155 ± 43.84) and the highest species richness and diversity in autumn (S = 19 ± 0.00; H′ = 12.55 ± 0.89). **BRUVS** also detected increased abundance in the FPA during autumn (75.50 ± 82.73), but with lower diversity (H′ = 6.13 ± 1.03) and Evenness (E = 0.40 ± 0.27), suggesting dominance by a few taxa. Comparing sound type abundance and richness across seasons and protection areas, **PAM** revealed marked variability. The BA showed higher abundances in spring (75.50 ± 66.52), whereas the FPA presented an increase in autumn (75.33 ± 34.39). Richness also peaked in the PPA during autumn (S = 10 ± 2.9). Regarding diversity indices, PAM generally showed low values across sites, with the FPA displaying particularly low diversity in spring (H′ = 3.33 ± 0.58; E = 0.48 ± 0.23). These patterns suggest seasonal and spatial trends that, although expressed in different units, align with those observed by visual methods.

Table 4.1: Mean values and standard deviations (SD) (mean ± standard deviation) of abundance (A), species richness (S), Shannon (H') Simpson's (1-D), and Evenness (E) for method (Underwater Visual Census (UVC), Baited Remote Underwater Visual System (BRUVS), Passive Acoustic Monitoring (PAM)) and protection level (Buffer Area (BA), Partial Protected Area (PPA), Full Protected Area (FPA)) and seasons (spring and autumn). The total number of samples for each method was UVC (n =25), BRUVS (n=25), PAM (n=24).

Method	Location	season	Abun	S	H'	1-D	E
UVC (N:25)	BA(n:8)	spring (n:5)	58,66 ± 38,85	10 ± 1,89	8,01 ± 1,12	0,85 ± 0,02	0,70 ± 0,05
		autumn (n:3)	51 ± 18,86	13,83 ± 2,14	10,15 ± 1,58	0,88 ± 0,02	0,61 ± 0,15
	FPA(n:8)	spring (n:4)	155 ± 43,84	16,5 ± 0,71	10,95 ± 0,21	0,89 ± 0,00	0,55 ± 0,01
		autumn (n:4)	106 ± 26,87	19 ± 0,00	12,55 ± 0,89	0,90 ± 0,01	0,51 ± 0,08
	PPA(n:10)	spring (n:5)	71,43 ± 28,49	12,86 ± 5,81	11,79 ± 2,46	0,80 ± 0,20	0,65 ± 0,11
		autumn (n:5)	110,75 ± 37,51	19,5 ± 3,87	13,05 ± 2,54	0,90 ± 0,03	0,53 ± 0,10
BRUVS	BA (n:5)	spring (n:5)	28 ± 10,39	12 ± 3,16	9,23 ± 2,46	0,85 ± 0,04	0,63 ± 0,17
	FPA (n:10)	spring (n:5)	25,20 ± 18,51	11 ± 4,90	8,68 ± 4,31	0,82 ± 0,11	0,70 ± 0,27
		autumn (n:5)	75,50 ± 82,73	12 ± 4,24	6,13 ± 1,03	0,74 ± 0,09	0,40 ± 0,27
	PPA(n:10)	spring (n:5)	23,66 ± 7,78	10 ± 2,90	6,95 ± 2,09	0,79 ± 0,07	0,51 ± 0,09
		autumn (n:5)	39 ± 9,90	9,5 ± 0,71	5,68 ± 0,57	0,76 ± 0,02	0,44 ± 0,01
PAM	BA (n:8)	spring (n:4)	75,50 ± 66,52	8 ± 1,15	5,43 ± 1,41	0,76 ± 0,07	0,57 ± 0,17
		autumn (n:4)	70,25 ± 28,32	9,25 ± 1,70	6,10 ± 1,61	0,77 ± 0,11	0,56 ± 0,26
	FPA(n:8)	spring (n:4)	30,50 ± 17,99	6 ± 2,45	3,33 ± 0,58	0,58 ± 0,08	0,48 ± 0,23
		autumn (n:4)	75,33 ± 34,39	7 ± 1,73	3,50 ± 0,78	0,58 ± 0,12	0,38 ± 0,14
	PPA(n:8)	spring (n:4)	41 ± 22,62	7,5 ± 3,54	4,95 ± 2,62	0,69 ± 0,18	0,50 ± 0,07
		autumn (n:4)	23,66 ± 7,78	10 ± 2,90	6,96 ± 2,09	0,79 ± 0,07	0,51 ± 0,09

c) General Linear Mixed Model

Figure 4.4 shows results for GLMM estimated marginal means (emmeans) and associated error bars for four ecological indices—Abundance, Richness, Shannon, and Simpson — across three protection levels (BA, FPA, PPA), two seasons (spring and autumn), and three monitoring methods (UVC, BRUVS, PAM).

UVC consistently recorded the highest values across most biodiversity indices. For example, in spring, it estimated the highest **abundance** in the FPA (~5.1), compared to

BRUVS (~3.2) and PAM (~3.4). **Richness** followed a similar trend, with UVC recording values near 2.8–2.9 across protection levels, while BRUVS and PAM ranged from ~1.8 to 2.4. **Shannon diversity (H′)** peaked in autumn at PPA for UVC (~13), whereas PAM reached its lowest value in FPA (~3.5). **Simpson's index (1–D)** was also highest for UVC (ranging ~0.88–0.90), while PAM showed reduced and more variable values, notably dropping to ~0.60 in spring at the FPA. Finally, **Evenness (E)** was more stable in UVC and BRUVS (generally between ~0.55–0.70), whereas PAM revealed pronounced spatial and seasonal variation, with the lowest values occurring in the FPA (~0.38–0.40). In summary, GLMM statistics confirmed that the 3 methods did not show similar trends across sites and seasons. These findings highlight the strong influence of monitoring methods on perceived community structure, with UVC detecting greater diversity and abundance overall, while PAM exhibited more sensitivity to spatial variation.

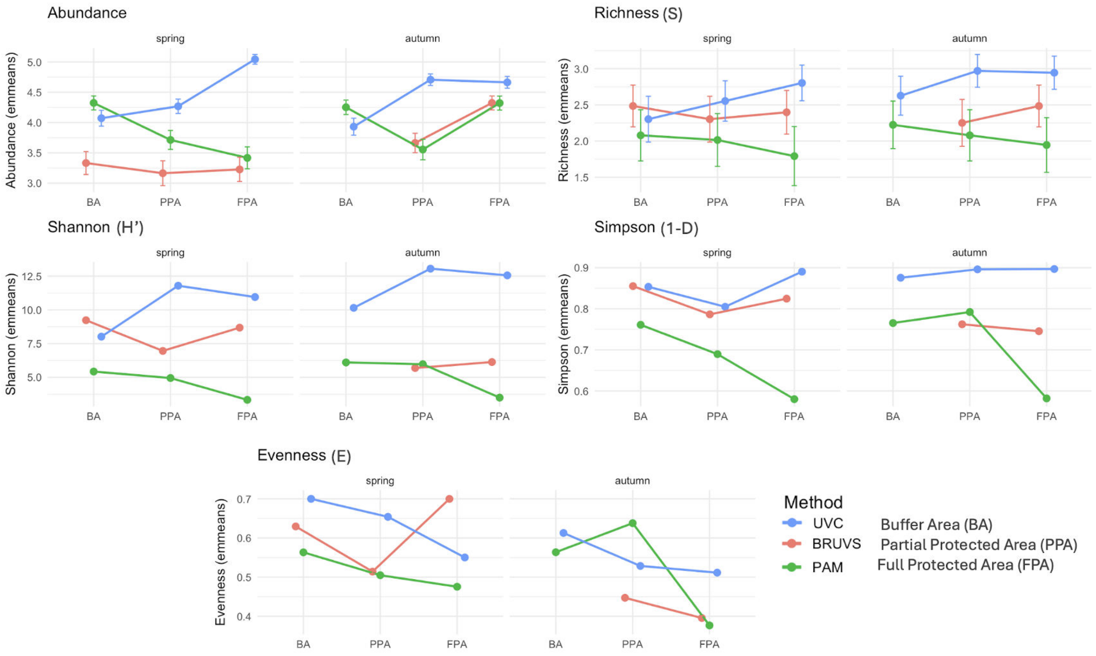


Figure 4.4 GLMM estimated marginal means (emmeans ± SE) for Abundance, Richness, Shannon, Simpson diversity and Evenness indices across three protection levels - Buffer Area (BA), Partial Protected Area (PPA), Full Protected Area (FPA), and two seasons (autumn and spring), as recorded by three monitoring methods - UVC Underwater Visual Census (blue), BRUVS Baited Remote Underwater Visual System (red), PAM Passive Acoustic Monitoring (green).

d) Ecoacoustic versus diversity indices

Pairwise correlations between the ecoacoustic indices (ACI, H and SPL) and diversity indices calculated on sound types were performed with Spearman correlation tests (N=6 average data of 2 seasons x 3 protection areas) **(Figure 4.5)**. ACI and H presented significant correlations with some of the diversity indices. ACI presented positive correlation values for Richness (R=0.99, p<0.0001), Shannon (R=1, p<0.0001), Simpson (R=0.93, p<0.001). The H index presented a negative correlation with Richness (R=-0.93, p<0.001), Shannon (R=-0.94, p<0.01) and Simpson (R=-0.93, p<0.001). ACI also presented a negative correlation with H (R=-0.94, p<0.01). SPL did not present a significant correlation with any of the ecological diversity indices, calculated on sound types.

Across correlations, ACI and H were the most informative indices, particularly for Shannon, Richness, and Simpson indices. Nonetheless, the limited sample size (N=6) constrained statistical power and results should be seen with caution.

The correlation plot revealed strong relationships among the diversity indices, particularly between Shannon and Simpson. Shannon showed strong correlations with Richness (R = 0.99, p=0.0001), reflecting the interdependence between species richness and the distribution of individuals among species. Moreover, Shannon and Simpson indices were positively correlated (R = 0.93, p=0.001), indicating they provide similar information about community diversity. Simpson also presented correlation with Richness (R = 0.88, p=0.01). Likewise, Evenness was positively correlated with Simpson (r = 0.93, p=0.001) suggesting an even distribution of abundance among species.

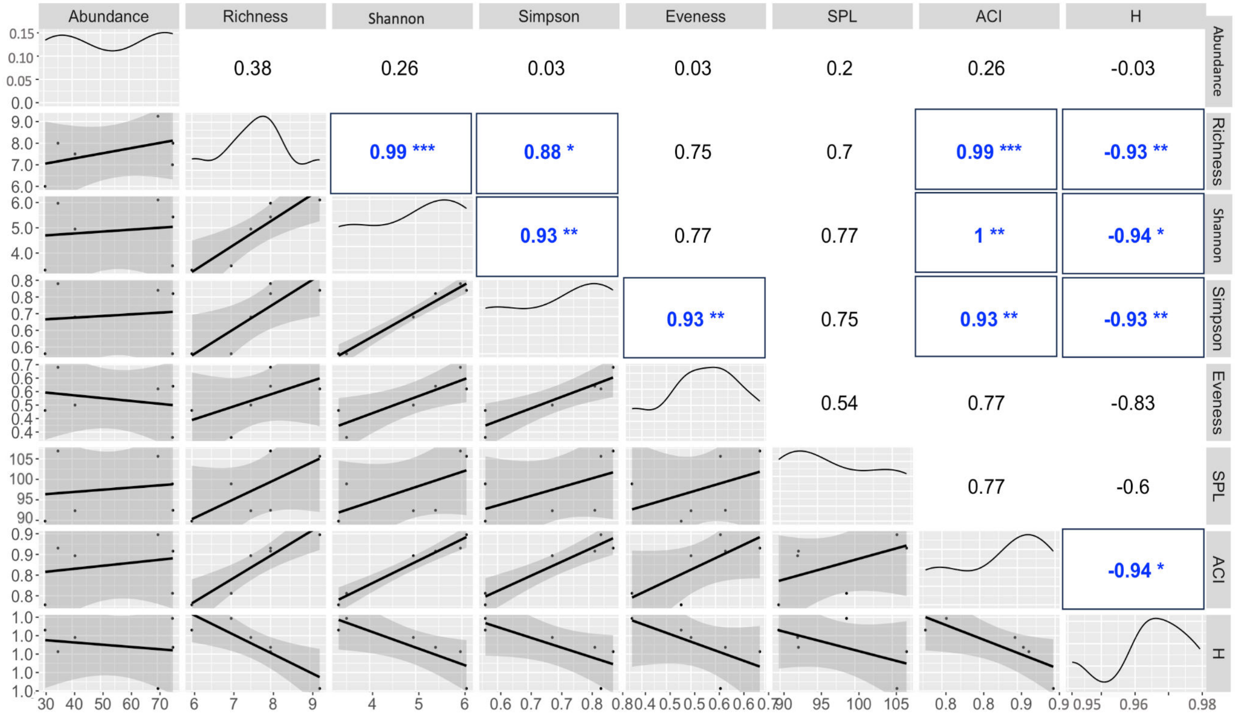


Figure 4.5. Pairwise correlation matrix showed the relationships between acoustic and ecological indices. The **main diagonal** displayed the individual distribution of each variable, as a smoothed density plot. The **lower triangle** showed scatterplots with fitted linear regression lines and confidence intervals, indicating the direction and strength of the relationships. In the **upper triangle** each cell displayed the Spearman correlation coefficient between pairs of variables, with scatterplots and fitted regression lines shown in the lower triangle. Asterisks denote significance levels ($p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***). Correlation values ranged from -1 to 1, where values closer to -1 or 1 indicate stronger negative or positive relationships, respectively.

4.5. Discussion

Effective marine conservation relies on robust monitoring to detect ecological changes and inform management decisions (Mackelworth et al. 2024). This study highlights clear differences in the effectiveness of monitoring methods in capturing fish diversity. While each technique has its own strengths and limitations, our findings suggest that combining multiple methods provides a more comprehensive assessment of fish biodiversity. UVC consistently recorded higher values for abundance, species richness, and other biodiversity indicators compared to BRUVS and PAM. In contrast, PAM relies on acoustic metrics derived from fish vocalisations, which may reflect only a subset of the community (soniferous species) but has the advantage of detecting nocturnal species that are often overlooked by visual methods. These results are particularly relevant given the limited number of comparative studies between acoustic and visual monitoring methods in MPAs.

4.5.1 Species accumulation curves and methodological efficiency

Species accumulation curves revealed contrasting efficiencies among the monitoring techniques, with each method targeting different taxonomic groups and community components (**Table S1**; Ríos et al. 2025). UVC detected a greater number of species with a smaller sampling effort, likely reflecting the direct and active nature of visual surveys, which allow observers to record both mobile and cryptic species in real time (Cappo et al. 2004, MacNeil et al. 2008). Observers can actively search for cryptobenthic species and transects often span heterogeneous areas that include multiple habitat types and depth zones within a single survey (Irigoyen et al. 2013). As a result, **UVC** captured the highest species richness overall, particularly among reef-associated and visually conspicuous taxa such as Blenniidae, Gobiidae, Labridae (*Centrolabrus exoletus*, *Ctenolabrus rupestris*, *Symphodus spp.*), Serranidae and Sparidae. In contrast, BRUVS performed robustly but recorded fewer species than UVC for a comparable effort, likely reflecting its specificities.

Diversity detected by **BRUVS** is influenced by species-specific responses to bait and the limited field of view of the cameras. Nevertheless, BRUVS were more effective in detecting mobile and schooling species, including pelagic carangids (*Trachurus spp.*) and sparids (*Boops boops*, *Diplodus spp.*), Labridae and Mugilidae, highlighting their strength in sampling highly mobile components of the fish community. These factors affect detectability depending on behavioural traits and ecological preferences (Whitmarsh et al. 2016, Langlois et al. 2020). In a Costa Rica study, BRUVS have been shown to detect a wider range of species than UVC; however, the overlap in species detected by both methods was only 66%, highlighting notable differences in the assemblages captured by each technique (Eisele et al. 2020). For example, BRUVS have been effective in assessing fish assemblages across various habitats, such as seagrass meadows, where they detected higher species richness than UVC and were better at capturing mobile predatory species (Zarco-Perello & Enríquez 2019). They were also useful for detecting spatial variation in fish density. For example, in the New Zealand Marine

Reserve, BRUVS produced more consistent estimates of *Pagrus auratus* abundance than UVC (Willis et al. 2000).

PAM yielded more limited species-level identifications, as the majority of acoustic detections could not be directly attributed to specific taxa. Nevertheless, several known soniferous species were identified as the probable sound sources, including *Cynoscion regalis*, *Scorpaena* spp., *Serranus* spp., *Epinephelus marginatus*, *Chromis chromis*, and members of the Triglidae family, demonstrating its ability to detect sound-producing fishes that are often underrepresented in visual surveys (**Table S1**). Assuming one sound type per species, PAM was at least as effective as visual methods in detecting species richness. In this study, 43 fish species were detected using UVC and 39 using BRUVS out of a total of 150 species (Serrão et al. 2021), representing a detection rate of 28.7% and 26% of the total species richness, respectively. In turn, PAM detected 25 distinct sound types, corresponding to 25 of the 29 confirmed soniferous species corresponding to a detection rate of 96.2%. When considering also the potentially vocal species (99 species) the detection rate would still be 25.3%, which is comparable to the rate obtained by the visual methods (Ríos et al. 2025). Despite likely overestimating species richness, the “one sound type per species” assumption, commonly used in marine PAM studies (e.g., Bertucci et al. 2016, Desiderà et al. 2019, Boyle et al. 2022, Hawkins et al. 2025), facilitates comparisons across sites and methods. Indeed, the number of distinct sound types has been shown to reflect species richness (Desiderà et al., 2019) and to serve as a useful proxy for acoustic diversity (Staaterman et al. 2017, Boyle et al. 2022). Moreover, PAM has been validated by comparing detected sound types with known soniferous species from visual observations or capture records (Amorim et al. 2023). This underscores the value of PAM for monitoring fish diversity, providing complementary insights to visual surveys and effectively detecting cryptic or nocturnal species often missed by traditional methods (Staaterman et al. 2017, Bolgan et al. 2023). For instance, Bolgan et al. (2023) showed that PAM revealed the widespread presence of *Ophidion rochei* across Mediterranean habitats and depths more effectively than visual or capture-based methods.

Nevertheless, other studies have demonstrated greater congruence among monitoring approaches than observed here. Desiderà et al. (2019), for example, found that acoustic and visual methods revealed parallel daily temporal dynamics in Mediterranean fish assemblages while Carriço and colleagues 2020 reported that annual sound diversity patterns derived from

PAM at the Azorean seamount were broadly consistent with fish community structures revealed by conventional surveys. Buscaino et al. (2023) showed that combining video and acoustic data improves fish biodiversity assessment, as each method captures different aspects of the community despite weak correlations, with both revealing a declining abundance trend over time.

4.5.2 Spatial and seasonal variation between methods

Our comparative analysis across UVC, BRUVS and PAM underscores that the choice of monitoring method profoundly influences observed community patterns. Seasonal and protection-related patterns in fish assemblages varied considerably across survey methods, revealing how biodiversity information depends on the chosen monitoring approach.

UVC proved particularly sensitive to protection areas, consistently distinguishing the FPA from other zones by recording the highest abundances, richness, and diversity during spring and autumn, likely due to its ability to detect resident and site-attached species benefiting from long-term protection. Variation in protection areas encompassed different habitat types, which may have influenced fish assemblage composition, highlighting the importance of considering habitat and protection area when interpreting UVC results (Pais et al. 2014). These findings are consistent with Prato et al. (2017), who reported that expanded transect designs in Mediterranean MPAs better captured species richness, particularly among large, shy predators, and with Holmes et al. (2013), who observed that UVC recorded significantly higher species richness than stereo-video methods, especially in complex tropical habitats.

BRUVS showed less pronounced spatial variation but captured notable seasonal differences, likely reflecting the mobility and seasonal foraging of bait-attracted species (Scott et al. 2017). Higher abundance of certain taxa in autumn suggests BRUVS is particularly responsive to seasonal predator activity, while diversity was generally higher in spring, with several sites recording greater species richness and evenness than in autumn. This may explain the lower evenness and diversity observed in our study, especially in the FPA. Seasonal trends in habitat use have also been reported in coastal BRUVS surveys (Hall et al. 2021). Although effective for mobile, bait-attracted species, BRUVS tended to underrepresent overall diversity, showing bias towards top predators and planktivorous, even when capturing higher species richness than UVC in Mediterranean MPAs (La Manna et al. 2021).

PAM produced the lowest diversity and evenness indices but revealed notable spatial and seasonal variation. In our study, the acoustic technique did not show significant differences between spring and autumn, contrasting with previous findings suggesting that PAM can be sensitive to temporal variation in vocal activity—for example, in seamount fish communities in the NE Atlantic (Carriço et al. 2020), artificial reefs in the Gulf of Mexico (Boyle et al., 2022), and Mediterranean coralligenous reefs (la Manna et al. 2024)—as well as general reviews highlighting the temporal dynamics of fish choruses (Siddagangaiah et al. 2022, Hawkins et al. 2025). Acoustic monitoring recorded higher abundance, richness, and diversity in the BA and PPA than in the FPA, likely reflecting habitat differences. BA and PPA feature complex rocky structures with diverse algal cover, whereas the FPA is dominated by a rock–sand interface, which may limit acoustic diversity. Soniferous fish are often associated with structurally complex habitats that provide shelter or nesting sites, important for territorial defence and reproduction, where acoustic communication typically occurs (Ladich 2004, Amorim 2023).

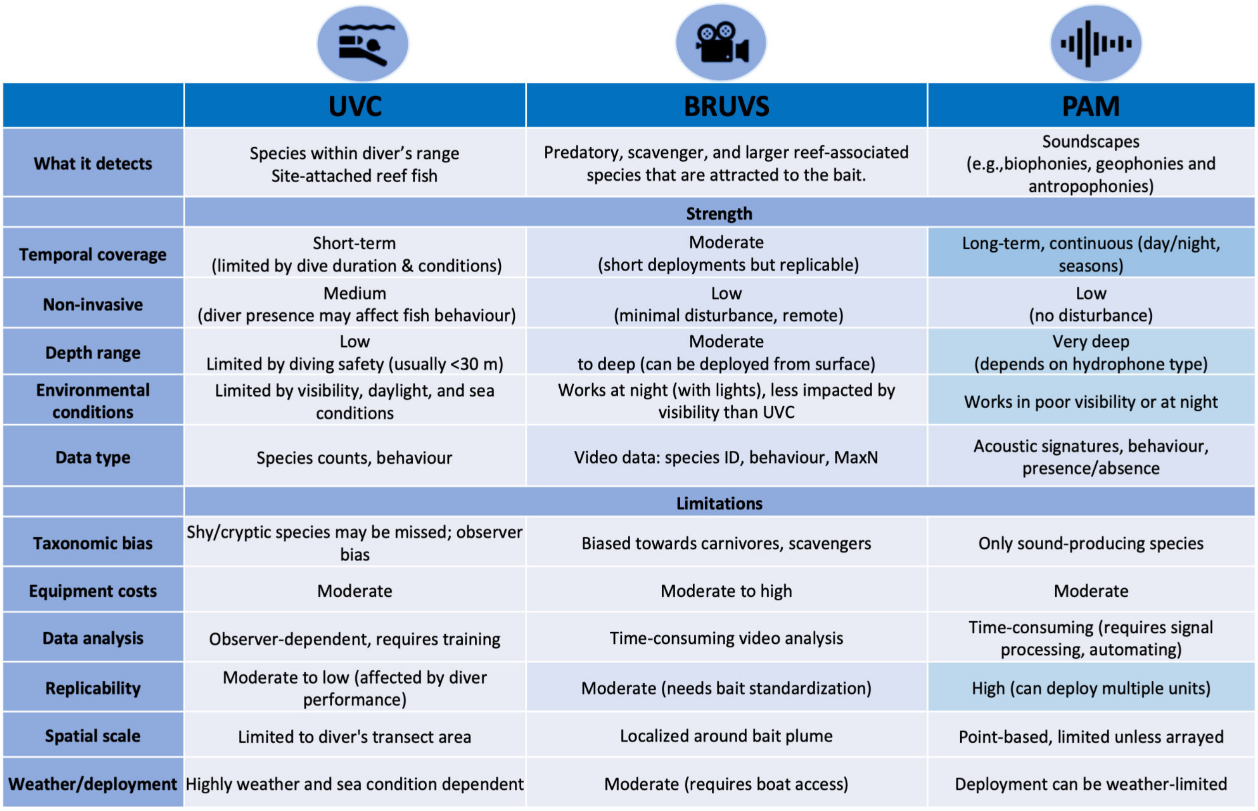
Many fish only produce sounds at specific life stages, often after reaching maturity, which can limit detectability in nursery habitats like the FPA (Amorim & Hawkins 2005, Kasumyan 2008, Vasconcelos & Ladich 2008). A study highlights PAM's strength for tracking temporal dynamics of soniferous fish, though community-level diversity estimates are influenced by vocal behaviour (Hawkins et al. 2025) and overall acoustic activity rather than true species richness (McGeady et al. 2023). Hydrophone number, orientation, and directivity critically affect spatial coverage and data reliability, with inadequate placement or sensor artefacts potentially biasing results in heterogeneous habitats (Tyack et al. 2021, Azofeifa-Solano et al. 2025).

Temporal mismatches between methods, including a two-year gap and differences in diel sampling, need also to be considered when interpreting our results on community patterns. While such differences warrant caution, comparing methods remains valuable, as each provides complementary insights into fish assemblages and contributes to a more comprehensive understanding of biodiversity.

4.5.3 Biases and complementarity

Beyond their effectiveness in assessing biodiversity, each method has strengths and limitations, which are summarised in **Table 4.2**. For example, PAM, although limited to soniferous species, offers key advantages such as affordability, the capacity for extended, continuous monitoring largely independent of weather conditions, and access to remote areas that are difficult to survey with other methods (Carriço et al. 2020). In contrast, active methods tend to detect more mobile, reef-associated species, often yielding higher estimates than passive or indirect approaches (Murphy & Jenkins 2010). Active visual surveys, such as UVC, are particularly effective in shallow reef systems where visibility and species behaviour favour detection (Kulbicki 1998, MacNeil et al. 2008, Colton & Swearer 2010). However, UVC often reports higher species richness and biomass than remote methods, reflecting potential observational biases linked to diver presence and survey methodology (Edgar et al. 2014). UVC may also overestimate fish abundance, particularly for large, conspicuous, or highly mobile species, as some species are attracted to divers while others become more elusive (Ward-Paige et al. 2010, Pais & Cabral 2017, Kulbicki 1998, Pais & Cabral 2018). The ability of reef communities to acclimatise to human presence can further influence these observations depending on the history of diver activity (Titus et al. 2015).

Acoustic methods require careful consideration, as a single species may produce multiple sound types depending on behaviour, as in *Halobatrachus didactylus*, which can increase sound diversity without reflecting species diversity (Amorim et al. 2008) or show sex-biased variation (Kéver et al. 2012). Conversely, multiple species may produce similar or identical sounds, complicating species-level identification (Raick et al. 2022). Even unique sounds can be difficult to distinguish in PAM due to overlapping temporal and spectral features. Species such as gobiids can be detected in laboratory settings (Amorim 2007), but field recordings are limited by signal attenuation and environmental noise, as their sounds are low-intensity and short-range (Amorim et al. 2018). This study highlights that UVC, BRUVS, and PAM reveal contrasting patterns in richness, abundance, and diversity, reflecting each method's suitability for detecting different species groups. Integrating visual and acoustic approaches is therefore essential to capture the full complexity of fish communities.



	UVC	BRUVS	PAM
What it detects	Species within diver's range Site-attached reef fish	Predatory, scavenger, and larger reef-associated species that are attracted to the bait.	Soundscapes (e.g.,biophonies, geophonies and antropophonies)
	Strength		
Temporal coverage	Short-term (limited by dive duration & conditions)	Moderate (short deployments but replicable)	Long-term, continuous (day/night, seasons)
Non-invasive	Medium (diver presence may affect fish behaviour)	Low (minimal disturbance, remote)	Low (no disturbance)
Depth range	Low Limited by diving safety (usually <30 m)	Moderate to deep (can be deployed from surface)	Very deep (depends on hydrophone type)
Environmental conditions	Limited by visibility, daylight, and sea conditions	Works at night (with lights), less impacted by visibility than UVC	Works in poor visibility or at night
Data type	Species counts, behaviour	Video data: species ID, behaviour, MaxN	Acoustic signatures, behaviour, presence/absence
	Limitations		
Taxonomic bias	Shy/cryptic species may be missed; observer bias	Biased towards carnivores, scavengers	Only sound-producing species
Equipment costs	Moderate	Moderate to high	Moderate
Data analysis	Observer-dependent, requires training	Time-consuming video analysis	Time-consuming (requires signal processing, automating)
Replicability	Moderate to low (affected by diver performance)	Moderate (needs bait standardization)	High (can deploy multiple units)
Spatial scale	Limited to diver's transect area	Localized around bait plume	Point-based, limited unless arrayed
Weather/deployment	Highly weather and sea condition dependent	Moderate (requires boat access)	Deployment can be weather-limited

Table 4.2. Strengths and limitations of three monitoring methods used in marine biodiversity assessments: UVC (Harvey et al. 2004, Bernard et al. 2013, Pais & Cabral 2018), BRUVS (Harvey et al. 2013 Langlois et al. 2020), and PAM (Browning et al. 2017). Each technique offers distinct strengths and limitations depending on research objectives, target taxa, and environmental constraints. To facilitate comparison, the table employs a color-coded scale: light blue identifies a method's constraints for a given criterion, whereas dark blue highlights its superior capabilities or advantages.

Several studies have combined visual census methods with complementary monitoring tools to improve assessments of fish assemblages. For example, UVC has been paired with BRUVs to compare species composition and detectability, with Langlois et al. (2010) and Watson et al. (2005) showing that BRUVs often capture more predatory species. UVC has also been integrated with acoustic techniques; Boswell et al. (2010) combined hydroacoustic and visual transects in reef environments to estimate biomass and validate species composition. Non-visual methods such as eDNA have detected 66% more molecular operational taxonomic units than species observed on video (Marques et al. 2021) and combining UVC with eDNA doubled the number of species identified compared to either method alone (Valdivia-Carrillo et al. 2021). An Australian study combining BRUV, DOV, URUV, UVC, and trawl sampling found that trawls best detected canopy-resident species, while stereo video captured larger, supra-canopy species, emphasising the value of multiple methods for comprehensive seagrass fish assessments (French et al. 2021). Together, these examples highlight the importance of using multiple methods for improved biodiversity assessment.

4.5.4 Comparing diversity indices and ecoacoustic indices

Acoustic indices such as the ACI, H, and SPL are widely used in marine environments to assess soundscape dynamics and infer ecosystem health, with each index capturing different aspects of acoustic diversity. The ACI showed strong positive correlations with traditional diversity indices of sound types in this study, including the Shannon index. These results support previous findings indicating that ACI is sensitive to both sound diversity and abundance (Bolgan et al. 2018, Harris et al. 2016). However, ACI's interpretability can be challenging, especially in environments dominated by single sound-producing species like snapping shrimp, where ACI increases non-linearly with activity (Bohnenstiehl et al. 2018). In this study, ACI also showed a strong negative correlation with H, reinforcing the notion that the two indices capture complementary but still equivalent aspects of the acoustic landscape. The H, which integrates information on sound evenness and richness, exhibited consistent but generally negative correlations with biodiversity indices, including Shannon. This suggests that H may decrease when soundscapes are dominated by similar sounds, potentially reflecting reduced acoustic evenness due to high activity of specific species or anthropogenic noise. Previous studies have similarly found that H responds differently depending on dominant sound types, decreasing with increasing harmonic fish calls and shrimp snaps (Harris et al. 2016, Bohnenstiehl et al. 2018). The SPL does not present correlation with any traditional index. Nonetheless, SPL remains a valuable ecoacoustic metric due to its ease of automation and scalability for long-term monitoring (Halliday et al. 2020). It primarily reflects the intensity of acoustic activity rather than diversity, but its seasonal variation can align with abiotic or anthropogenic activity patterns, such as increased wind or vessel traffic in summer months (Vieira et al. 2021).

Overall, the correlation analysis underscores that while ACI aligns closely with conventional diversity metrics, it may overrepresent dominant sources. H, in contrast, captures evenness but usually declines with increased presence of biological sounds. Although they showed a strong negative correlation, given their different sensitivities and scopes (Bohnenstiehl et al. 2018), these indices should ideally be used in combination. Their integration, possibly alongside machine learning approaches (Buxton et al. 2018, Williams et al. 2022), can enhance the accuracy and ecological relevance of passive acoustic monitoring in marine systems.

The results revealed strong relationships among the diversity indices, particularly between Shannon and Simpson, indicating that both capture similar aspects of community structure. The close association between Shannon and Richness reflects the interdependence between the number of species present and the distribution of individuals among them. Likewise, the positive correlation between Evenness and Simpson suggests that communities with more equitable distributions of abundance are characterised by higher diversity. These patterns are consistent with previous studies showing that different diversity indices often convey overlapping but complementary information about ecosystem structure (Clarke & Warwick 2001, Duffy et al. 2003).

4.6. Conclusions

This study underscores the critical role of method selection in marine biodiversity monitoring as the observed spatial and temporal patterns depended on the methodological approach. In addition, the different survey methods detected different species with some degree of overlap. UVC detected a higher number of species with low effort, mostly site attached mobile species, while BRUVS and PAM offered valuable insights to detecting other species, namely bait attracted and soniferous species. Acoustic methods complemented visual techniques, with potential to improve as more sound sources are identified. Furthermore, these findings suggest that combining ecoacoustic indices with traditional diversity metrics can enhance our understanding of marine environments, offering a more nuanced view of community structure than either approach alone. As the calculation of ecoacoustic indices is a relatively rapid method it offers a scalable continuous tool to complement traditional visual methods.

Ultimately, integrating acoustic and visual techniques supports more adaptive, evidence-based management of MPAs. The strength of PAM and ecoacoustic indices offer the advantage to rapidly generate standardized, quantifiable data that can be easily automated, making them particularly well-suited for long-term and large-scale monitoring efforts. This study is an important step in understanding the complementarities between PAM and visual methods in marine environments. More comparisons of PAM and visual methods are needed to better understand their complementarity on fish ecology.

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4.8 Supplementary material

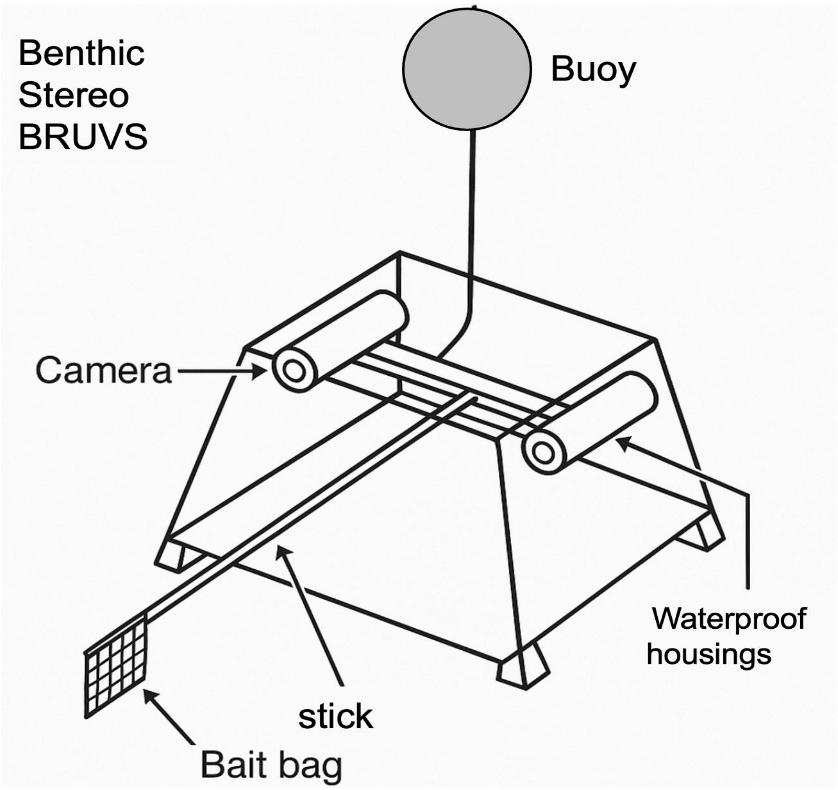


Figure 4.S1. Schematic representation of the Benthic Stereo BRUVS components, including buoy, waterproof housings, and structural stick, used for underwater video surveys in marine ecological studies.

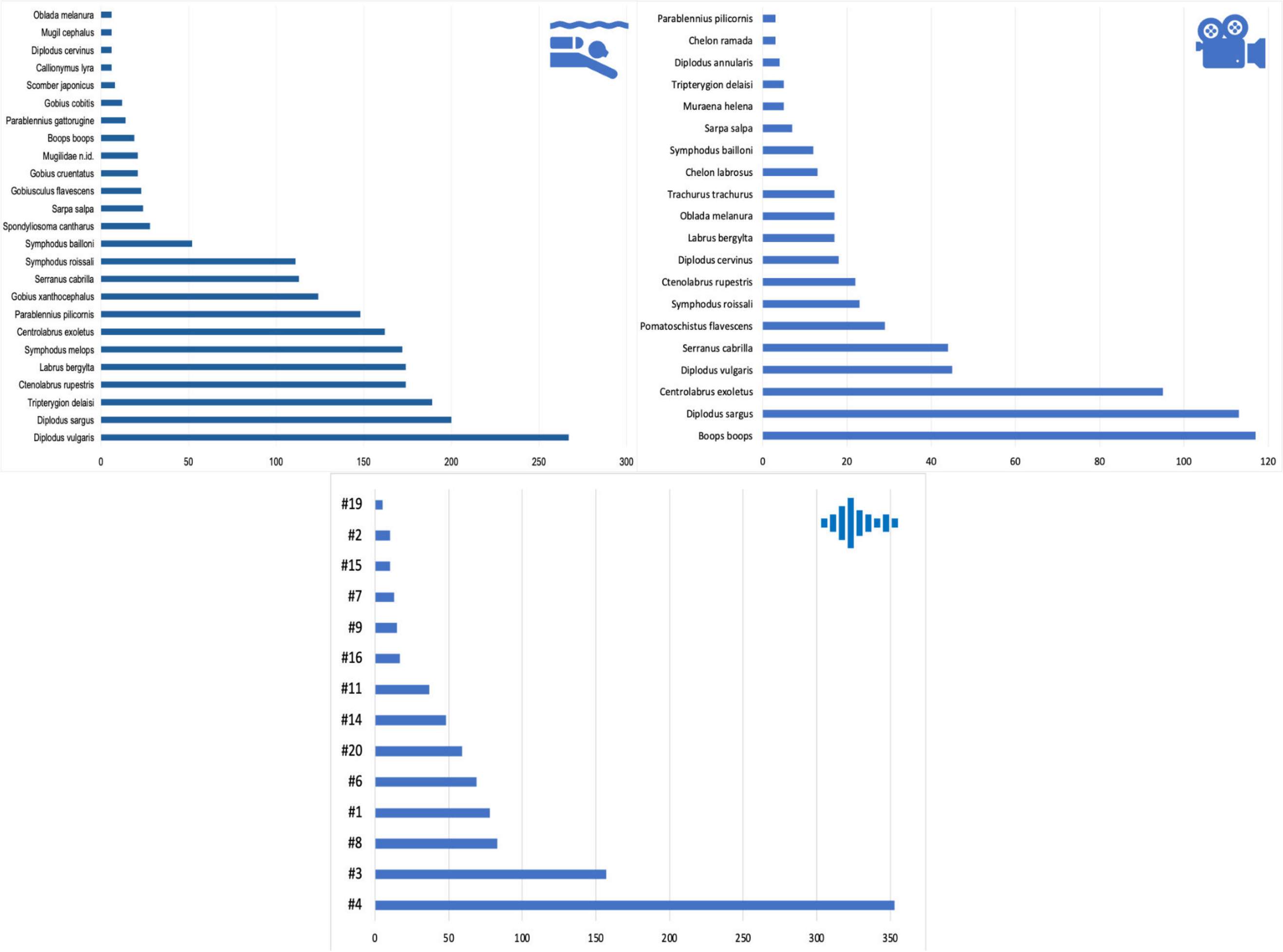


Figure 4.S2. This figure compares fish species observations/sound types recorded using three survey methods: underwater UVC (left), BRUVS (right), and PAM (down). Icons indicate the respective survey techniques. This comparison highlights methodological variations in biodiversity assessments.

Table 4.S1. Ichthyofauna found in the Marine Park Professor Luiz Saldanha. Detection in any of the three monitoring methods are shown. Soniferous status is also indicated as follows: soniferous (S), potentially soniferous (PS), or potentially non-soniferous (PNS) (Ríos et al. 2025). For PAM, most sound types detected were unidentified, only some potential sources are presented. Conservation status (CS), environment and depth range are also presented.

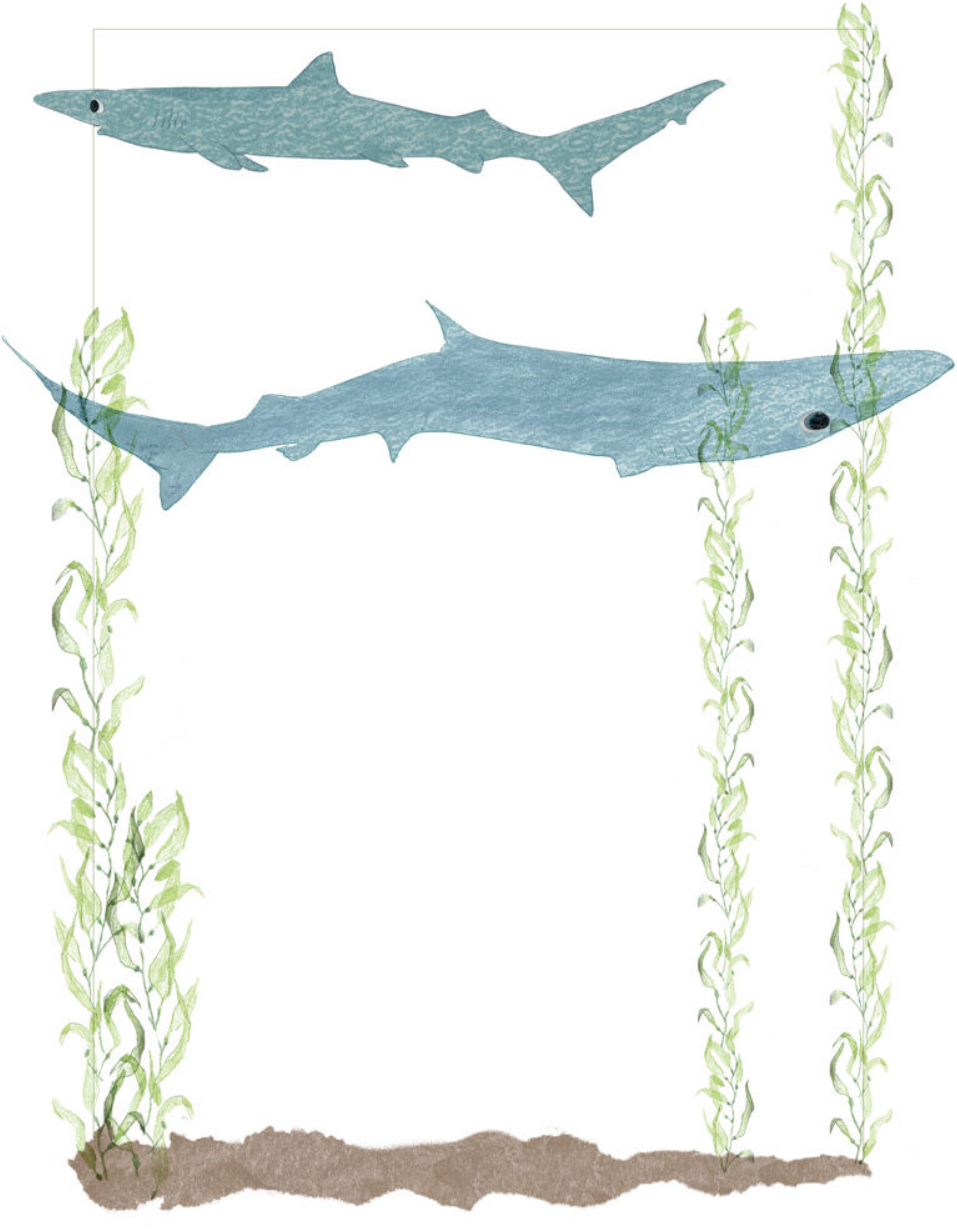
Species	Family	Cryptic	Status	Environment ^{b}	Depth range^b	Soniferous	UVC	BRUVS	PAM
Hyperoplus lanceolatus	Ammodytidae	Pelagic	LC	Demersal	5-30	PNS		1	
Atherina presbyter	Atherinidae	Pelagic	LC	Pelagic	1-64	PNS	4		
Balistes capriscus	Balistidae	Pelagic	DD	Demersal	0-100	**S**		1	
Parablennius gattorugine	Blenniidae	Cryptic	LC	Demersal	3-32	PNS	27	1	
Parablennius incognitus	Blenniidae	Cryptic	LC	Demersal	0-2	**PS**		1	
Parablennius pilicornis	Blenniidae	Cryptic	LC	Demersal	0-25	**PS**	245	3	
Parablennius sanguinolentus	Blenniidae	Cryptic	LC	Demersal	0-1	**PS**		1	
Callionymus lyra	Callionymidae	Cryptic	LC	Demersal	1-440	PNS	12		
Seriola dumerili	Carangidae	Pelagic	LC	Reef associated	1-385	**S**		1	
Trachurus mediterraneus	Carangidae	Pelagic	LC	Pelagic	0-500	**PS**		8	
Trachurus picturatus	Carangidae	Pelagic	LC	Benthopelagic	305-370	**PS**		6	
Trachurus trachurus	Carangidae	Pelagic	LC	Pelagic	0-1050	**PS**	3	23	
Conger conger	Congridae	Cryptic	LC	Demersal	0-1171	PNS		1	
Trisopterus luscus	Gadidae	Benthic	NE	Benthopelagic	30-100	**PS**	6		
Diplecogaster bimaculata	Gobiesocidae	Cryptic	LC	Benthopelagic	0-20	PNS	1		
Lepadogaster candolii	Gobiesocidae	Cryptic	NE	Demersal	3-6	PNS	6		
Lepadogaster lepadogaster	Gobiesocidae	Cryptic	LC	Demersal	3-6	PNS	1		
Gobius bucchichi	Gobiidae	Cryptic	LC	Demersal	1-3	**PS**	19		
Gobius cobitis	Gobiidae	Cryptic	NE	Demersal	8-35	**S**	2		
Gobius cruentatus	Gobiidae	Cryptic	LC	Demersal	15-40	**S**	53		
Gobius niger	Gobiidae	Cryptic	LC	Demersal	1-50	**S**	1		
Gobius paganellus	Gobiidae	Cryptic	LC	Demersal	0-15	**S**	28		
Gobius xanthocephalus	Gobiidae	Cryptic	LC	Demersal	3-36	**PS**	394		
Pomatoschistus flavescens	Gobiidae	Cryptic	LC	Reef associated	0-/	**PS**	11	29	
Pomatoschistus pictus	Gobiidae	Cryptic	LC	Demersal	1-55	**S**	113		
Centrolabrus exoletus	Labridae	Benthic	LC	Demersal	0-/	**PS**	778	4	
Ctenolabrus rupestris	Labridae	Benthic	LC	Reef associated	0-50	**PS**	475	22	
Labrus bergylta	Labridae	Benthic	LC	Reef associated	1-50	**PS**	269	17	
Labrus mixtus	Labridae	Benthic	LC	Reef associated	2-200	**PS**	6	1	
Symphodus bailloni	Labridae	Benthic	LC	Reef associated	1-50	**PS**	110	12	
Symphodus cinereus	Labridae	Benthic	LC	Demersal	1-/	**PS**		4	
Symphodus mediterraneus	Labridae	Benthic	LC	Demersal	1-50	**PS**		4	
Symphodus melops	Labridae	Benthic	LC	Reef associated	1-30	**S**	269	1	
Symphodus ocellatus	Labridae	Benthic	LC	Reef associated	1-30	**PS**		1	
Symphodus roissali	Labridae	Benthic	LC	Reef associated	1-30	**PS**	215	23	

Gaidropsarus vulgaris	Lotidae	Benthic	LC	Demersal	20-120	**PS**	1		
Dicentrarchus labrax	Moronidae	Pelagic	LC	Demersal	10-100	PNS	9	1	
Chelon auratus	Mugilidae	Pelagic	LC	Neritic	2-20	PNS	8		
Chelon labrosus	Mugilidae	Pelagic	LC	Demersal	0-120	PNS	13	13	
Chelon ramada	Mugilidae	Pelagic	LC	Neritic	2-20	PNS	8	3	
Mugil cephalus	Mugilidae	Pelagic	LC	Benthopelagic	0-120	PNS	30	31	
Mullus surmuletus	Mullidae	Benthic	LC	Demersal	5-409	**PS**	3	2	
Muraena helena	Muraenidae	Cryptic	LC	Reef associated	1-801	PNS	1	5	
Phycis phycis	Phycidae	Benthic	LC	Benthopelagic	13-614	**PS**	3	1	
Chromis chromis	Pomacentridae	Pelagic	LC	Reef associated	2-40	**S**	3	2	#42 (Lobel and Mann 1995, Amorim 2006, Picciulin et al., 2018)
*Cynoscion regalis**	Sciaenidae	Benthic	NE	Demersal	8-26	**S**			#15 (Connaughton et al., 2002, Amorim et al., 2023)
Scomber japonicus	Scombridae	Pelagic	LC	Neritic	0-300	PNS	9		
Scorpaena notata	Scorpaenidae	Cryptic	LC	Demersal	10-700	**PS**	5		#43 (Bolgan et al., 2018)
Scorpaena porcus	Scorpaenidae	Cryptic	LC	Demersal	/-800	**S**	5	1	#43 (Bolgan et al., 2018)
Scorpaena scrofa	Scorpaenidae	Cryptic	LC	Demersal	20-500	**PS**	1		#43 (Bolgan et al., 2018)
Epinephelus marginatus	Serranidae	Benthic	EN	Reef associated	8-300	**S**			#4,#8,#33 (Bertucci et al., 2015, Wilson et al., 2020, Viera et al., 2024)
Serranus atricauda	Serranidae	Benthic	DD	Demersal	3-150	**PS**		1	#4,#8 (Viera et al., 2024)
Serranus cabrilla	Serranidae	Benthic	LC	Demersal	5-500	**PS**	199	44	#4,#8 (Viera et al., 2024)
Serranus hepatus	Serranidae	Benthic	LC	Demersal	5-100	**PS**	6		#4,#8 (Viera et al., 2024)
Boops boops	Sparidae	Pelagic	LC	Demersal	0-350	**PS**	89	117	
Diplodus annularis	Sparidae	Benthic	LC	Benthopelagic	0-90	**PS**	1	4	
Diplodus cervinus	Sparidae	Benthic	LC	reef associated	30-300	**PS**	15	18	
Diplodus sargus	Sparidae	Benthic	LC	Demersal	0-50	**PS**	349	113	
Diplodus vulgaris	Sparidae	Benthic	LC	Benthopelagic	0-160	**PS**	478	45	
Oblada melanura	Sparidae	Benthic	LC	Benthopelagic	/-30	**PS**	39	17	
Pagrus auriga	Sparidae	Benthic	LC	Benthopelagic	/-170	**PS**		1	
Sarpa salpa	Sparidae	Benthic	LC	Benthopelagic	5-70	**PS**	617	7	
Sparus aurata	Sparidae	Benthic	LC	Demersal	1-150	**PS**		1	
Spondyliosoma cantharus	Sparidae	Benthic	LC	Benthopelagic	5-300	**PS**	53	2	
Syngnathus acus	Syngnathidae	Cryptic	LC	Demersal	0-110	**PS**	4	1	
Chelidonichthys cuculus	Triglidae	Benthic	LC	Demersal	15-400	**S**			#10 (Amorim et al., 2004)
Chelidonichthys lastoviza	Triglidae	Benthic	LC	Demersal	10-140	**S**			#10 (Amorim et al., 2004)
Chelidonichthys lucerna	Triglidae	Benthic	LC	Benthic	20-318	**S**			#10 (Amorim et al., 2004)
Chelidonichthys obscurus	Triglidae	Benthic	LC	Demersal	20-170	**PS**			#10 (Amorim et al., 2004)

Lepidotrigla cavillone	Triglidae	Benthic	NE	Demersal	30-450	**PS**			#10 (Amorim et al., 2004)
Tripterygion delaisi	Tripterygiidae	Benthic	LC	Demersal	0-40	PNS	32	5	

CHAPTER V

Characterising the behaviour of bait-attracted blue shark *(Prionace glauca)* using pelagic drift video



Chapter V: *Characterising the behaviour of bait-attracted blue shark (Prionace glauca) using pelagic drift video*

Noelia Ríos, María Jimenez, Gustavo Franco, Gonçalo Ramos, Miguel Pessanha Pais, Emanuel J. Gonçalves, Maria Clara P. Amorim, Gonçalo Silva

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5.1 Abstract

Baited pelagic underwater videos are increasingly being used to assess ecological indices but they can also be effective in recording the behaviour of pelagic species attracted to the bait. In this study, the behaviour of 79 blue sharks *Prionace glauca* was recorded using drifting pelagic baited remote underwater video system (BRUVS) rigs, deployed outside the Professor Luiz Saldanha Marine Park, Portugal. Juveniles were more frequently sighted over epipelagic (depth between 60 and 200 m) and mesopelagic zones (200–1000 m), while adult sightings were more common further offshore, particularly over canyons (1200–2000 m). Importantly, juvenile sightings were more frequent in spring (breeding season), suggesting that the study area is likely an important nursery habitat. Blue sharks primarily exhibited inspection activities around the BRUVS. Generalised linear models indicated that visibility, distance to the shore, bathymetry and temperature influenced their behavioural patterns. Moreover, juveniles interacted with the BRUVS for a longer time (mean duration: 0.4 min) than adults (0.2 min). A preliminary analysis of blue sharks' reaction to boat presence suggests that boat noise decreased both the duration of interaction with the BRUVS and the range of observed behaviours. This study provides valuable insights into the behaviour of this species in its natural environment, which is relevant for management and conservation efforts.

5.2 Introduction

Underwater videos are a commonly used non-invasive and non-extractive tool for monitoring marine environments and species in situ (Mallet & Pelletier 2014, Vaudo et al. 2023). One such technique, the baited remote underwater video system (BRUVS), is widely used for underwater sampling of invertebrates (Jones et al. 2020) and fish assemblages (Cappo et al. 2007), including elasmobranchs (Osgood et al. 2019, Bruns & Henderson 2020). Globally, BRUVS are implemented for estimating biodiversity, abundance, species richness, community assemblages and their dynamics, individual identification and biomass (Wraith et al. 2013, Griffin et al. 2016, Letessier et al. 2022). Pelagic BRUVS are commonly used worldwide to study top predator assemblages in offshore areas (Bouchet & Meeuwig 2015, Fukuba et al. 2015, Letessier et al. 2019, Cambra et al. 2021, Leonetti et al. 2024). More specifically, they have been used to study pelagic sharks in their natural environment, providing insights into their foraging behaviour (Lester et al. 2022), predator–prey interactions (Loiseau et al. 2016), social behaviour (Sabando et al. 2020) and responses to anthropogenic pressures (Roberts et al. 2016, Chapuis et al. 2019). Although the behaviour of species observed around BRUVS may not be entirely natural, as these are anthropogenic structures, BRUVS provide an effective tool to study animal behaviour in the natural marine environment.

Studying the distribution and behaviour of sharks is crucial, as many pelagic shark species play a vital role in the stability and functioning of marine ecosystems (Bornatowski et al. 2014, 2018). Commercial longline fisheries are depleting pelagic shark populations worldwide (Queiroz et al. 2019, Pacoureau et al. 2021), causing an imbalance in the ecological network that impacts both the environment and coastal livelihoods (Grubbs et al. 2016, Jaiteh et al. 2017, Shiffman et al. 2021).

The blue shark Prionace glauca is a pelagic carcharhinid distributed globally in temperate and tropical waters. In the North Atlantic Ocean, blue sharks exhibit highly migratory behaviour (Veríssimo et al. 2017, Coelho et al. 2018) and can be found in the water column from the surface to a depth of 1000 m (Lessa et al. 2003, Megalofonou et al. 2009, Weigmann 2016). This species is one of the most predominantly caught shark species worldwide (FAO 2023), and in 2019, it was classified as Near Threatened on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Rigby et al. 2019). Data from Portuguese landings also revealed that the blue shark is the most frequently caught species in pelagic longlines (Alves et al. 2020).

In addition to fisheries, other anthropogenic activities, such as noise pollution, may pose a threat to blue shark populations. Noise pollution has increased from coastal areas to the deep sea due to shipping, harbour development, fishing activities, marine traffic, wind farms and wave energy generation (Erbe et al. 2018, Duarte et al. 2021, Vieira et al. 2021). Underwater sound can lead to significant alterations in fish behaviour (Holles et al. 2013, Simpson et al. 2016), cause auditory masking and increase stress levels among other impacts (Erbe et al. 2019). Due to its deleterious effects, anthropogenic sound is included in the United Nations Convention on the Law of the Sea (United Nations 1994) and in European legislation such as the Marine Strategy Framework Directive 56/2008 CE (Tasker et al. 2010). Shark hearing relies on the detection of the particle-motion component of an acoustic field (Nedelec et al. 2016, Popper & Hawkins 2018). Their hearing range varies from 40 to 1500 Hz, which overlaps with low-frequency anthropogenic sounds like vessel sound (Myrberg 2001, Chapuis & Collin 2022, Nieder et al. 2023). Although elasmobranchs are one of the least studied groups of animals in terms of the effect of anthropogenic sound, there is evidence that noise pollution causes changes in their behaviour (Chapuis et al. 2019, de Vincenzi et al. 2021, Rider et al. 2021).

A previous study using pelagic drift BRUVS around the Professor Luiz Saldanha Marine Park (PLSMP), Portugal, showed that the blue shark was the most frequently observed megafauna species in the water column (Serrão et al. 2021), offering a unique opportunity to observe and investigate the foraging behaviour of this species in its natural habitat. This study thus aimed to (1) describe the behavioural patterns of blue sharks when attracted to bait; (2) evaluate whether differences in the occurrence and duration of behaviours are independent of temporal (spring, summer and autumn), spatial (bathymetric profile), biological (sex and life stage) and oceanographic factors (temperature, visibility, distance from shore and wind direction); and (3) provide a preliminary assessment of the effects of boat noise on this species' behavioural patterns.

5.3 Material & Methods

5.3.1. Study area

The study area is located on the west coast of Portugal, 10 nautical miles (nmi, ~18 km) outside the limits of PLSMP, a marine park that extends for approximately 38 km of the coastline (**Figure 5.1**). It is also located near the fishing town of Sesimbra, whose fish market has one of the highest elasmobranch landings in the country, including the blue shark (Henriques et al. 2021).

One important characteristic of the study area is the topographic variability provided by the Lisboa-Setúbal submarine canyon. The canyon is divided into the Lisbon branch, west of the PLSMP, with the canyon head extending along a south–north axis, and the Setubal branch, south of the PLSMP, which extends from west to east (**Figure 5.1**). In this area, bathymetry ranges between 60 and 2500 m (Lastras et al. 2009). The Sado estuary significantly impacts the area's physical and chemical conditions (Reid & Wood 1976), and the region is also affected by the coastal current, which moves southward due to prevailing northern winds that promote upwelling conditions during the spring and summer along the coast (Peliz et al. 2002). During autumn and early winter, the pre- vailing winds invert, affecting the coastal current and ceasing upwelling conditions (Ambar & Fiúza 1994).

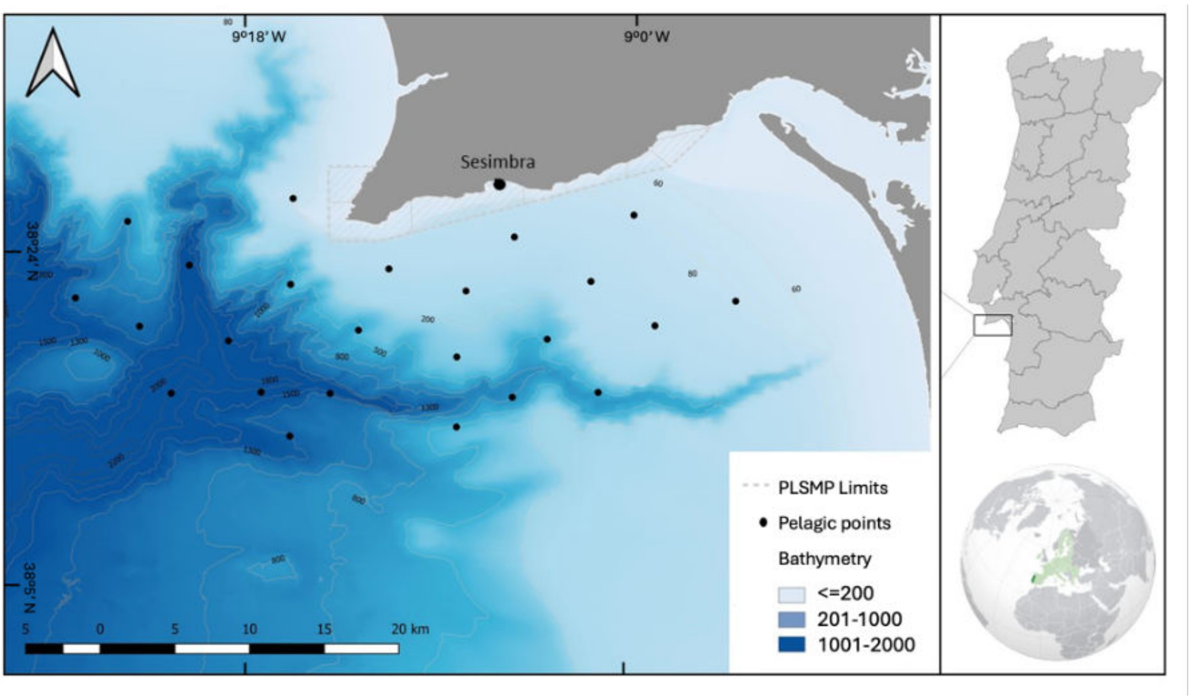


Figure 5.1. Map of the study area, showing the Lisboa-Setúbal submarine canyon system around Professor Luiz Saldanha Marine Park (PLSMP) in Portugal. The sampling points (black dots) and bathymetric levels (shades of blue: epipelagic ≤ 200 m, mesopelagic 201-1000 m, bathypelagic 1000 - 2600 m) are represented around the 10 nautical miles surrounding the park.

5.3.2. Structure setup and experimental design of BRUVS

The BRUVS setup was composed of 3 individual stereo BRUVS rigs mounted in series, connected by a 250 m nylon floating string and deployed adapting the scheme of Bouchet & Meeuwig (2015). BRUVS were suspended with a large buoy at a depth of ~12 m, and one tracking buoy (WAMBLEE, W880 Longline HF Radio Buoy) was connected to each end of the setup to provide information about its location (**Figure 5.S1** in the Supplement at www.int-res.com/articles/suppl/ m753p137_supp/). This setup, adapted from Letessier et al. (2013), consisted of a stainless-steel frame with 2 GoPro HERO 5 Black cameras, set to 1080 p resolution, 60 fps (frames per second) and a medium FOV (field of view) for video recording. Concerning acoustic recordings, each GoPro is equipped with 3 internal microphones that can register sound with a sampling rate of 48 kHz. Following Chapuis et al. (2021), we disabled the automatic audio adjustments on the GoPro cameras and recorded raw audio files in WAV format using the protune settings instead of automatic gain and advanced audio coding. The acoustic characteristics of all cameras were tested with white noise and showed similar results (**Figure 5.S2**). The 2 GoPro cameras were mounted in stereo, approximately 80 cm apart, with an inward convergence angle of 4°, allowing for an optimal field of view up to 10 m. GoPro cameras were encased in Sea- GIS waterproof housings (https://www. seagis.com.au/). A bait canister was placed 1.5 m at the cross-section in front of the cameras. We used 2–3 kg of chopped mackerel *Scomber spp*. as bait in every structure, according to standard BRUVS practices of 1 kg of bait per 60 min sampled (Harvey et al. 2013), for deployments between 160 and 180 min.

We used a stratified random sampling design based on the bathymetry sampling area, obtained from the European Marine Observation and Data Network portal (EMODnet Bathymetry Consortium 2018). Bathymetry was classified into 3 levels: epipelagic (60–200 m), mesopelagic (201–1000 m) and bathypelagic (1001–2600 m) (**Figure 5.1**). At each bathymetric zone, 8 random points were generated, forming a total of 24 sample sites at a minimum distance of 5 km, using the Accuracy Assessment of Thematic Maps of QGIS software (QGIS Development Team 2020). The samples included 24 data points from 3 stereo BRUVS over 2 seasons in 2019 and 2020 (accounting for potential camera failures or material loss). The BRUVS sets were deployed during the day, at least 1 h after sunrise and recovered no later than 1 h before sunset to avoid fish crepuscular behaviour (Axenrot et al. 2004). The BRUVS deployment was oriented perpendicular to the surface current direction and drifted freely with local currents. The research boat maintained a safe distance of 150–250 m from the BRUVS setup in accordance with guidelines for observing marine fauna in the wild (Lewis & Walker 2018). For safety reasons, we chose not to turn the engines on and off multiple times a day while drifting 10 nmi (~18 km) offshore.

5.3.3 Video analyses

Videos with the presence of blue sharks were analysed to identify individuals and behaviour patterns and to estimate distribution. Pictures suitable for photo identification (photo ID) were extracted from the video footage for each individual during every BRUVS deployment. Identification was based on sex (female, male or not defined), size and body marks. Animals were sexed by verifying the presence or absence of claspers (present only in males).

The size of the animals was calculated with Event Measure software using the stereo-camera recordings (SeaGIS; https://www.seagis.com.au/event.html). The age of each individual was estimated based on total length (TL; length of a fish as measured from the tip of the snout to the tip of the tail), following the length–age data of Skomal & Natanson (2003) for the North Atlantic region considering adult animals older than 3 yr (>140 cm). The accuracy of length measurements was verified using the data set from Skomal & Natanson (2003), which is the largest database in the North Atlantic region, accounting for regional variations. A length growth model was developed to assess the animals' life stage. Age classes were classified as: 0+ yr (size: 50–96 cm), 1+ yr (97–125 cm), 2+ yr (126– 142 cm), 3+ yr (143–170 cm), 4+ yr (171–200 cm), 5+ yr (201–225 cm) and 6+ yr (up to 240 cm).

Body marks were also used for photo ID. Scar markings (wounds, nicks, scratches and other marks) can change over time in elasmobranchs, but it typically takes around 6 mo for them to recover from injuries (Marshall & Bennett 2010, Anderson et al. 2011, Marshall & Pierce 2012). Therefore, body marks were used for individual identification within a single season. The maximum number of distinct individuals photo identified in a single video or season (MaxID) was used during the analysis of social interactions (see Section 2.5).

5.3.4. Observation of behavioural patterns

To establish behavioural categories for analysis, 10% (2.8 h) of video footage was observed ad libitum, using the behavioural categories defined in other shark studies (Sperone et al. 2010). The videos were analysed and annotated using BORIS software (Friard & Gamba 2016) to estimate the number of occurrences and duration of behavioural patterns. In total, 9 behaviours within 4 broader categories were described (**Table 5.1**). The categories considered were swimming, BRUVS interaction, social behaviour and feeding; we also considered out of frame (when animals are out of view) (see **Video 5.S1** at www.int-res.com/articles/suppl/m753p137_supp/)

Videos were analysed using focal analysis with instantaneous sampling (annotating observed behaviours from each individual at a regular interval) (Martin & Bateson 1993). The occurrence of each behaviour was scored at 10 s intervals since the time at first sighting. Inter-observer reliability (between 2 observers) was assessed by calculating the mean percentage of agreement, ensuring concordance above 90 %.

Table 5.1. Ethogram of blue shark Prionace glauca with 5 behavioural categories and 9 behaviours. Notice that the ‘out of frame’ category was included to account for the periods when the animal is not visible in the baited remote underwater video system (BRUVS); it encompasses 2 behaviours based on the duration of absence.

Behavioural category	**Behaviour**	**Description**
Swimming	*Approach*	Shark swims slowly, approaching the BRUVS at a considerate distance 5-10 m from the BRUVS. The animal is passing by without any interaction.
BRUVS interaction	*Structure inspection*	Shark swims around the BRUVS, closely observing the structure, camera, and/or bait. Usually with frequent eye movements.
	Vertical swimming	Shark swims vertically to the BRUVS.
	Physical Contact	Shark makes physical contact with the BRUVS with different parts of the body but head principally. Usually accelerates after contact.
Feeding	*Biting*	Shark bites the bait box or pole.
Social interaction	*Parallel swimming*	Both sharks swim parallel in the same or opposite direction.
	Following	One shark is following the other.
	Chasing	One of the sharks follows the other while accelerating, aggressively.
	Being chased	One shark is being chased and swims away.
Out of frame	*No visual*	Shark is not in frame but is still assumed to be close by.
	Time out	Shark not in frame for a long period (more than 30 s).

5.3.5. Social interactions and effects of boat sound

Social interactions

To study intraspecific interactions during bait attraction, we analysed 8 videos, each with a group of 2 or more individuals (MaxID ≥ 2) engaging in different interactions. Focal analysis with continuous sampling was used to observe and record these interactions (Martin & Bateson 1993).

Effect of boat sound on behaviour patterns

During field operations, the sound emanating from our boat was captured in the video footage from a significant number of samples that also showed the presence of blue sharks.

This allowed for a preliminary analysis of the potential impact of boat sound on blue shark behaviour around BRUVS. Furthermore, we verified the proximity of other boats to the structure by annotating and calculating their positions. The distance from our boat to the structure was determined using data from the WAMBLEE W880 Longline HF Radio Buoy and the boat navigation system (http://www.wamblee.it/w880; **Figure 5.S1**). During video recordings containing sharks, no other boats were in the vicinity. Hence, the only source of boat sound was our research vessel, which was present during fieldwork to prevent interference between the BRUVS setup and fishermen's buoys or from boats crossing. Our boat was making either continuous sound, which occurred when we were moving steadily, or intermittent sound, which occurred when we were manoeuvring the boat. Sound spectrograms of continuous and intermittent boat sounds are shown in **Figure 5.S3**.

Soundtracks were extracted from the videos and inspected both aurally and visually (using spectrograms and oscillograms) for the presence of boat sound, using Raven Pro software (v.1.6) (Cornell Laboratory of Ornithology 2023). The boat sounds were characterised in the frequency and time domains to evaluate their duration and whether they were continuous or intermittent. The boat sound was typically between 500 and 1500 Hz (**Table 5.S1**).

To assess the impact of boat sound on shark behaviour, 15 videos with the presence of sharks (23 individuals) and boat sound were inspected. The observation was divided into 3 periods: before noise (BN; the period before any boat sound was captured), during noise (DN; when boat sound was detected by the camera microphone) and after noise (AN; once boat sound was no longer detectable). Only video segments in which a shark was visible were considered. The available duration of each of the 3 periods varied greatly per video, with most videos not including all 3 periods (**Table 5.S2**).

An analysis was performed to compare the number and average duration of behaviours between experimental (with boat sound and only considering the DN period) and control animals (without boat sound).

From the experimental group, we selected 12 videos with a minimum DN duration of 2 min (range: 0:02:02– 0:17:25 min) for further analysis. We also considered another 12 videos for the control group (range: 0:04:04– 0:31:30 min) from which we selected random clips of similar duration to the considered DN videos (**Table 5.S2**). Each video segment featured only one visible shark, allowing us to study individual responses to boat sound. All individuals (12 experimental + 12 control) in the selected videos were thus different animals.

To further explore the reaction of sharks to boat sound, a within-individual comparison was conducted. As very few individuals were visible in all 3 periods (Table S2), only BN and DN were used to compare the number of behaviours between periods. From the individuals with BN and DN periods available for analysis, only 6 met the minimum time length criteria of 2 min in each of these 2 periods. A 2 min subsample from both the BN and DN periods was used to compare the behaviour within these individuals (selected individuals are shown in **Table 5.S2**). When possible (i.e. when longer periods were available), a random 2 min subsample was chosen. In all videos, shark behaviour was measured by focal analysis with continuous sampling. For these analyses, only foraging behaviours were considered: approaching, structure inspection, vertical swimming, physical contact and biting.

5.3.6. Statistical analysis

To assess which variables (temporal, spatial, biological and oceanographic) influence the behaviour of blue sharks, the average of the total number of behaviours per minute was calculated for every individual. Generalised linear models (GLMs) (McCullagh & Nelder 1989) were used to investigate which parameters could explain the variability in the observed behaviour. For each response variable (behaviour), a set of categorical and continuous explanatory variables were used. The categorical explanatory variables were bathymetric zones (bathypelagic, mesopelagic and epipelagic), seasons (autumn, spring and summer), the sex of the individuals (male, female or not defined) and life stage (juvenile or adult). We also considered duration (the length of time when the animals were visible) as a continuous variable. Biogeochemical oceanographic attributes, such as sea surface temperature (SST), were obtained from the EU Copernicus Marine Service Information platform as NetCDF files (https://resources. marine.copernicus.eu/). Other considered variables were visibility measured with a Secchi disc (depth, m), distance to the shore (m) obtained from EMODnet (https://emodnet.ec.europa.eu/en) and wind intensity (knots) obtained from the Portuguese Institute for Sea and Atmosphere (IPMA) database (https://www.ipma.pt/en/oipma/quem/ipma/). The response variables were the selected behaviour patterns: structure inspection, physical contact, vertical swimming, approach and biting (**Table 5.1**). Social interactions such as parallel swimming, following, chasing and being chased were not considered in the analyses as the sample size was too low. All response variables were analysed using a Poisson distribution (McCullagh & Nelder 1989).

The Poisson density function is expressed in terms of a dispersion parameter Ø = 1 and a canonical link $\theta = \log(\mu)$; therefore, the Poisson variance $V(\mu) = \mu$, and iii a logarithmic-link function $\log(\mu i)$ was used for these variables. Stepwise procedures were performed by backward elimination to provide a set of comparable models from the full ones. Akaike's information criterion was used to check which model best fit the data (Akaike 1973, Burnham et al. 2011). Normal probability plots of the residual components of the deviance versus quantiles as well as the null and residual deviances were also evaluated for the selected models. Analysis of deviance was performed to evaluate the significance of the variables and interactions for all selected models. Note that although each longline had 3 BRUVS, the same blue shark individual was never observed on more than one camera. As such, behavioural data obtained from videos of different BRUVS and used in statistical tests were considered independent, as they were collected from different individuals. Furthermore, Bouchet et al. (2018) indicated that data from pelagic BRUVS are independent when the devices are deployed 200–500 m apart.

We compared the total number of behaviours per minute and the average duration of all behaviours of each shark, for individuals exposed to boat sound (DN, n = 12) and control individuals (sharks from videos without boat sound; control, n = 12), using unpaired 2-sample Wilcoxon tests. Additionally, we compared the total number of behaviours per minute exhibited by each of 6 blue sharks between the BN and DN periods with a paired Student's t-test. Note that we did not consider the average duration of all behaviours observed per shark because the considered periods were only 2 min long, rendering duration less informative.

All statistics were carried out using R (v.4.2.2) (R Core Team 2022) with the packages 'devtools' (Wickham et al. 2022) and 'ggpubr' (Kassambara & Mundt 2020).

5.4 Results

A total of 248 BRUVS deployments (24 sets of 3 BRUVS in 2 seasons) were conducted within the scope of the INFORBIOMARES project (108 BRUVS in 2019 and 140 BRUVS in 2020; https://www.lpn.pt/conservacao-da-natureza/projetos-cofinanciados-pela-ue/inforbiomares) resulting in 374 h of video footage from 84 samples. From these, 45 videos (21 epipelagic, 16 mesopelagic and 8 bathypelagic) recorded the presence of blue sharks, resulting in 28 h of video analyses.

5.4.1 Sex–life-stage–age occurrence

In total, 79 blue sharks were identified in the 45 videos, varying from 1 to 4 MaxID per video. In terms of gender, nearly 48.1% were females, 29.11% were males and 22.78% were not defined. In terms of life stage, 81% (64 individuals) were classified as juveniles and 18.9% (15 individuals) as adults. A total of 40 juveniles were aged 0+ yr (50.6%), 21 were aged 1+ yr (26.6%) and 3 were aged 2+ yr (3.8%). Ten adults were aged 3+ yr (12.7%), 4 were aged 5+ yr (5.1%) and one was older than 6 yr (1.3%). Approximately 50.6% of the total individuals were juveniles and were observed during the spring in the epipelagic and mesopelagic zones (**Figure 5.S4**), whereas 10.2% of total individuals were adults and were more common in the autumn and in the offshore bathypelagic zone (near canyons) (**Figure 5.2**). A total of 18 individuals could not be sexed either due to the low number of recorded frames, poor positioning relative to the cameras or their small size; 15 individuals were under 120 cm TL.

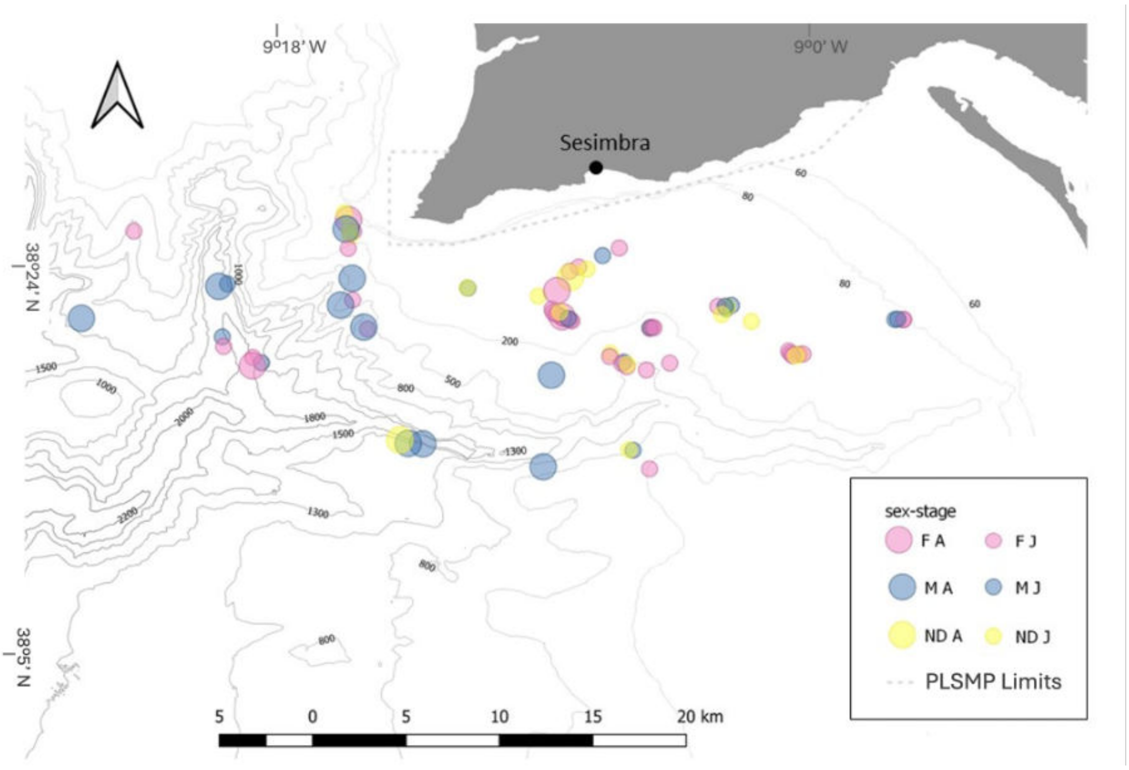


Figure 5.2. Sex-stage geographic distribution of blue sharks in the study area in the surroundings of the PLSMP. Distribution is determined by the coordinates of the radio buoy when sharks are detected. F- female (pink), M - male (blue), ND - No Identified (yellow), A - adults (large dots), J - juveniles (small dots).

5.4.2. Observation of behavioural patterns

Of the 79 individuals observed, the average duration of individual presence in the videos was almost double for juveniles (0.38 ± [SE] 0.02 min throughout 25 h of footage) compared to adults (0.2 ± 0.04 min throughout 3 h 10 min of footage). The duration of the interaction ranged from a minimum of 10 s for both life stages to a maximum of 2 h 12 min for juveniles and 52 min for adults. The average of the total number of behaviours per life stage showed that juveniles interacted less (aver- age [±SE] no. of behaviours min–1: 1.84 ± 0.15) with the BRUVS rigs but they remained for longer periods around the structure than adults (average no. of behaviours min–1: 2.87 ± 0.31). See **Table 5.S3** for the duration and number of behaviours per minute for all individuals. Most of the observed behaviours were related to the BRUVS interaction category. Within this category, structure inspection (average no. of behaviours min–1: 2.36 ± 0.44) was the most common behaviour, followed by physical contact (average no. of behaviours min–1: 0.84 ± 0.03) and vertical swimming (average no. of behaviours min–1: 0.066 ± 0.02) (**Table 5.2, Figure 5.3 a–d**).

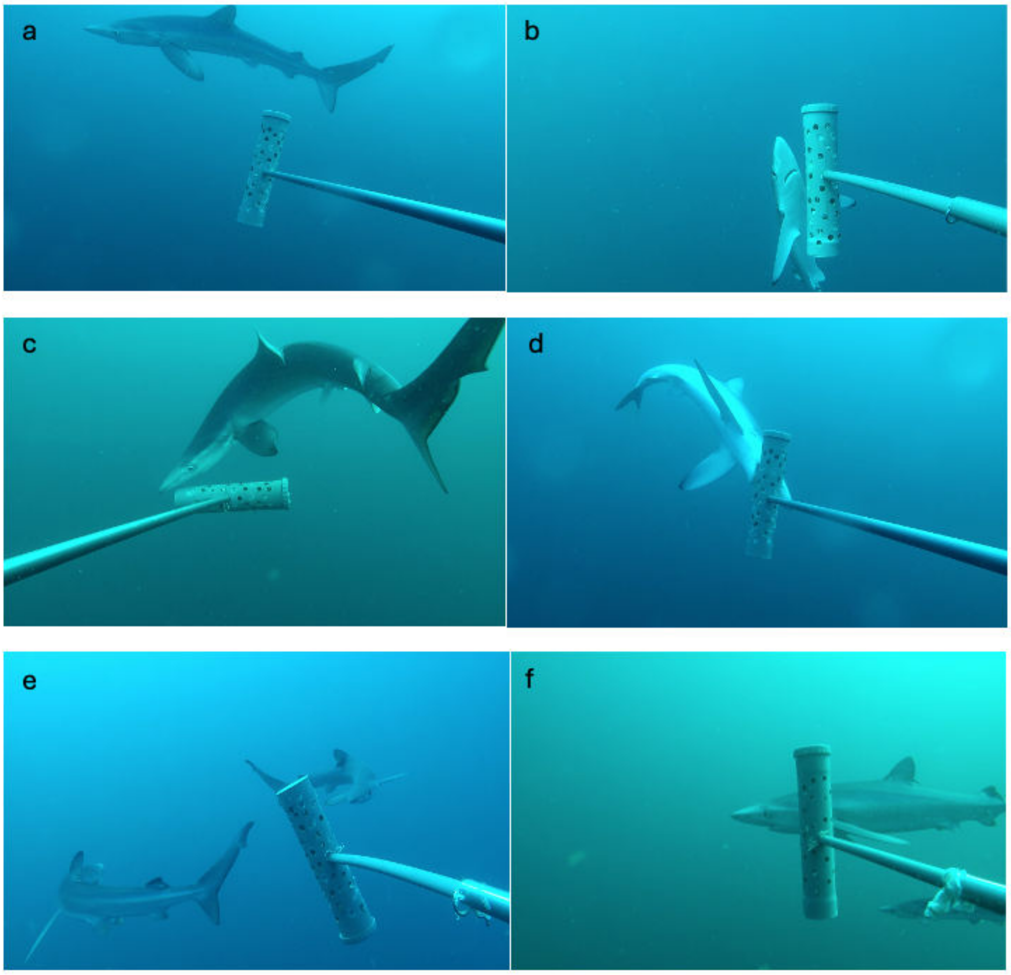


Figure 5.3. Video frames showing examples of behaviours within the category of BRUVS interaction for blue shark: a) structure inspection b) vertical swimming, c) physical contact, d) biting, and social interactions e) parallel swimming, f) following.

Table 5.2. Average of total number of behaviours per minute ± standard error, number of individuals presenting this behaviour, min and maximum occurrence of the behaviours observed over 28 h of video by instantaneous sampling (every 10 s scanning).

	Behaviour	**Average ± SE**	**No. of individuals**	**Min**	**Max**
BRUVS Interaction	*Structure inspection*	2.366 ± 0.443	78	0	410
	Physical Contact	0.084 ± 0.027	34	0	22
	Vertical swimming	0.066 ± 0.021	29	0	16
Feeding	*Biting*	0.012 ± 0.004	10	0	5
Social Interaction	*Being chased*	0.002 ± 0.002	1	0	1
	Chasing	0.001 ± 0.001	1	0	1
	Parallel swimming	0.011 ± 0.006	7	0	2
Swimming	*Approach*	0.184 ± 0.077	48	0	12

Sharks were more likely to interact with the BRUVS when in the epipelagic (depth: <200 m; distance to shore: 3113–13 464 m) and mesopelagic (depth: 201– 1000 m; distance to shore: 6338–19 353 m) zones; note that BRUVS deployments were conducted at a depth of approximately 12 m over areas of these 3 ranges of bathymetry. A greater diversity of behaviours (average of the total number of behaviours per season) was observed during spring and summer, which corresponded with the seasons when animal occurrence was also higher (**Figure 5.4**). In the epipelagic zone, BRUVS interaction was more frequent in spring for adults and in summer and early autumn for juveniles. However, in the mesopelagic zone, BRUVS interaction was more frequent for adults in autumn and for juveniles in the summer period. In the bathypelagic zone (depth: 1000– 2000 m), adults exhibited a higher number of BRUVS interaction behaviours in autumn, whereas juveniles interacted more with the BRUVS in spring.

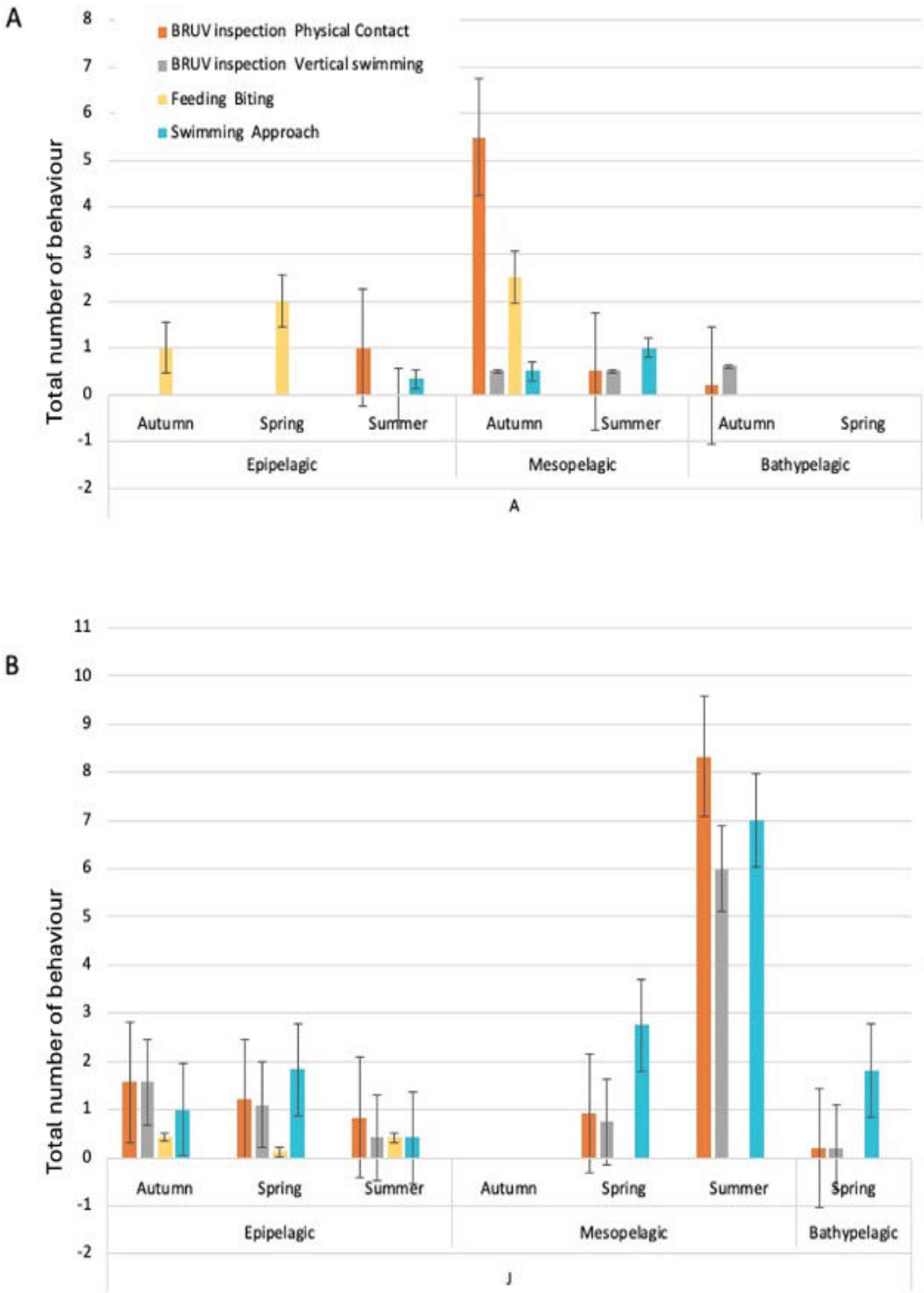
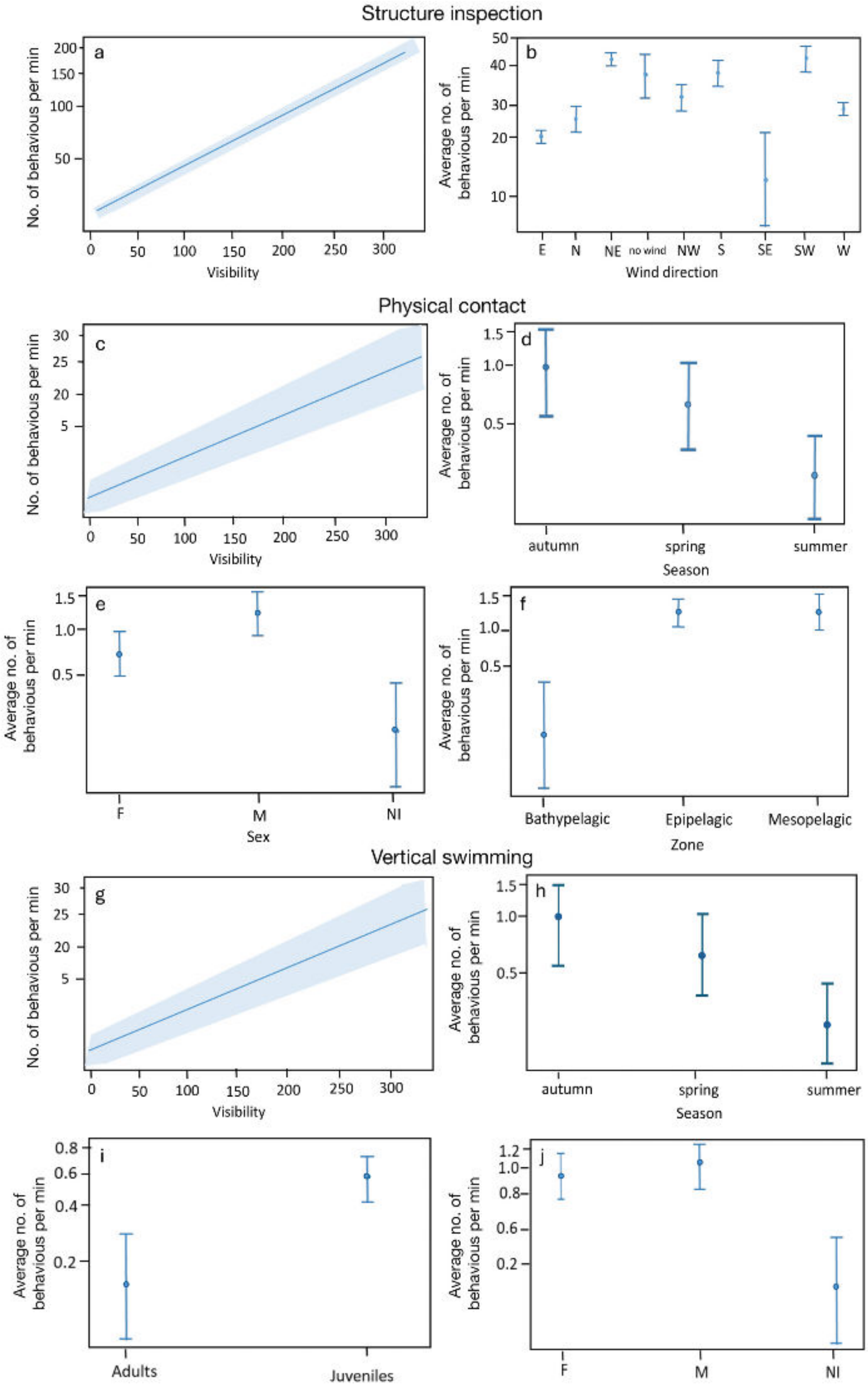


Figure 5.4. Average number of behaviours per minute by maturity stage (**A**-adult, **B**-juvenile) and season (autumn, spring, summer) presented on every bathymetric area (epipelagic, mesopelagic and bathypelagic). Error bars denote the standard deviation.

The GLMs (**Figure 5.5**) showed that structure inspection was mostly influenced by north and south winds. Furthermore, structure inspection, physical contact and vertical swimming increased with visibility. In addition, physical contact and vertical swimming were influenced by season and sex. Physical contact and vertical swimming were more frequent during autumn and spring and less frequent during summer. Sex differences in behaviour were evident, with males exhibiting behaviours such as physical contact and vertical swimming more frequently than females. Nevertheless, vertical swimming was more frequent in juveniles than in adults. Biting was correlated with temperature; this behaviour was also more frequent in autumn and spring and more commonly observed in adult females. **Table 5.3** shows the analysis of deviance for the GLMs.


Structure inspection
a
No. of behavious per min
200
150
100
50
0
50
100
150
200
250
300
Visibility
b
Average no. of behavious per min
50
40
30
20
10
E
N
NE
no wind
NW
S
SE
SW
W
Wind direction
Physical contact
c
No. of behavious per min
30
25
20
5
0
50
100
150
200
250
300
Visibility
d
Average no. of behavious per min
1.5
1.0
0.5
autumn
spring
summer
Season
e
Average no. of behavious per min
1.5
1.0
0.5
F
M
NI
Sex
f
Average no. of behavious per min
1.5
1.0
0.5
Bathypelagic
Epipelagic
Mesopelagic
Zone
Vertical swimming
g
No. of behavious per min
30
25
20
5
0
50
100
150
200
250
300
Visibility
h
Average no. of behavious per min
1.5
1.0
0.5
autumn
spring
summer
Season
i
Average no. of behavious per min
0.8
0.6
0.4
0.2
Adults
Juveniles
j
Average no. of behavious per min
1.2
1.0
0.8
0.6
0.2
F
M
NI

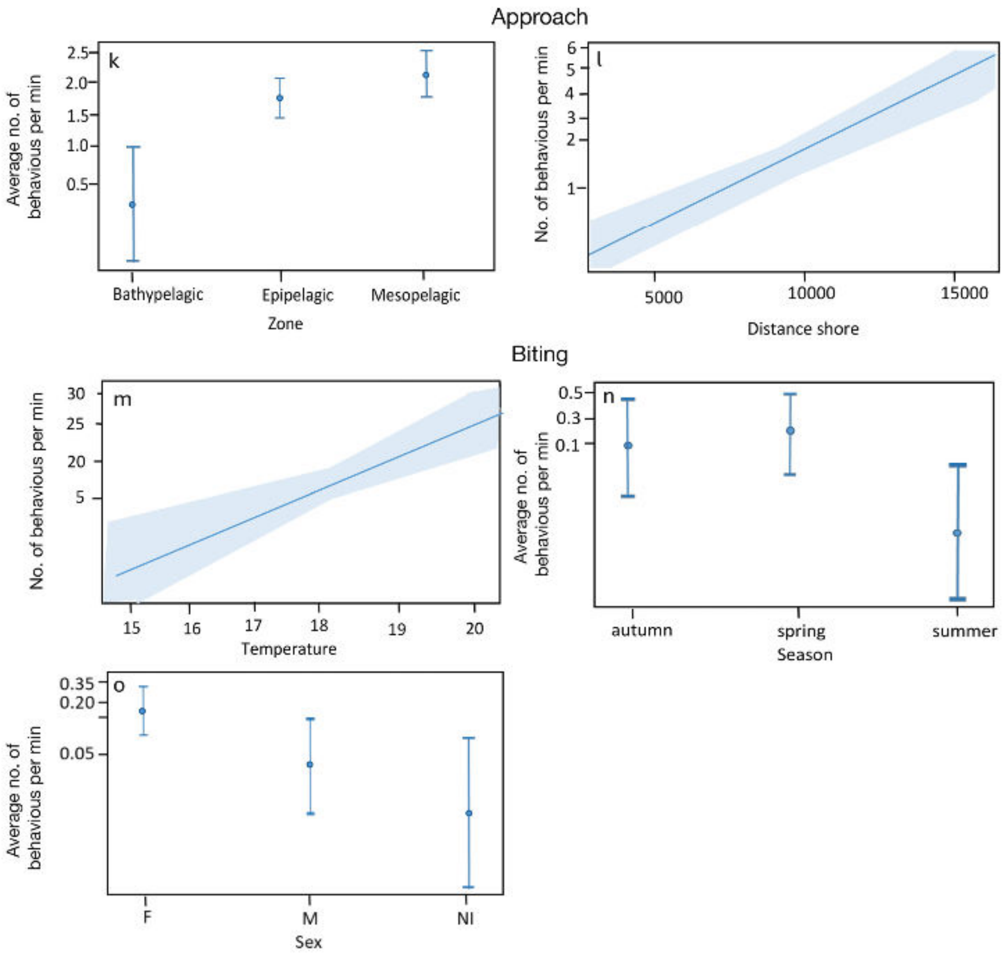


Figure 5.5. General linear mixed model fitting the response of the average number of behaviours exhibited by blue sharks per minute in relation to continuous variables (depth, visibility, wind direction, distance to the coast, temperature) and categorical variables zone (epipelagic, mesopelagic, bathypelagic), season (autumn, spring, summer), sex (female: F; male: M; not defined: ND) and life stage (juvenile: J; adult: A). Shading and error bars: 95% confidence intervals. Number of structure inspections based on (a) visibility and (b) wind direction; physical contacts based on (c) visibility and (d) season; physical contacts by (e) sex and (f) zone; vertical swimming activities based on (g) visibility and (h) season; vertical swimming activities by (i) stage and (j) sex; approaches (k) by zone and (l) distance to shore; and biting incidents based on (m) temperature, (n) season and (o) sex.

Table 5.3. Analysis of deviance table for generalised linear models to analyse the response of the average number of behaviours to the continuous variables: duration, depth, visibility, wind direction, distance to the coast, temperature, and categorical variables: zone, season, sex and stage. Significant results were highlighted in bold.

Structure inspection

	df	Deviance Resid	Residual df	Resid. Dev	p
NULL			71	3584.6	
duration	1	1582.29	70	2002.3	**< 0.001 *****
depth	1	155.95	69	1846.3	**< 0.001 *****
visibility	1	800.39	68	1045.9	**< 0.001 *****
Wind direction	8	181.21	60	864.7	**< 0.001 *****

Physical Contact

	df	Deviance Resid.	Residual df	Resid. Dev	p
NULL			76	265.18	
duration	1	48.416	75	.21676	**< 0.001 *****
visibility	1	63.864	74	152.90	**< 0.001 *****
season	2	4.676	72	148.22	0.097
zone	2	19.090	70	129.13	**< 0.001 *****
sex	2	12.777	68	116.36	**0.0017 ****

Vertical swimming

	df	Deviance Resid.	Residual df	Resid. Dev	p
NULL			76	203.63	
duration	1	34.930	75	168.70	**< 0.001 *****
visibility	1	46.932	74	121.77	**< 0.001 *****
season	2	7.731	72	114.04	**0.021 ***
stage	1	3.656	71	110.38	0.056
sex	2	7.930	69	102.45	**0.019 ***

Approach

	df	Deviance Resid.	Residual df	Resid. Dev	p
NULL			78	192.99	
duration	1	88.244	77	184.17	**0.0030 ****
distance shore	1	259.712	76	158.20	**< 0.001 *****
zone	2	160.463	74	142.15	**< 0.001 *****

Biting

	df	Deviance Resid.	Residual df	Resid. Dev	p)
NULL			78	86.201	
duration	1	77.547	77	78.446	**0.0054 ****
temperature	1	163.384	76	62.108	**< 0.001 *****
season	2	80.838	74	54.024	**0.018 ***
sex	2	73.666	72	46.657	**0.025 ***

5.4.3. Social interactions

From the 45 video recordings with shark presence, only 8 videos had a MaxID of more than one individual (the number of individuals varied between 2 and 4). During the study, 19 individuals presented social interactions in 8 different interaction groups (**Table 5.S4**). Of these individuals, only one was a female adult (life stage 3+ yr); the other 18 were juveniles (12 females, 6 males and 2 not defined). Furthermore, of the 18 juveniles, only one was 2+ yr, while the others were younger (0+ and 1+ yr). Some of the individuals in the group appeared at the same time but did not interact (Group 2; see **Table 5.S5**). The highest proportion of social interaction (89%) was observed in spring due to the higher occurrence of sharks in this season, while a lower proportion (21%) was observed in autumn and summer.

Continuous sampling of these 19 individuals resulted in a total of 872 total behaviours, of which 149 were classified as social. The remaining behaviours included BRUVS interaction (structure inspection, vertical swimming and physical contact), swimming and biting (**Figure 5.S6**). Social interactions appeared only in the epipelagic and mesopelagic zones, which were areas with a higher number of shark observations. The average number of social interactions per minute in the 8 different interaction groups showed that parallel swimming and following were the most frequent social behaviours (**Figures 5.3 e, f, & 5.6**).

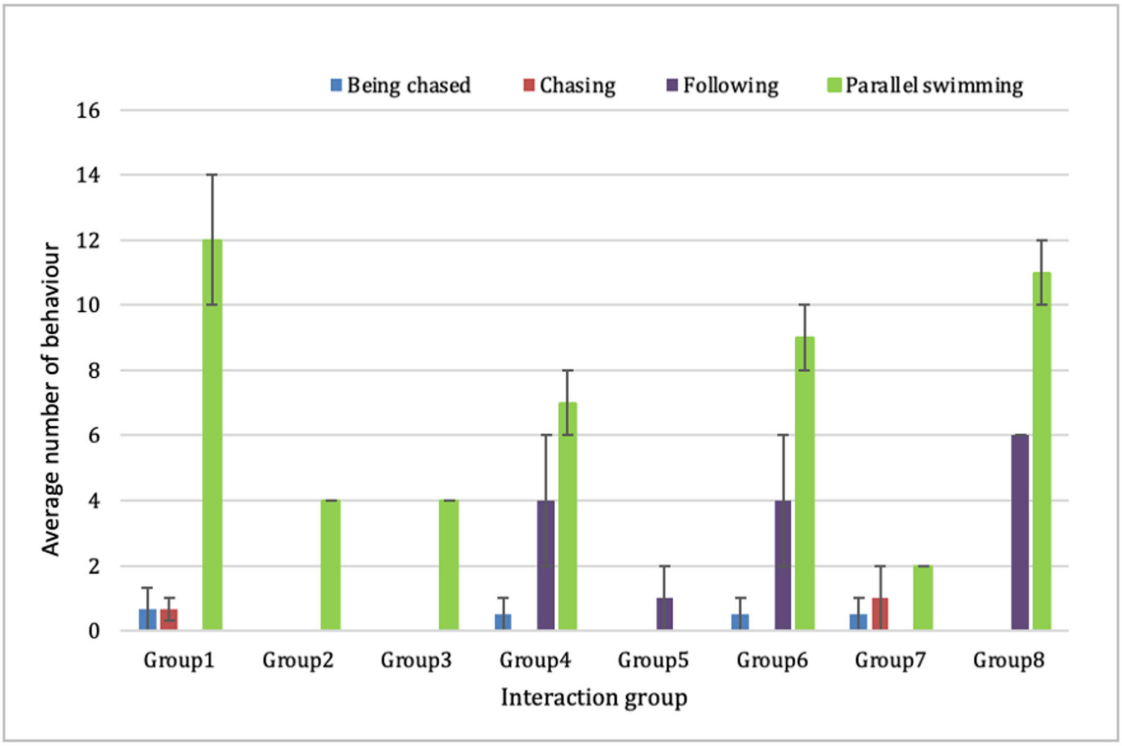


Figure 5.6. Average number of social interactions per minute and group of interaction, with error bars representing the standard deviation. Interaction groups sex stage; Group 1: three individuals - 1 adult female and two juvenile females, Group 2: three juvenile females, Group 3: two juvenile female/ND (not defined), Group 4: two juvenile male/female, Group 5: two juvenile females, Group 6: two juvenile female/male, group 7: two juvenile male/ND, group 8: two juvenile female/ND. (More detail about time of interaction on **Table 5.S2** of the Supplementary Material.)

5.4.4. Noise-related behaviour

In this study, 23 sharks detected in 15 videos were exposed to boat engine sound. Of these, 7 individuals appeared only during boat sound exposure (i.e. DN) (ID1, ID10, ID11, ID12, ID27, ID 70 and ID2), while 8 individuals were present both before and after the boat sound (i.e. BN and AN) (ID20, UD21, ID26, ID39, ID56, ID74, ID58 and ID75) but not in the DN period (**Figure 5.7**). Twelve individuals had a minimum duration of boat sound exposure of 2 min (ID10, ID12, ID18, ID27, ID30, ID58, ID63, ID66, ID64, ID70, ID4 and NID2) (see **Table 5.S2**).

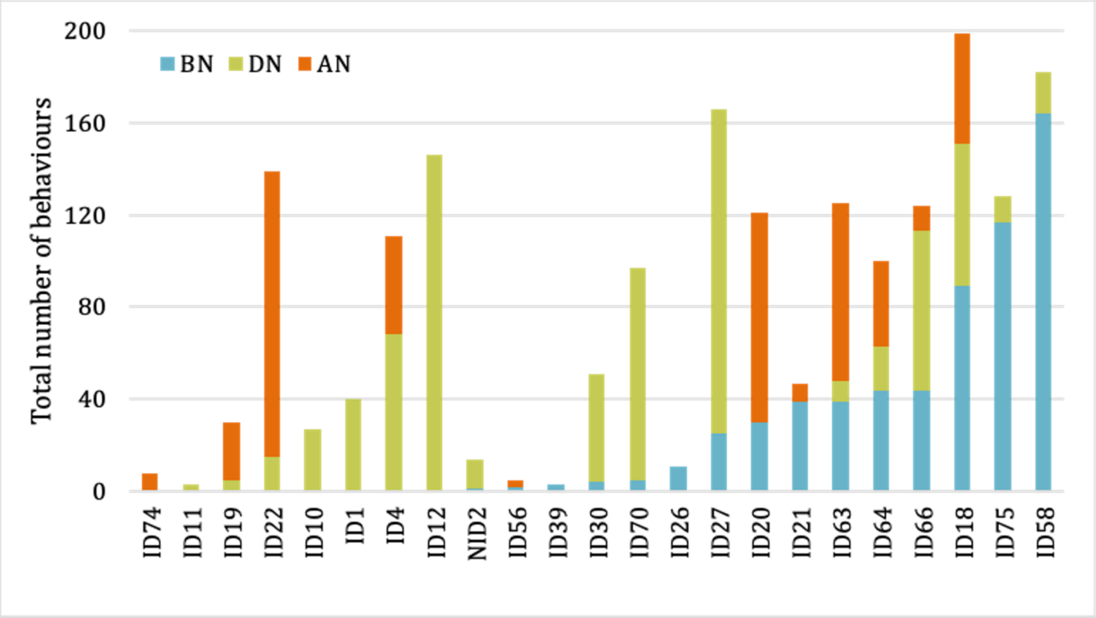


Figure 5.7. Number of behaviours observed for 23 individuals (individual ID is shown) in videos with boat sound representing the three periods; before (BN), during (DN), and after (AN) the boat noise.

Unpaired Wilcoxon tests showed no effect of boat sound on either the total number of behaviours per minute or the duration of all behaviours between sharks exposed to noise (DN, n = 12) and those not exposed (control, n = 12) (no. of behaviours min–1: W = 69, p = 0.4; duration: W = 33, p = 0.012; **Figure 5.8a**, **Table 5.S2**). However, when comparing behaviour between the BN and DN periods within individuals (paired t-tests), sharks exhibited a significantly higher number of behaviours per minute in BN than in DN periods (n = 6; t = 6.52, df=5, p=0.001; **Figure 5.8b**).

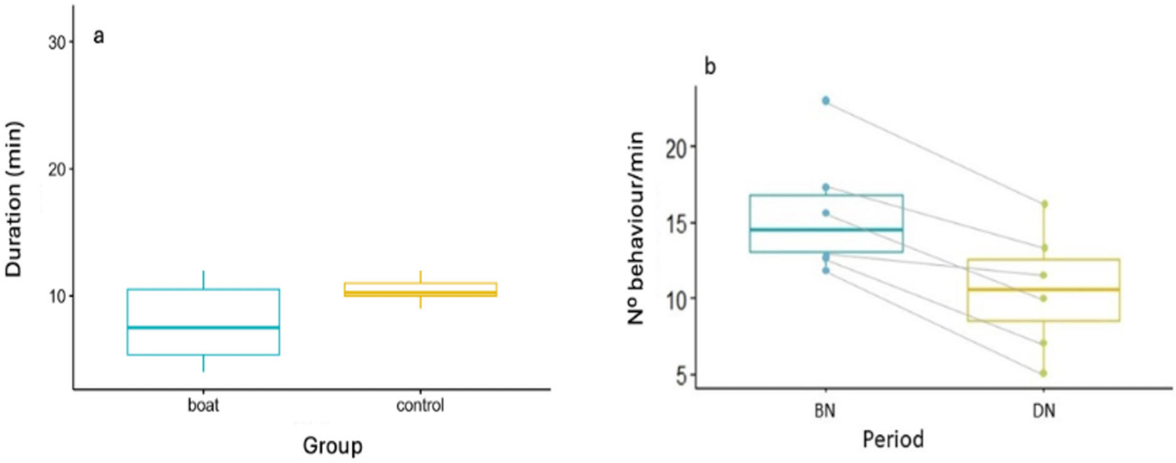


Figure 5.8. a) Total duration of behavioural states (*approach, structure inspection*, *vertical swimming*, *physical contact*, *biting*) experimental animals exposed to boat noise and control animals. **b)** Number of behaviours (*approach, structure inspection*, *vertical swimming*, *physical contact*, *biting*) per minute and per individual, before (BN) and during (DN) boat noise. Box plots depict median, quartiles and range.

5.5 Discussion

Improved understanding of the distribution and behaviour of blue sharks is crucial for the conservation of these animals (Mas et al. 2024). However, studies focussing on the behaviour of sharks in their natural habitats are rare and challenging (Klimley et al. 2023). Our study highlights differences in the occurrence and behaviour of blue sharks in relation to seasonal, spatial, bathymetric, biological (sex and life stage) and oceanographic factors in a temperate coastal region adjacent to a marine protected area. Importantly, the high density of juveniles observed during spring suggests that the study area is likely a nursery, making it relevant for conservation. This study describes the behaviour of blue sharks in relation to BRUVS and suggests that anthropogenic sounds may influence their foraging behaviour, highlighting the potential impacts of noise pollution. In sum, this study provides valuable insights relevant for the management and conservation of this species.

5.5.1 Sex–life-stage–age distribution

The spatio-temporal distribution of blue sharks in the study area exhibited certain trends in relation to the life stage and sex of the animals. Juveniles were more frequently observed in spring, while adults were more frequent in autumn. In addition, juveniles were sighted more frequently in the epipelagic and mesopelagic zones, and adults (mainly males) were mostly found in bathypelagic zones associated with the Lisbon and Setubal canyons.

Our results are consistent with previous distribution studies in the North Atlantic, which showed that the distribution of blue sharks in relation to water temperature varied by sex and life stage (Nakano & Stevens 2008). Data on blue shark landings in Europe (STECF 2015), the South Atlantic (Hsu et al. 2015) and several studies in the North Atlantic (Vandeperre et al. 2014, Howey et al. 2017, Coelho et al. 2018) suggest that larger blue sharks tend to prefer warmer regions, while smaller individuals are more commonly found in colder areas. In the North Pacific, blue shark distribution also seemed to be strongly influenced by SST (Maxwell et al. 2018).

Our data are also consistent with previous studies carried out in Portuguese waters. On the Portuguese coast, longlines mainly capture juvenile females, which comprise 61–77% of total blue shark catches (Queiroz et al. 2005). Moreover, juveniles and adult females are more frequent in shallow waters, whereas adult males prefer zones with active water dynamics, such as seamounts (Litvinov 2006). Spring is the breeding season for this species near Portuguese waters (Nakano & Stevens 2008), with nursery areas located off Portugal, north of Spain and near the Azores (Aires-da-Silva et al. 2008). Mature and pregnant females are found in African waters during winter, with mating and pupping likely occurring off the

Portuguese coast (Nakano & Stevens 2008). Our 2 yr study identified a potential nursery area for blue sharks based on high shark density, especially juveniles, prolonged presence and frequent use over multiple years, meeting the criteria outlined by Heupel et al. (2018). Many sharks show site fidelity to specific areas, such as nurseries, mating grounds and feeding areas, but it remains unclear whether oceanic sharks exhibit long-term site fidelity to particular areas such as nursery or feeding grounds (Hueter et al. 2005). Nevertheless, recent sightings of blue sharks in specific areas challenge the belief that they are purely oceanic wanderers, suggesting that blue sharks may exhibit residency or philopatry, returning to the same locations regularly (Fontes et al. 2024). If the study area is confirmed as a nursery area, this information could provide valuable insights for management, potentially supporting its designation as an Important Shark and Ray Area.

5.5.2. Observation of behavioural patterns

Foraging behaviour patterns indicated that juveniles exhibited greater curiosity towards the bait than adults. This could be due to their foraging instinct and/or limited experiences, causing them to linger near the stimuli for longer periods. Adults were less likely to stay near the bait for extended periods, given that the food-related cues did not lead to actual prey (the bait box only released scent and blood, not food). Despite spending less time around them, adults tended to bite the structures more frequently. Most behaviours were in the BRUVS interaction category, which indicates sharks were attempting to acquire information through visual (structure inspection), touch and electromagnetic senses (vertical swimming and physical contact). Naturally, olfactory cues are often the first utilised by aquatic animals searching for food (Gardiner et al. 2014). After olfactory attraction, visual stimuli also provide important information, followed by touch and electroreception (Lorenzini organs; Fields 2007). Indeed, sharks can follow multiple sensory cues simultaneously or alternate between them as they hunt or forage (Gardiner & Atema 2007, Gardiner et al. 2014).

Physical contact and vertical swimming were more frequent in autumn and spring. Furthermore, physical contact, vertical swimming and structure inspection behaviour increased with visibility. These observations are likely linked to the importance of visual cues for these pelagic predators. Structure inspection decreases and physical contact increases slightly with lower visibility and bathymetric zone. This could be explained by the fact that high turbidity impairs the use of vision as the main source of information, forcing sharks to rely more on tactile cues and electroreception (McFarland 1989).

Biting was significantly related to temperature, mostly at lower (15– 16°C) and higher (>19°C) temperatures, and occurred more frequently in autumn and early spring. Biting was also more frequent in adults than in juveniles. This may be correlated with coastal upwelling conditions in late spring and summer, which impact SST, and, in turn, create ideal feeding conditions for pelagic fish schools such as sardines and mackerel (Santos et al. 2002). As a result, this can trigger foraging behaviour in sharks.

5.5.3 Social interactions

Most of the social interactions observed in the study area were exhibited by juveniles. Juveniles tended to remain longer in response to food-related olfactory cues, which led to increased social interactions, especially in the spring when juvenile sharks were more abundant. None of the observed social interactions were considered aggressive, but rather intra-specific curiosity and exploratory behaviour. Although sharks are often seen as solitary predators, it has been observed that some shark species form aggregations when attracted to food stimuli or for reproductive purposes, often in response to environmental changes (Jacoby et al. 2012, Micarelli et al. 2020). In these cases, they can be observed to show intraspecific interactions. For example, although focussing on a different species, social studies based on direct observation and video footage of white sharks attracted to bait suggest that they exhibit repeated, specific behaviours in interactions with conspecifics (Micarelli et al. 2023).

Interestingly, on 2 occasions during the present study, a mako shark *Isurus oxyrinchus* was observed near blue sharks, but smaller individuals of both species avoided interaction by leaving the area. Indeed, it has often been observed that animals avoid interspecific or intraspecific interactions with larger individuals when competing for a common resource (Thompson 1988).

While social interactions are poorly understood in sharks, this study provided a good opportunity to gain some preliminary insights. However, more targeted behavioural studies are needed.

5.5.4 Boat sound effect on behaviour

The comparison between the animals exposed to boat sounds and the control animals showed no significant difference in the duration of foraging behaviours, although the duration was on average 20% longer in the control group than in noise-exposed sharks. However, when comparing the number of behaviours per minute performed by the same animal before and during sound exposure, we found a significant decrease in behaviour frequency in the presence of boat sound. These observations should be considered preliminary and interpreted with caution due to the small sample size and constraints faced in this study, namely the differences in available time periods and the duration of observable footage. Future studies on the effects of boat noise on this species should thus attempt to minimise or eliminate these constraints by using larger observation areas, complementary methods or ex situ experimental trials.

Although our results are preliminary, they suggest that anthropogenic sounds can affect the behaviour of this species. This could have potential implications for their distribution and/or foraging activity and deserves further investigation. Boat sounds are within the hearing range of sharks (Chapuis & Collin 2022) and other aquatic animals (Kasumyan 2005) and are known to cause measurable impacts (Chapuis et al. 2019, Alves et al. 2021, Amorim et al. 2022). Boat sounds other than those from our research vessel were not observed through direct observation of the spectrograms. However, this does not necessarily mean that other disturbances were not heard by the sharks. Nevertheless, nearby sounds are more likely to be perceived as a threat and therefore to impact behaviour (Myrberg et al. 1978, Rider et al. 2021). Research focussing on elasmobranchs has found mixed results in terms of noise effects. For example, some studies have exposed captive and wild elasmobranchs to artificial sounds without observing changes in their feeding behaviour (Ryan et al. 2018). By contrast, escape behaviour was observed in stingrays during exposure to anthropogenic sounds (Mickle et al. 2022). Consistent with our study, a BRUVS based study in a coastal reef showed that sharks decreased in number of individuals and interactions within the video structure when artificial sound (with 95% of its energy below 1 kHz) was continuously played (Chapuis et al. 2019). Furthermore, alterations in swimming behaviour have been observed in small-spotted catsharks *Scyliorhinus canicular* exposed to loud sounds at 80 and 200 Hz (de Vincenzi et al. 2021). Together, these studies suggest that anthropogenic noise has the potential to negatively impact elasmobranch behaviour.

Despite the numerous reports on behavioural alterations in sharks exposed to short-term sounds, habituation may occur when exposure is recurrent. A study comparing the presence and abundance of 3 shark species with variable boat density and boat traffic (Rider et al. 2021) suggested habituation of sharks to high levels of recurrent boat activity within the study area. Boat noise may even attract sharks, particularly when sharks associate boat noise with food (Mitchell et al. 2018). Nevertheless, habituation or attraction to boat noise may still have negative consequences, such as increased physiological stress (Amorim et al. 2022, Di Franco et al. 2023), increased probability of bycatch (Mitchell et al. 2018) and reduced foraging success due to masking of auditory cues produced by prey (Codarin et al. 2009, Nedelec 2023).

5.6 Conclusions

Juvenile sharks were observed more frequently around BRUVS in the study area, and both juveniles and adults presented distinct seasonal occurrence patterns. Juveniles were more abundant in spring, coinciding with the breeding season, which hints at a potentially important nursery habitat in the study area. Results provided some insights into the spatial preferences and distribution of this species as well as the influence of the environment on behavioural pat- terns. Despite the aforementioned constraints related to observation times and sample sizes, which are difficult to avoid in a field study, our results support the use of BRUVS for studying foraging and social behaviours in pelagic predators such as blue sharks. This study also demonstrated that boat noise seems to disturb behavioural patterns in blue sharks, potentially affecting their foraging efficiency. There is a significant lack of acoustic and behavioural studies on sharks, yet such research is critical to strengthening our understanding and predictive capacity regarding the effects of anthropogenic stressors on sharks. Filling knowledge gaps on the behaviour of this threatened species is key to better planning and predicting the outcome of targeted conservation measures.

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5.8 Supplementary material

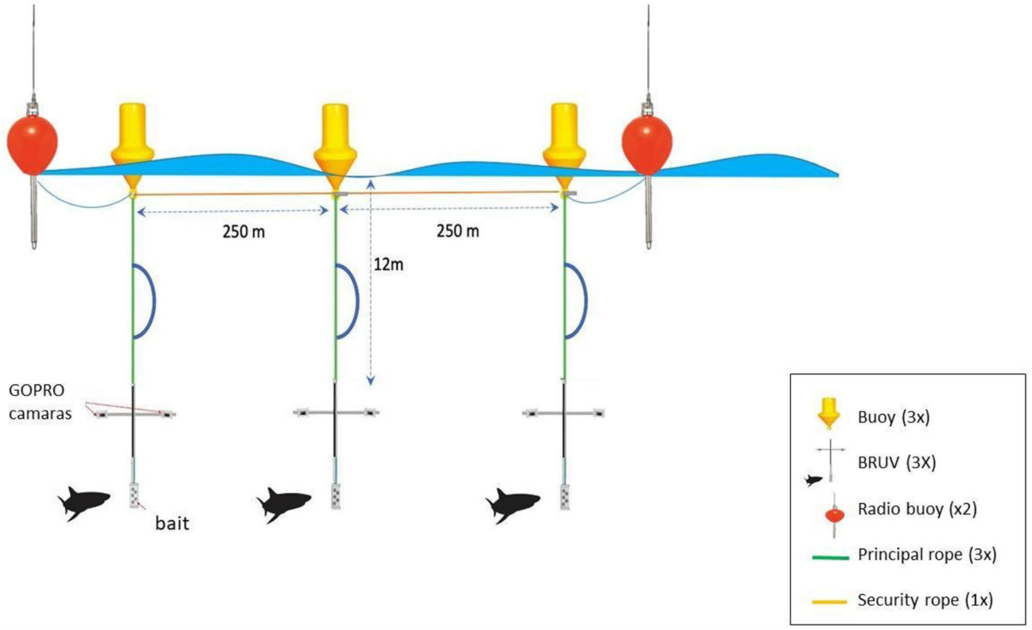


Figure 5.S1. The diagram illustrates the pelagic drift setting structure, featuring three security buoys for each BRUV (yellow), three BRUV rigs, two signalling position tracking buoy (WAMBLEE, W880 Longline HF Radio Buoy) (red), a main rope (green) connecting BRUVs to the security buoy and additional ropes connecting the BRUV rigs (yellow).

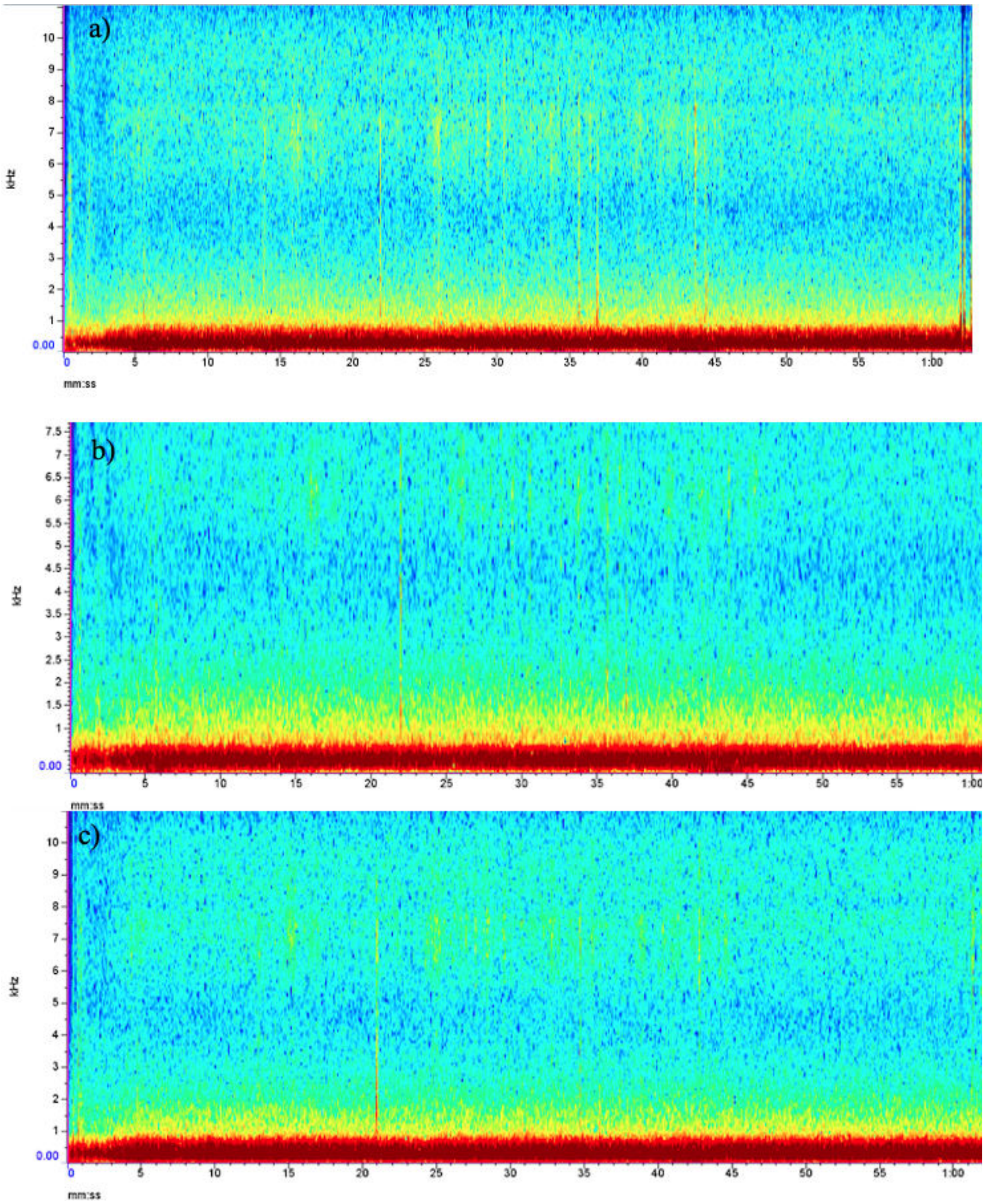


Figure 5.S2. Spectrogram of white noise captured by three synchronised GoPro cameras (a, b, c). The recordings were made simultaneously under identical conditions to assess the recording capacity of the cameras. Spectrogram (FFT 256, Hanning window, frequency scale linear to between 7500-10000 kHz) made using the Raven software.

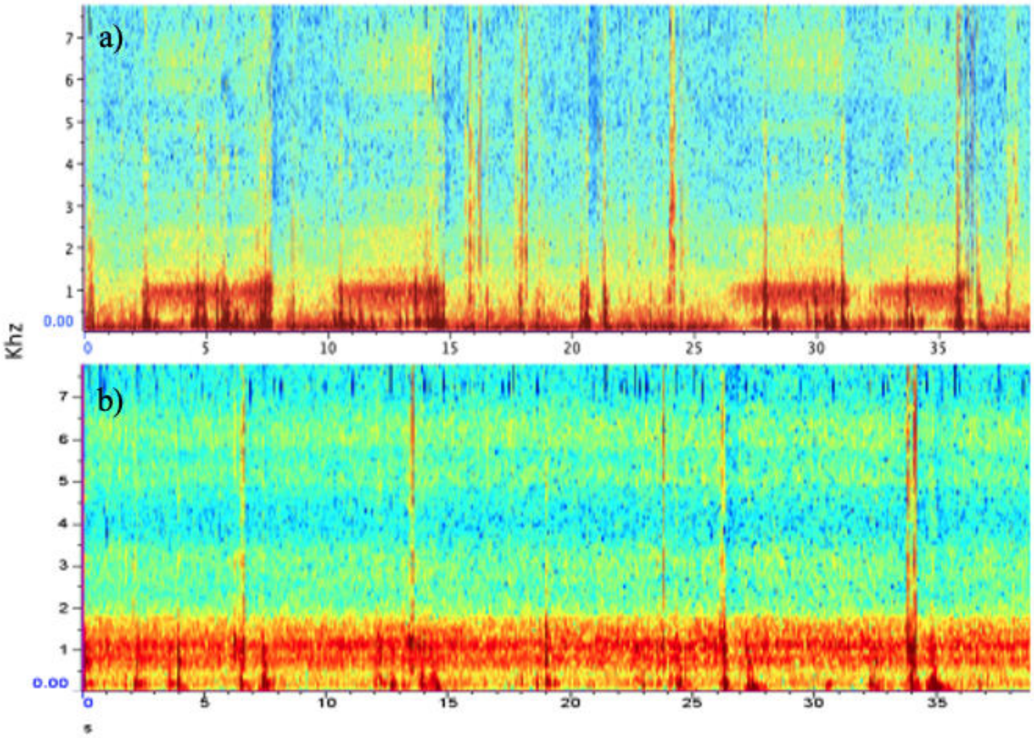


Figure 5.S3. Examples of boat sound categories (a) intermittent and b) continuous) found in the videos. Spectrogram (FFT 256, Hanning window, frequency scale linear to 7 Khz) made using the Raven software.

Table 5.S1. Acoustic characteristics of boat noises that appear in the 23 individuals of 15 videos with the presence of sharks and boats. Identification number (ID) of the 23 individuals are present in the first column. Frequency 25% (Hz), frequency 75% (Hz), boat sound duration (hour:minutes:seconds) independent of shark presence, and sound types (continuous or intermittent) are described in the table. Sounds were recorded by the GoPro Hero 5 black cameras that registered the videos, using raw audio mode.

ID *P. glauca*	**Freq 25% (Hz)**	**Freq 75% (Hz)**	**Boat sound duration (h:m:s)**	**Sound type**
ID_1	172 266	430 664	02:57:00	intermittent
ID_10	602 930	861 328	01:10:48	continuous
ID_11	602 930	861 328	01:10:48	continuous
ID_12	258 398	775 195	01:10:48	continuous
ID_18	258 398	1 205 859	00:17:42	intermittent
ID_19	258 398	1 205 859	00:17:42	intermittent
ID_20	344 531	1 119 727	00:17:42	continuous
ID_21	775 195	1 205 859	01:06:48	continuous
ID_22	775 195	1 205 859	01:02:48	continuous
ID_26	689 062	1 119 727	00:04:42	continuous
ID_27	172 266	1 119 727	00:14:42	intermittent
ID_30	258 398	861 328	00:10:42	intermittent
ID_39	861 328	1 378 125	00:13:42	continuous
ID_4	689 062	947 461	01:10:48	continuous
ID_56	775 195	1 291 992	00:47:06	continuous
ID_58	258 398	1 205 859	00:05:18	continuous
ID_63	258 398	1 033 594	00:56:30	intermittent
ID_64	258 398	861 328	00:36:06	intermittent
ID_66	258 398	1 378 125	00:53:06	intermittent
ID_70	172 266	1 033 594	00:48:06	intermittent
ID_74	689 062	861 328	00:42:06	intermittent
ID_75	602 930	1 205 859	00:10:42	intermittent
NID_2	258 398	861 328	00:10:42	intermittent

Table 5.S2: Table displaying identification codes for sharks (ID), including duration time (hours:minutes:seconds) of footage for the control of individuals and individuals exposed to boat noise. For the boat noise group, there are different periods: before noise BN, during noise DN, after noise AN, and the total duration. *Indicates individuals with time in BN and DN periods used for paired analyses.

ID control	Total duration	**ID noise**	BN duration	DN duration	AN duration	Total duration
ID17	0:28:40	**ID10**	0:00:00	0:10:41	0:00:00	0:10:41
ID35	0:09:30	**ID12**	0:00:00	0:17:25	0:00:00	0:17:25
ID34	0:09:50	**ID18***	0:05:03	0:04:38	0:03:00	0:12:41
ID41	0:31:30	**ID27***	0:02:58	0:10:32	0:00:00	0:11:30
ID42	0:10:28	**ID30**	0:00:00	0:08:14	0:00:00	0:08:14
ID45	0:10:04	**ID58***	0:12:15	0:02:02	0:00:00	0:14:17
ID47	0:10:07	**ID63***	0:03:24	0:05:18	0:14:42	0:23:24
ID49	0:10:50	**ID66**	0:02:23	0:04:12	0:01:02	0:07:37
ID52	0:11:19	**ID64***	0:05:10	0:07:15	0:08:49	0:21:14
ID57	0:09:11	**ID70***	0:02:18	0:12:14	0:00:00	0:14:32
ID60	0:04:04	**ID4**	0:00:00	0:09:16	0:01:43	0:10:59
ID71	0:10:13	**NID2**	0:00:00	0:06:01	0:00:00	0:06:01

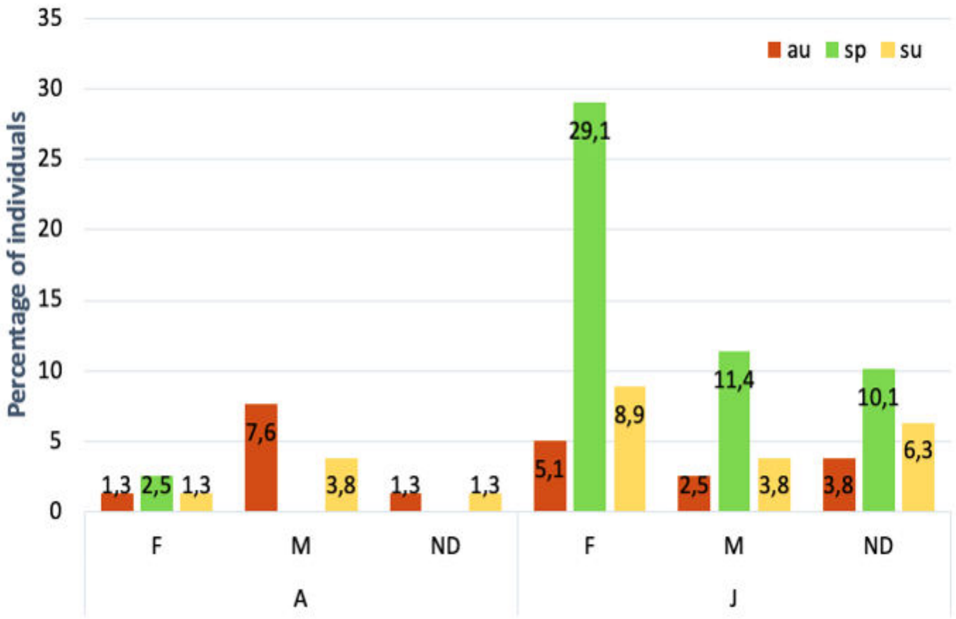


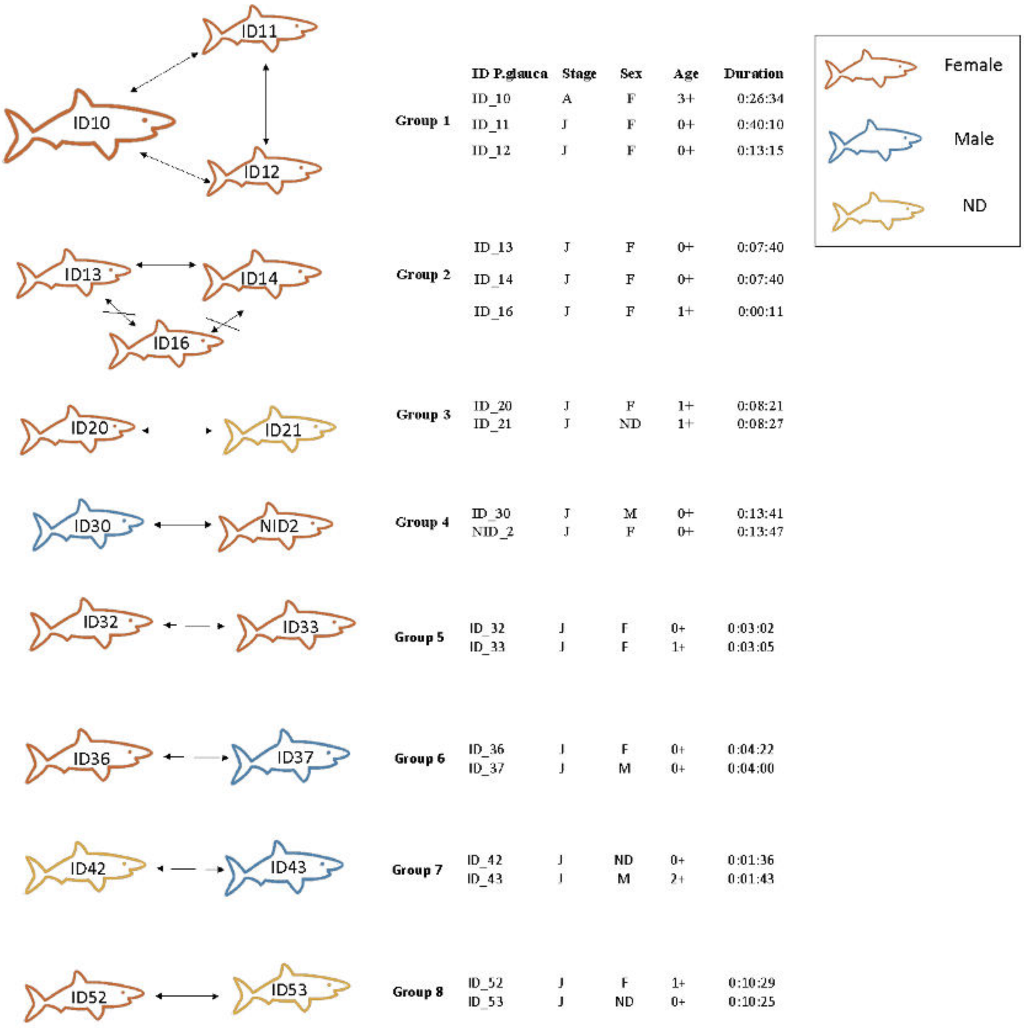
Figure 5.S4. Blue shark (*Prionace glauca*) population breakdown by stage (adults and juveniles), sex (F - Female, M - Male, ND - No identified), and season (autumn, spring, summer). From the total of 79 individuals, juveniles accounted for 81% of the population, and adults while adults made up 19%. Juveniles were more prevalent in spring (50.6%), while adults were more common in autumn (10.2%).

Table 5.S3. The table displays the identification code (ID), stage, length in mm, sex, age, and duration of analysis for each of the 79 blue sharks in the study. The start and end time of interaction, and duration of footage for each individual are also included in the table.

Individual	Sex	Age	Stage	Average	Nºbeh	Duration analysis	beh/min	Average beh/min
ID_1	ND	1+	J	0,89	8	0:05:30	1,45	0,05
ID_10	F	3+	A	5,78	52	0:18:40	2,80	0,31
ID_11	F	0+	J	7,11	64	0:46:40	1,37	0,15
ID_12	F	0+	J	7,78	70	0:26:40	2,63	0,29
ID_13	F	0+	J	2,22	20	0:12:58	1,54	0,17
ID_14	F	0+	J	1,11	10	0:14:51	0,67	0,07
ID_15	F	0+	J	1,22	11	0:22:00	0,50	0,06
ID_16	F	1+	J	1,67	15	0:29:30	0,51	0,06
ID_17	M	0+	J	8,22	74	0:28:40	2,59	0,29
ID_18	M	0+	J	10,89	98	0:35:20	2,78	0,31
ID_19	ND	0+	J	0,67	6	0:01:50	3,16	0,37
ID_2	F	1+	J	1,22	11	0:15:10	0,73	0,08
ID_20	F	1+	J	2,78	25	0:18:22	1,37	0,15
ID_21	ND	1+	J	1,89	17	0:13:22	1,29	0,14
ID_22	F	0+	J	3,56	32	0:09:50	3,23	0,36
ID_23	F	0+	J	2,44	22	0:12:40	1,75	0,20
ID_24	F	0+	J	2,89	26	0:17:10	1,52	0,17
ID_25	F	1+	J	0,33	3	0:01:20	2,50	0,18
ID_26	ND	0+	J	0,22	2	0:02:30	0,80	0,10
ID_27	M	1+	J	14,22	128	0:47:30	2,71	0,30
ID_28	F	0+	J	2,22	20	0:08:30	2,35	0,26
ID_29	ND	0+	J	0,56	5	0:02:40	1,92	0,23
ID_3	F	1+	J	6,00	54	1:21:22	0,67	0,07
ID_30	M	0+	J	6,78	61	0:33:30	1,82	0,20
ID_31	F	0+	J	1,56	14	0:12:50	1,09	0,12

ID_32	F	0+	J	2,44	22	0:29:30	0,75	0,08
ID_33	F	0+	J	1,33	12	0:05:20	2,31	0,26
ID_34	F	0+	J	5,00	45	0:17:00	2,65	0,29
ID_35	M	0+	J	2,67	24	0:09:30	2,53	0,28
ID_36	F	1+	J	2,78	25	0:19:49	1,28	0,14
ID_37	F	0+	J	1,67	15	0:03:50	3,85	0,43
ID_38	F	0+	J	10,78	97	0:50:40	1,92	0,21
ID_39	F	1+	J	3,78	34	0:26:40	1,29	0,14
ID_4	M	0+	J	0,89	8	0:24:00	0,33	0,04
ID_40	M	0+	J	10,22	92	0:38:00	2,42	0,27
ID_41	M	0+	J	1,78	16	0:30:20	0,53	0,06
ID_42	M	0+	J	5,89	53	0:14:47	3,56	0,41
ID_43	ND	0+	J	1,11	10	0:05:20	1,92	0,21
ID_44	F	0+	J	10,44	94	0:51:00	1,84	0,20
ID_45	F	1+	J	2,11	19	0:30:20	0,63	0,07
ID_47	M	3+	A	7,56	68	0:52:00	1,31	0,15
ID_49	ND	1+	J	7,11	64	0:18:20	3,52	0,39
ID_5	M	0+	J	4,11	37	0:46:07	0,80	0,09
ID_51	F	1+	J	9,67	87	0:38:00	2,29	0,25
ID_52	M	2+	J	22,89	206	1:33:04	2,21	0,25
ID_53	F	1+	J	5,33	48	0:37:04	1,30	0,14
ID_54	F	0+	J	5,22	47	0:17:20	2,73	0,30
ID_55	ND	0+	J	0,11	1	0:00:10	2,00	0,11
ID_56	ND	3+	A	0,11	1	0:00:10	2,00	0,11
ID_57	M	3+	A	3,67	33	0:09:10	3,63	0,40
ID_58	F	0+	J	50,00	450	2:11:50	3,42	0,38
ID_59	F	0+	J	3,00	27	0:09:40	2,87	0,32
ID_6	F	0+	J	1,00	9	0:40:30	0,22	0,02
ID_60	F	3+	A	2,44	22	0:16:00	1,38	0,15
ID_61	ND	1+	J	0,11	1	0:00:10	2,00	0,11

ID_62	ND	1+	J	0,22	2	0:04:00	0,50	0,06
ID_63	ND	0+	J	4,11	37	0:33:10	1,12	0,12
ID_64	ND	1+	J	7,56	68	0:27:50	2,52	0,27
ID_65	M	3+	A	2,89	26	0:09:40	2,71	0,30
ID_66	F	2+	J	2,78	25	0:08:00	3,13	0,35
ID_67	M	3+	A	2,56	23	0:16:50	1,36	0,15
ID_68	M	1+	J	1,89	17	0:13:50	1,26	0,14
ID_69	F	3+	A	1,78	16	0:05:10	3,14	0,35
ID_7	M	0+	J	0,22	2	0:00:30	0,40	0,44
ID_70	ND	0+	J	5,33	48	0:12:40	3,87	0,42
ID_71	F	0+	J	5,78	52	0:14:20	3,66	0,41
ID_72	F	1+	J	4,89	44	0:15:00	2,93	0,33
ID_73	M	1+	J	4,00	36	0:19:00	1,89	0,21
ID_74	M	/	A	0,22	2	0:00:40	0,33	0,37
ID_75	M	3+	A	16,89	152	0:44:20	3,44	0,38
ID_76	M	5+	A	0,67	6	0:01:20	5,45	0,56
ID_77	M	6+	A	0,11	1	0:00:10	2,00	0,11
ID_78	M	5+	A	1,89	17	0:05:20	3,27	0,36
ID_79	ND	5+	A	0,22	2	0:10:40	0,19	0,02
ID_8	F	3+	A	0,22	2	0:00:21	10,00	0,22
ID_80	ND	1+	J	1,33	12	0:09:40	1,25	0,14
ID_9	F	0+	J	0,44	4	0:05:10	0,78	0,09
N.ID_1	ND	2+	J	4,22	38	0:25:20	1,51	0,17
NID_2	ND	0+	J	1,44	13	0:19:40	0,66	0,07



	ID P.glauca	Stage	Sex	Age	Duration
Group 1	ID_10	A	F	3+	0:26:34
	ID_11	J	F	0+	0:40:10
	ID_12	J	F	0+	0:13:15
Group 2	ID_13	J	F	0+	0:07:40
	ID_14	J	F	0+	0:07:40
	ID_16	J	F	1+	0:00:11
Group 3	ID_20	J	F	1+	0:08:21
	ID_21	J	ND	1+	0:08:27
Group 4	ID_30	J	M	0+	0:13:41
	NID_2	J	F	0+	0:13:47
Group 5	ID_32	J	F	0+	0:03:02
	ID_33	J	F	1+	0:03:05
Group 6	ID_36	J	F	0+	0:04:22
	ID_37	J	M	0+	0:04:00
Group 7	ID_42	J	ND	0+	0:01:36
	ID_43	J	M	2+	0:01:43
Group 8	ID_52	J	F	1+	0:10:29
	ID_53	J	ND	0+	0:10:25

Table 5.S5. Interactions between blue sharks involve social behaviours that are organized into interaction groups. The information provided includes the stage of the shark (only ID10 is an adult), sex, age, and duration of the interaction for each group of sharks.

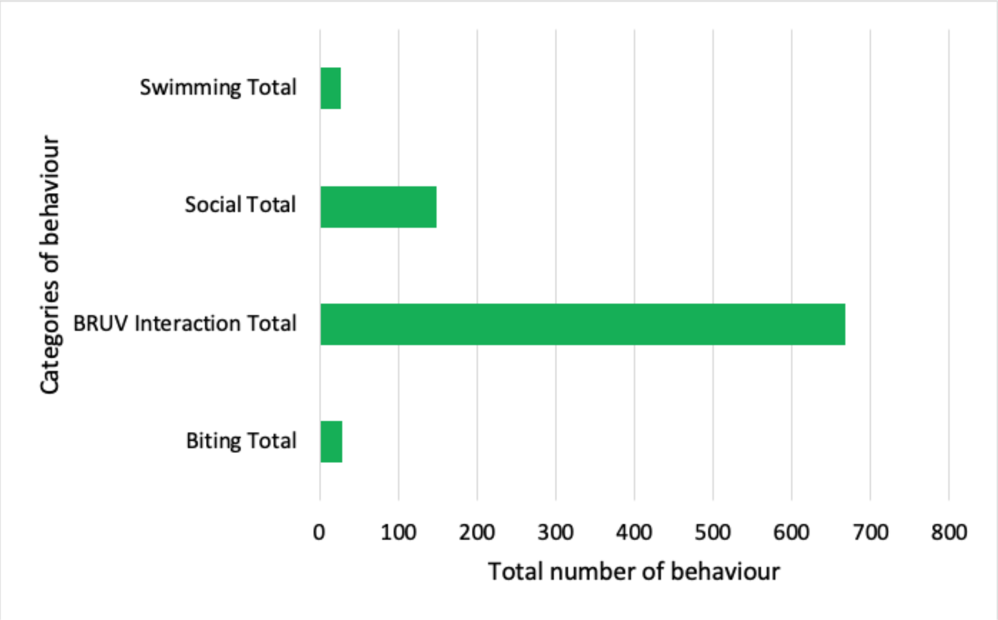
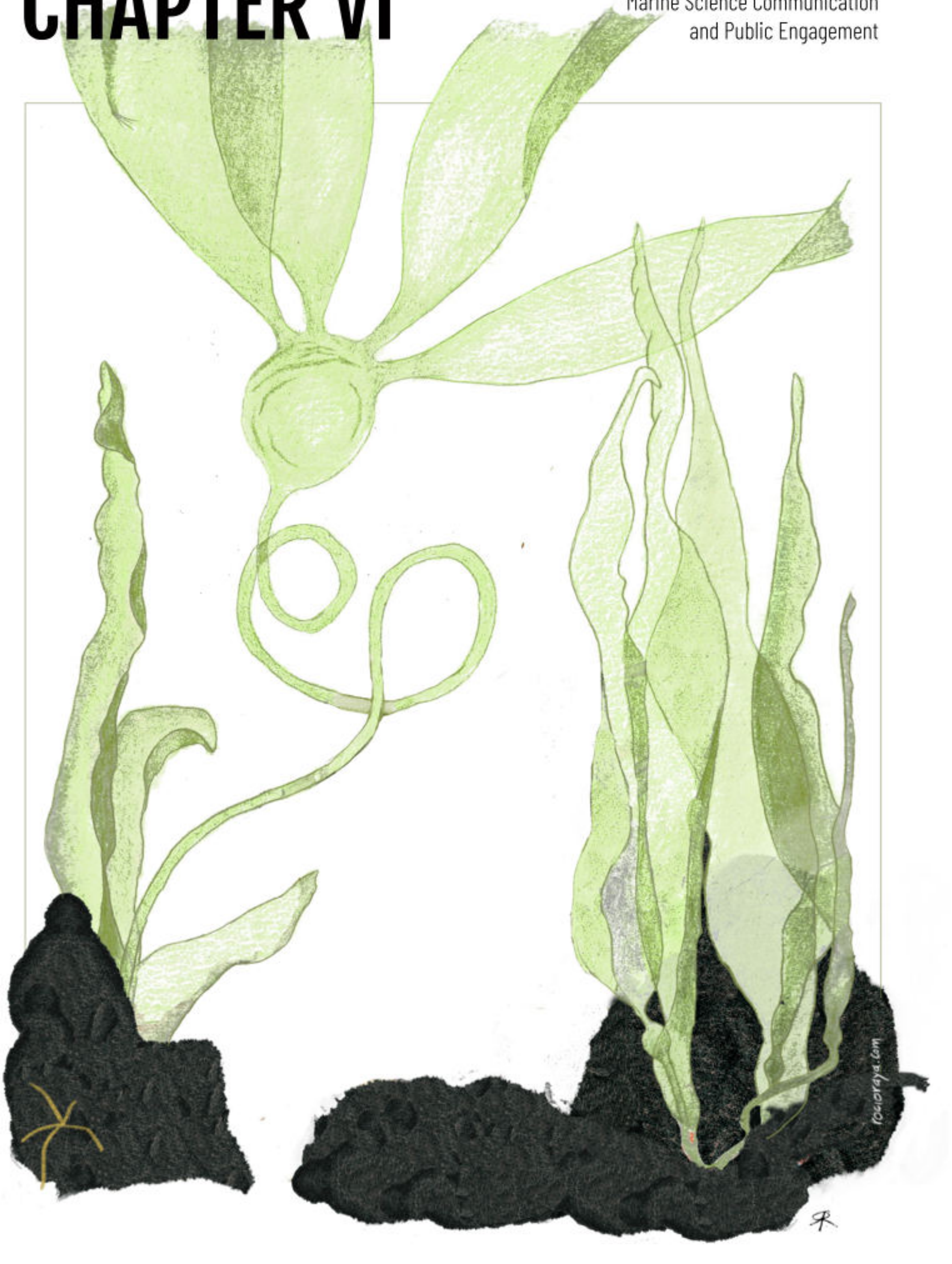


Figure 5.S5. Total number of behaviours per category presented for blue sharks during social interactions.

Link to behaviour video:
https://drive.google.com/file/d/1HalwvO_onmGhmAhHq9nZOIzUlRTB_yjK/view?usp=drive_link

CHAPTER VI

Marine Science Communication and Public Engagement



Chapter VI: *Marine science communication and public engagement*

Outreach is the way to connect research with society, encompassing activities like public engagement, communication and promoting participation in conservation activities. It can be a fulfilling aspect of PhD research, allowing you to share your passion, inspire others, and enhance your research through public commitment. However, simplifying complex research and making it accessible to a wide audience can be difficult, especially when translating marine science into content that remains accurate while engaging people with different levels of knowledge. The target audience included scientific peers and the general public, from children to adults with limited knowledge of marine ecology. Enhancing the ability to communicate complex research clearly can significantly benefit an academic career. It improves the effectiveness of teaching and supervision, increases the chances of securing competitive funding through compelling grant applications, and facilitates interdisciplinary collaboration by making findings accessible to researchers from other fields. To implement the outreach strategy, clear explanations were combined with visual and interactive elements fostering understanding and interest. Well-communicated research is more likely to be widely disseminated, cited, and used in evidence-based policy and public engagement, enhancing both academic progress and societal impact. Different communication channels were chosen to reach different audience segments, including social media, public talks, workshops, and educational materials. The objective of this chapter is to communicate marine science in the different levels (both scientific audiences and members of the general public across diverse age groups) to reach a broad audience.

6.1 Importance of public engagement

Marine conservation should prioritize outreach, as effective protection depends on understanding which involves communicating scientific information to the public (Kelly et al. 2018). A significant gap exists between academic knowledge and public understanding of ocean issues, which poses a challenge to effective conservation (Lotze et al. 2018, 2020). Traditionally, scientists have attributed public indifference to a lack of knowledge; however, research indicates that simply increasing knowledge does not necessarily lead to behavioural change (Varner 2014). Despite the fact that motivation to act is driven by factors such as knowledge, personality, and skills, actual behaviour is shaped by a broader range of situational and contextual influences (Heimlich & Ardoin 2008). To bridge this gap, fostering two-way communication between scientists and the public is essential. Such dialogue can build trust, promote participation, and create shared understanding, ultimately increasing the likelihood of meaningful action (Varner, 2014, Zaelzer, 2020). An increasing recognition exists of the importance of involving the public in science, ranging from simply providing information to active participation through citizen science initiatives. This growing awareness has sparked all kinds of collaborations between different fields like scientists, environmental educators and teachers (Heimlich & Ardoin 2008, Lesen et al. 2016).

6.2 Importance of awareness of fish sound

Many marine species rely on sound for vital life processes, including communication, navigation, foraging, and reproduction (Montgomery & Radford 2017). However, underwater noise pollution can disrupt these processes, ultimately threatening the conservation of affected species (Gelcich et al. 2014). Public awareness of this issue remains limited or, in many cases, entirely absent. As the saying goes, we can only protect what we know and with which we have developed an emotional connection.

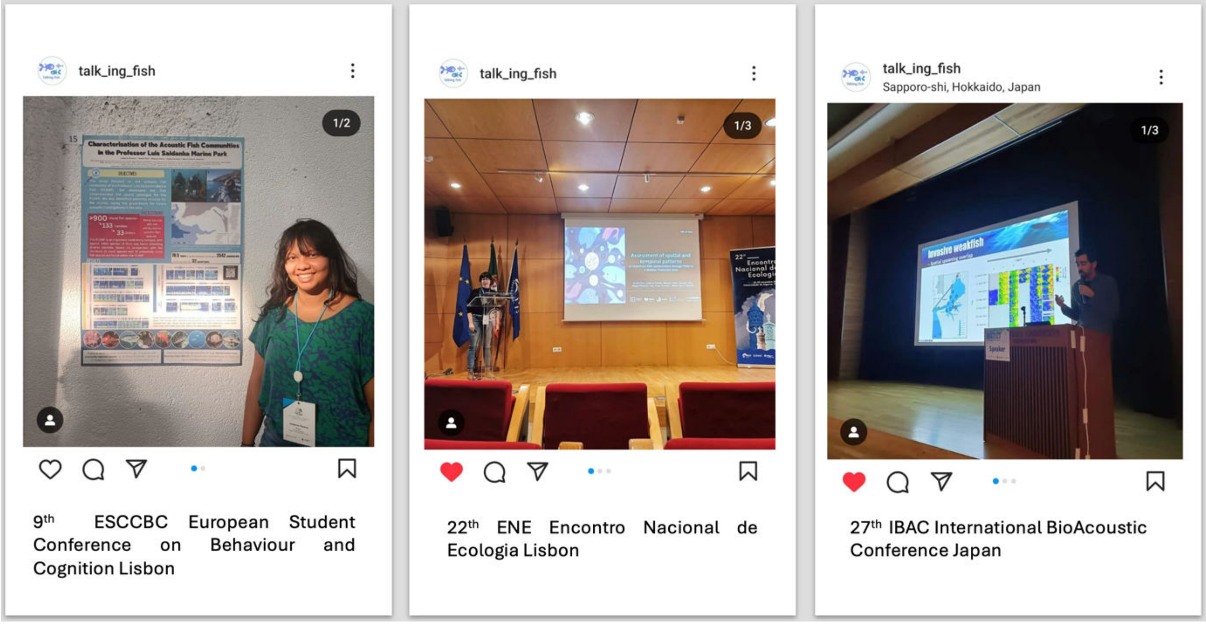
Furthermore, raising awareness among society, policymakers, and relevant authorities about the role of sound in the underwater environment, and the use in spatial planning, and biodiversity management, can help to promote conservation efforts and the sustainable use of the oceans. Indeed, popular media often highlights soniferous marine mammals as they are considered flag species, as their vocalisations are more conspicuous and humans generally relate more closely to mammals than to fish. However, it may surprise many people that over 1,000 fish species are also capable of producing sounds (Looby et al. 2022). Acknowledging the importance of managing ocean soundscapes is essential for safeguarding marine ecosystems and supporting the long-term health and resilience of ocean environments (Duarte et al. 2021). Noise pollution represents a significant concern, as the ecological consequences of altered ocean soundscapes on marine organisms remain comparatively understudied relative to the well-documented effects of anthropogenic noise in terrestrial ecosystems. While solutions to mitigate anthropogenic noise are emerging through policy measures (e.g. Marine Strategy Framework Directive) and technological advancements, addressing this issue remains challenging.

This section presents the dissemination activities of this thesis, aimed at communicating findings to both the scientific community and the general public to foster awareness and support for marine conservation.

6.3 Science communication activities

6.3.1 Dissemination within the Scientific Community

Throughout the thesis development, the findings have been shared at national and international conferences and scientific meetings (**Figure 6.1**) as listed below. The results have been published or submitted to scientific journals to engage the scientific community, and they are referenced at the beginning of the thesis as **Chapter II** and **Chapter V**. These recent studies demonstrate growing interest in marine monitoring. Research on bait-attracted blue sharks (*Prionace glauca*) using pelagic drift video (Research Interest Score 6.4; 176 reads) highlights predator behaviour in open-ocean ecosystems and boat noise impact over foraging behaviour patterns. The article of acoustic fish communities (Score 7.6; 130 reads) underscore the value of PAM for understanding species composition and dynamics.



talk_ing_fish

1/2

9th ESCCBC European Student Conference on Behaviour and Cognition Lisbon

talk_ing_fish

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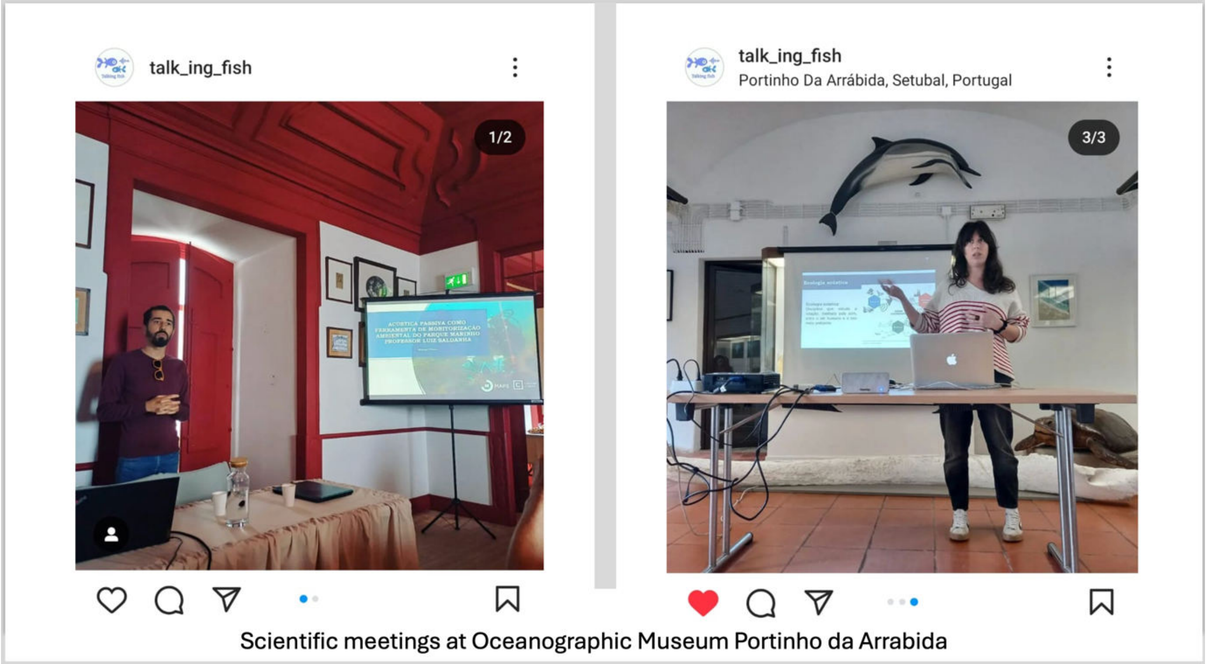
22th ENE Encontro Nacional de Ecologia Lisbon

talk_ing_fish

Sapporo-shi, Hokkaido, Japan

1/3

27th IBAC International BioAcoustic Conference Japan



Scientific meetings at Oceanographic Museum Portinho da Arrabida

Figure 6.1 Examples of activities at conferences and meetings, also divulged in social media.

Presentations and posters at international and national congresses

Oral presentations

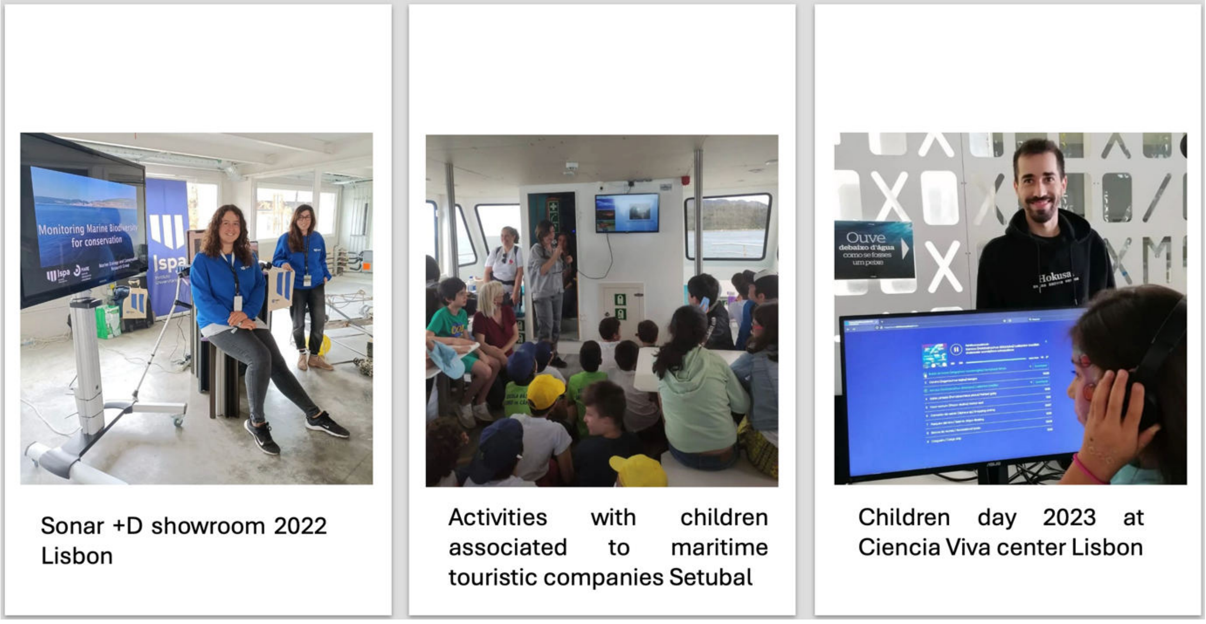
- Vieira M, **Ríos N**, Muñoz-Duque N, Pereira J, Carriço R, Fernandez M, Monteiro JG, Pais MP, Quintella BR, Silva G, Silva RP, Fonseca PJ & MCP Amorim (2024). Playing Who's Who? The Challenge of Undocumented Sound Production in Marine Acoustic Communities. XXI Congress of the Portuguese Ethological Society, Lisbon, Portugal.
- **Rios N**, J Pereira, M Vieira, G Silva, MP Pais, PJ Fonseca & MCP. Amorim (2023). Assessment of spatial and temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area (Portugal). IBAC 2023: XXVIII International Bioacoustics Congress, Sapporo, Japan.
- **Rios N**, J Pereira, M Vieira, G Silva, MP Pais, PJ Fonseca & MCP. Amorim (2023). Assessment of spatial and temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area (Portugal). ESCBC 2023 - 9th European Student Conference on Behavior & Cognition, Lisbon, Portugal.

Posters

- Vieira, M, **N Rios,** S Muñoz-Duque, J Pereira, R Carriço, M Fernandez, JG Monteiro, MP Pais, BR Quintella, G Silva, RP Silva, PJ Fonseca & MCP Amorim (2024). Playing Who's Who?: The Challenge of Undocumented Sound Production in Marine Acoustic Communities. XXI Congress of the Portuguese Ethological Society, Lisboa, Portugal.
- **Ríos N**, M Jimenez, G Franco, G Ramos, MP Pais, EJ Gonçalves, MCP Amorim, G Silva (2023). Characterizing the behaviour of bait-attracted *Prionace glauca* using pelagic drift video. XX Congress of the Portuguese Ethological Society, Vila do Conde, Portugal.
- **Ríos N**, J Pereira, M Vieira, G Silva, MP Pais, PJ Fonseca, MCP Amorim (2023). Assessment of spatial and temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area. XX Congress of the Portuguese Ethological Society, Vila do Conde, Portugal.
- **Ríos N**, J Pereira, M Vieira, G Silva, MP Pais, PJ Fonseca, MCP Amorim (2023). Assessment of spatial and temporal patterns of nearshore fish communities through passive acoustic monitoring in a Marine Protected Area. Ciências Research & Innovation Day 2023, FCUL, Lisboa, Portugal.
- Pereira J, **N Ríos,** M Vieira, PJ Fonseca, MCP Amorim (2023). Characterization of the Acoustic Fish Communities in the Professor Luis Saldanha Marine Park (Portugal). ESCBC 2023 - 9th European Student Conference on Behaviour & Cognition, Lisbon, Portugal

6.3.2 General public divulgation

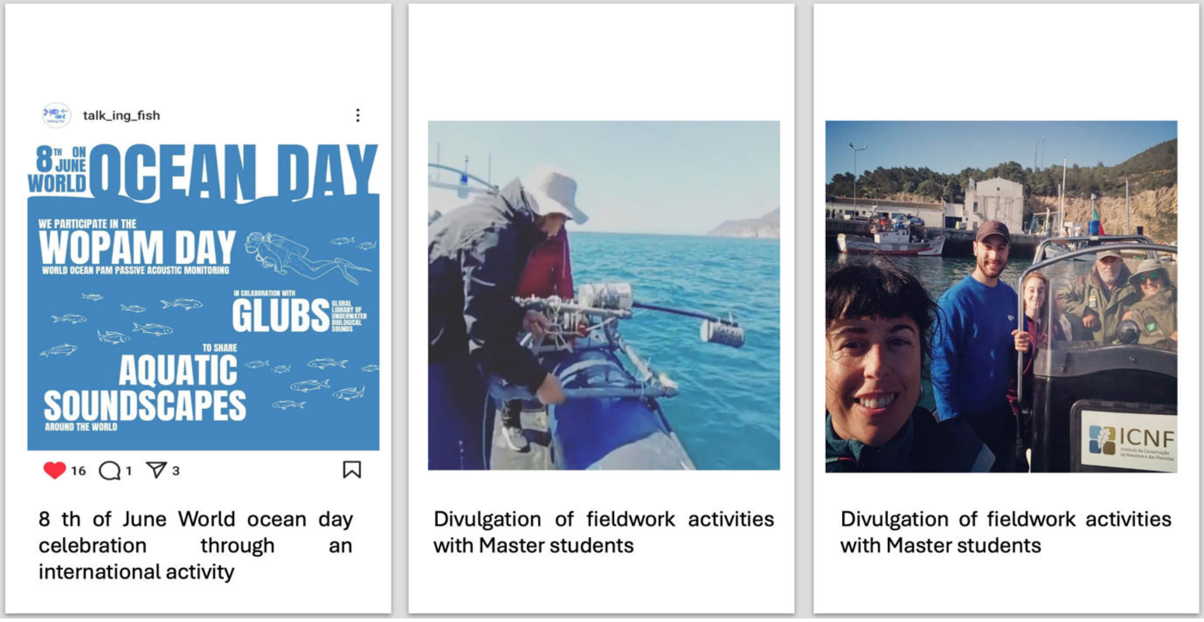
Outreach activities to the general public focussed on environmental and bioacoustics topics targeting different age groups, offering educational activities for children and adults. The activities included cultural music festivals like Sonar (https://www.sonarlisboa.pt/pt), special events for Children's Day (https://www.pavconhecimento.pt/dia-da-crianca/2024) and also summer activities with children organized by maritime tourism companies. Moreover, we participate in World Ocean Day (https://www.iqoe.org/articles/world-oceans-passive-acoustic-monitoring-day-wopam-day).



Sonar +D showroom 2022 Lisbon

Activities with children associated to maritime touristic companies Setubal

Children day 2023 at Ciencia Viva center Lisbon



8 th of June World ocean day celebration through an international activity

Divulgation of fieldwork activities with Master students

Divulgation of fieldwork activities with Master students

Figure 6.2 Examples of activities to the general public and students, also divulged in social media.

In addition, following the publication of the two thesis-related scientific articles, press releases were issued, resulting in several journalistic pieces, interviews, and news coverage on major TV channels see list below.

In addition, the published articles have been broadcast by newspapers and TV programs.

Divulgation of chapter III:

https://www.fishbioacoustics.pt/news

SICNoticiaspt
https://e-global.pt/noticia/vida/ciencia/estudo-pioneiro-revela-diversidade-sonora-dos-peixes-na-costa-portuguesa/
https://www.diariodigital.pt/noticias/regioes/mare-lanca-primeiro-catalogo-sonoro-de-peixes_682eafc494d88003940c57cf
https://observador.pt/2025/05/21/identificados-33-tipos-de-sons-diferentes-de-peixes-em-investigacao-pioneira/
https://www.rtp.pt/noticias/pais/identificados-33-tipos-de-sons-diferentes-de-peixes-em-investigacao-pioneira_n1656287
https://www.cmjornal.pt/sociedade/detalhe/peixes-emitem-33-tipos-de-sons
https://www.ambientemagazine.com/mare-lanca-primeiro-catalogo-acustico-da-costa-portuguesa/
https://open.spotify.com/episode/6xtDshqrQR6zzxatNmBCAs?si=631366094cb04f05

Divulgation of chapter V:

https://www.ipressjournal.pt/estudo-revela-a-importancia-da-protecao-do-tubarao-azul/
https://anacao.sapo.pt/estudo-sobre-o-tubarao-azul-sublinha-a-importancia-da-protecao-e-da-preservacao-dos-canhoes-submarinos/
https://www.instagram.com/p/DF5Xs68tNja/?igsh=MXd0Yjlvenc5dmVwag%3D%3D
https://www.diarioleiria.pt/2025/02/10/estudo-sobre-tubarao-azul-destaca-necessidade-de-protecao-dos-canhoes-submarinos/
https://www.youtube.com/watch?v=O35MOP6z4Zs
https://sustentix.sapo.pt/estudo-sobre-tubarao-azul-destaca-necessidade-de-protecao-dos-canhoes-submarinos/
https://www.facebook.com/share/1AHQ4eYppF/
https://www.mare-centre.pt/pt/bercario_tubaraoazul
https://greensavers.sapo.pt/na-arrabida-pode-esconder-se-um-bercario-de-tubaroes-azuis/
https://www.fishbioacoustics.pt/news

Instagram Sci-Comm Strategy (2022–25)

All the activities have been divulgated through social media on Instagram @Talk_ing_fish (1142 followers) (**Figure 6.1** and **6.2**). Between March 2022 and March 2025, 48 Instagram posts were published (both images and videos) as part of a structured science-communication strategy. The objective was to amplify public engagement with marine ecology research by showcasing field work, research highlights, and outreach events. The account posted on an almost monthly basis throughout the period, supplemented by 111 Instagram stories featuring fieldwork activities, publications, and other scientific content, each aimed at promoting new posts and maintaining engagement.

The main goals of this section included building a scientifically interested follower base. To achieve this, I linked my personal network, followed relevant associations and interest-based accounts, used targeted hashtags (#Marinebiology, #fishsounds #marineecology, #PLSMP, #MPA #Ocenaconservation, #Acousticecology #behaviour), tagged collaborators and institutions (ICNF, MARE, ISPA), and cross-posted across channels. Effective use of Instagram in academic settings can enhance engagement and communication strategies (Carpenter 2020, Cabyová et al. 2024) The strategy also featured collaborations via tagging partners (e.g. conference organisers such as SPEA, IBAC, MARE, outreach days *Kids Day*, or WOPAM) and occasionally inviting guest contributions or takeovers, as recommended for science outreach accounts.

Overall, the engagement data reflect the impact of videos, with intrinsic view counts peaked at 4 818 views in October 2022, 1 831 in April 2023, and 1 208 in March 2025, while image posts generated consistent likes in the 20–62 range. Insights metrics (views/accounts reached) for later months show reach between 425 and 1 208, with 52%–66% of followers reached. These trends mirror broader Instagram benchmarks: for small accounts, reels typically drive more impressions, while images yield slightly lower engagement than video.

By tracking key metrics—follower growth, engagement rate, reach, impressions and comments—I could analyse which content formats resonated most and refine the approach accordingly. Altogether, this multi–format, collaborative, and data-driven approach enabled steady follower growth, enhanced public visibility, and contributed significantly to my scientific outreach goals.

A video was also made to explain Passive Acoustic Monitoring (PAM) and their application on the Professor Luiz Saldanha Marine Park and was divulgated in several media, Instagram, youtube, and fishbioacoustics web. Link of the video https://www.instagram.com/reel/CyVdGyDtsOK/?igsh=dncwcHR6djUyZ3lv

Besides, fish sounds and other scientific material were divulged through web links like https://www.fishbioacoustics.pt, https://www.wo-pam.com/, and https://www.glubs.org/.

6.4 Acoustic knowledge survey for PLSMP users

To assess public perceptions of underwater sounds and their relevance for fish acoustic communication, a structured online opinion survey with eleven questions was conducted in Portuguese and/or in English. Two versions of the survey were randomly distributed online through a link: one including a short educational video and one without, to test for differences in responses. The target audience focused primarily on users (diving centres, tourists, workers) of the Professor Luiz Saldanha Marine Park (PLSMP). The survey was open during August and September 2023 and disseminated via social media (Instagram), email, and posters with QR codes displayed in maritime tourism centres in Sesimbra, adjacent to the Marine Park. Questions Q1-Q4 were related to demographic information (age, sex, and profession), while questions Q6 to Q10 assessed people's perception of underwater sound using Likert levels, multiple choices. Q11 was a free question that was used to extract keywords from the answers. All responses were collected individually and anonymously. Participation was voluntary, and respondents were informed about the purpose of the survey, its duration, and how the data would be used. In compliance with the European General Data Protection Regulation (GDPR; European Parliament & Council, 2016), data were processed lawfully, fairly, and transparently, solely for the purposes of this study. No personally identifiable information was recorded, and participants were free to withdraw at any time without providing a reason. All data were stored securely in password-protected files accessible only to the research team. As the survey collected anonymous data and no sensitive personal information, formal Ethics Committee approval was not required under GDPR guidelines.

The survey was completed by a total of 90 participants. Demographic data were collected to characterise the sample population. The age of participation in the survey ranged between 18 to 75 years old (Q1). The sex ratio of participation was 51 women and 36 men (Q2). From the 38 different professions of the participants, 70,1% did not have an ocean-related profession, while 29,9% had ocean related professions (Q4). In the former, the most common were biologists (n =12), teachers (n =9), followed by consultants, engineers and students (n = 6) (Q3). Regarding the level of appreciation of the sea (scale of 1 to 5, being 5 the maximum), 83,4% selected the maximum level (Q5). The question addressed if the underwater world is silent, 33% agreed or were neutral, while 66,7 % disagreed (Q6). 80% of participants agreed or strongly agreed that sound could be used to assess the biodiversity of marine environments (Q7) and 98% agreed or strongly agreed with marine traffic affecting marine life (Q8). When questioned on which animals produce sound (whales, invertebrates, fish), the lower percentage was for fish (22%), while whales represented 52% of the answers (Q9). 98 % of the participants stated they felt very good when listening to the ocean. Open-ended responses about what participants hear while swimming most frequently included the following keywords: waves, boats, nothing, bubbles, and stones. Results are represented in **Figure 6.3**.

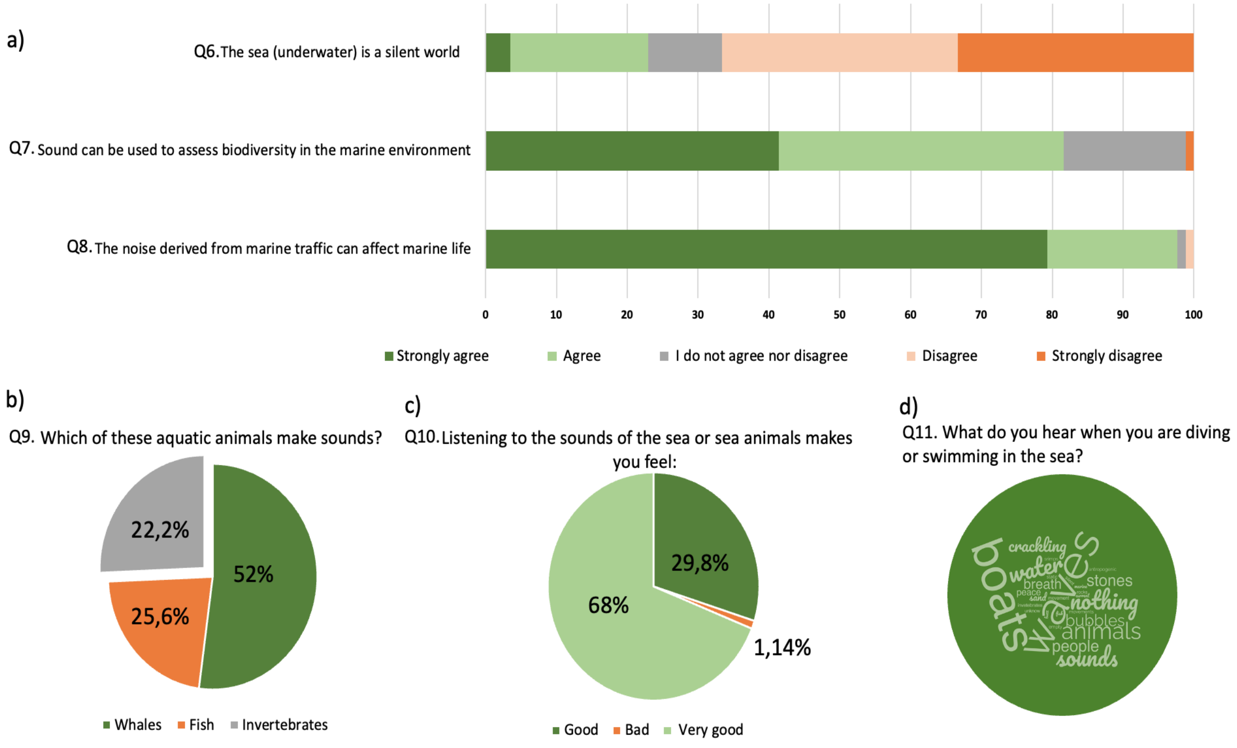


Figure 6.3. Questionnaire results for questions Q6-Q8 (a), Q9 (b), Q10 (c) and for the open-ended question Q11 (d). Results are shown as percentages except for (d) where a word cloud is shown.

The survey indicated that most participants (80%) acknowledged that sound can be utilised to assess marine biodiversity, but only 25.6% of participants identified fish as sound-producing animals. However, 98% of the participants recognised the impact of marine traffic noise on marine life. These findings align with broader research highlighting the detrimental effects of anthropogenic noise on marine ecosystems (Duarte et al. 2021). This aligns with studies emphasising that fish sound could be impacted by anthropogenic noise that can interfere with vital activities like reproduction, feeding or communication. Nevertheless, the mechanisms by which fish avoid this interference are not well understood (Radford et al. 2014). Besides, a Portuguese study over seahorses indicates that boat noise causes stress and site abandonment in seahorses, and this study supports calls to explore navigation exclusion zones to protect coastal populations (Palma et al. 2019).

Participants also reported positive emotional responses to natural underwater sounds, with 98% stating they felt "good" or "very good" when listening to the ocean. This is in line with research indicating that natural soundscapes contribute to psychological well-being and stress recovery (Shu 2023).

The findings indicate that, although there is a general awareness of underwater acoustics among the public, particularly regarding the negative impact of anthropogenic noise, there remains a need for focused educational efforts to improve understanding of fish bioacoustics and the potential effects of anthropogenic noise on acoustic communities. Increasing public awareness of underwater acoustics can play a crucial role in conservation by strengthening the connection between people, the ocean, and its biodiversity, fostering a greater appreciation of marine ecosystems, and promoting behaviours that support the protection of acoustic habitats and overall marine health (NOAA Fisheries 2025, Ocean Research Institute 2025). Advantages of positive emotional connections that individuals have with natural marine sounds could be instrumental in fostering greater support for acoustic conservation in marine environments.

In conclusion, this chapter emphasises the vital role of public engagement in marine conservation. The study not only contributes to the national research base but also raises awareness of fish bioacoustics and the impacts of underwater noise in the PLSMP. Through targeted science communication, the programme aims to inform stakeholders, foster environmental literacy, and encourage community participation in conservation initiatives.

Such efforts enhance public understanding of marine ecosystems and support their protection, reflecting the principle that we can only conserve what we truly understand.

CHAPTER VII

General Discussion and Conclusion

Chapter VII: *General Discussion and Conclusion*

Acoustic research has been conducted to study acoustic fish communities in the coastal waters of Portugal, specifically in the Azores and Madeira archipelagos (Carriço et al. 2019, Muñoz-Duque et al. 2024), as well as in estuary environments (Vieira et al. 2021). This thesis is the first to apply acoustic monitoring to investigate fish assemblages in a MPA in mainland Portugal, specifically in Professor Luiz Saldanha Marine Park (PLSMP), where the park has been monitored since 2007 using visual and other monitoring techniques.

This study focuses on the soundscapes, specifically examining fish acoustic communities, their temporal variability, and the impact of anthropogenic noise on key species. The research is organised into chapters that explore various monitoring methods used to assess fish acoustic diversity and evaluate the ecological conditions of the PLSMP. **Chapter I** introduces main concepts that are needed for the understanding of the thesis. To provide a basis for assessing the fish acoustic community in mainland Portuguese coastal areas, fish sounds were identified and classified, leading to the creation of the first marine fish sound library for this area. Potential sound sources were then investigated through a literature review of soniferous species, combined with the integration of PAM and BRUVS methodologies (**Chapter II**). The temporal, spatial and environmental factors that influence fish sound occurrence in PLSMP were studied, providing a baseline to monitor future changes in these fish communities (**Chapter III**). Furthermore, the effectiveness of acoustic (PAM) and visual methods (BRUVS and UVC) in providing ecological and acoustic indicators were compared, highlighting the potential of PAM for assessing marine environmental diversity (**Chapter IV**), while also showing the strengths and limitations of each method for monitoring marine environments. The last part of the study assessed behaviour patterns of endangered species, such as the iconic blue shark and the impact of anthropogenic pressure (boat noise) on this species (**Chapter V**).

Furthermore, outputs from this thesis were used in divulgation activities to promote ocean literacy and raise public awareness about the importance of monitoring the marine environment, particularly through the use of PAM (**Chapter VI**). The final section presents a general discussion and conclusion, synthesizing the main findings of the thesis, comparing and integrating results across chapters, and outlining directions for future research (**Chapter VII**).

7.1 Acoustic fish community (Chapter II)

Chapter II was published in ICES Journal of Marine Science (2025) Doi: https://doi.org/10.1093/icesjms/fsaf027. This study identified soniferous fish species within the PLSMP, a MPA in the Portuguese mainland coast. Of the 150 fish species recorded in the park, 28 were confirmed as soniferous, while 71 were classified as potentially soniferous. A total of 33 distinct fish sound types were identified, contributing significantly to the marine soundscape in the area. Fish sounds were first categorised into two principal groups: *pulsed* and *continuous* sounds. Pulsed sounds were divided into *low* and *high* frequency sounds and continuous sounds were split into *tonal* and *noisy* subcategories, and then further divided by frequency bands. The use of such a dichotomous key in fish bioacoustics is relatively recent but has shown significant potential for standardising the categorisation of diverse acoustic repertoires and have been used in other areas (Muñoz-Duque et al. 2024, Puebla-Aparicio et al. 2024). These findings suggest that the number of vocal fish species along the Portuguese coast may be higher than currently documented, as many species remain acoustically unstudied (Carriço et al. 2019).

The sound type richness in PLSMP is comparable to other regions, including tropical habitats: for example, Bertucci et al. (2020) discovered 22 fish sounds in a Polynesian coral reef, while Raick et al. (2023) detected 45 fish sound types across various Polynesian islands and water depths. In coral reefs in islands in Mozambique and Hawaii, 47 and 85 sound types were respectively identified (Tricas & Boyle 2014, Puebla-Aparicio et al. 2024). In subtropical and mediterranean climates, Muñoz-Duque and colleagues (2024) documented 43 fish sounds from a total of 188 fish species, including 35 soniferous and 102 potentially soniferous species in the Macaronesia region, while in the Tavolara-Punta Coda Cavallo MPA (Mediterranean Sea) 12 fish sound types from 53 fish species were identified (Desiderà et al. 2019). Other habitats can be extremely species-rich, a pattern often reflected in the high diversity of detected fish sound types. For example, Wang and colleagues (2017) identified 66 types of fish sounds in the Pearl River estuary, an area renowned for its high fish biodiversity. Although 834 species were reported historically, this number declined to 287 species by 2008 and may be smaller at present. These data are represented in **Figure 7.1.**

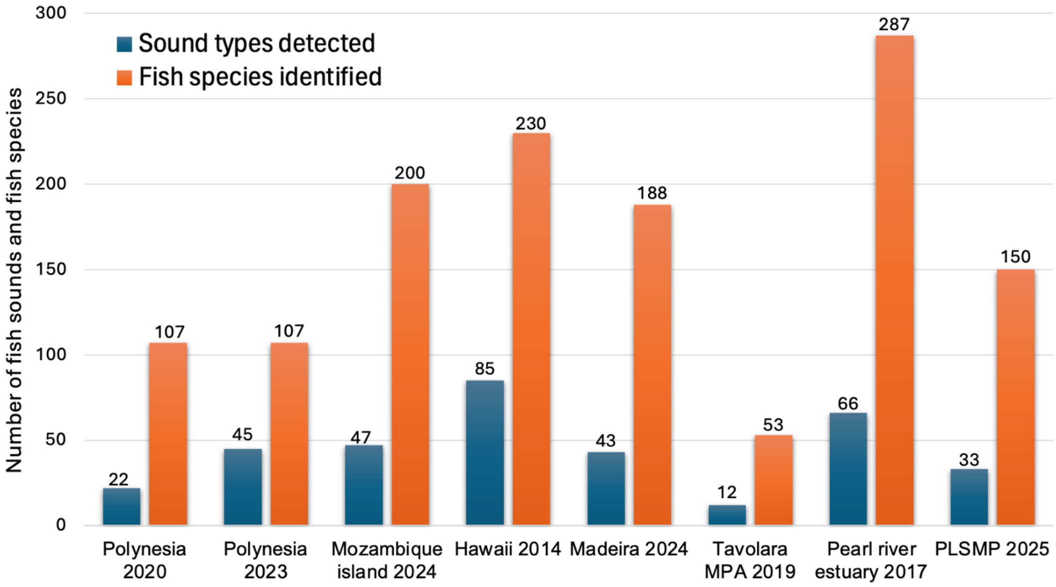


Figure 7.1. Comparison of the number of fish sound types detected and the total number of fish species identified in different marine regions and habitats. Data sources: Polynesia – 22 sound types (Bertucci et al. 2020); Polynesia – 45 sound types and 107 species (Raick et al. 2023); Mozambique Island – 47 sound types and 200 species (Puebla-Aparicio et al. 2024); Hawaii – 85 sound types and 230 species (Tricas & Boyle 2014); Madeira, Macaronesia – 43 sound types and 188 species (Muñoz-Duque et al. 2024); Tavolara–Punta Coda Cavallo MPA (Mediterranean Sea) – 12 sound types and 53 species (Desiderà et al. 2019); Pearl River estuary – 66 sound types and 287 species (Wang et al. 2017); PLSMP – 33 sound types and 150 species (Ríos et al. 2024). Note that numbers are not corrected for sampling effort which differs between studies.

Comparative analyses with existing literature revealed strong similarities between recorded sounds and known vocalisations of specific fish species. For example, sound type #42 resembles vocalisations of the genus *Chromis*, sound type #43 is similar to the /kwa/ call of *Scorpaena* species in the Mediterranean, while sound type #15 is likely produced by the invasive weakfish (*Cynoscion regalis*) during its spawning activity. Sound type #10 is comparable to the grunt of the grey gurnard (*Eutrigla gurnardus*) during competitive feeding and sound types #4 and #8 are potentially produced by Serranidae species, particularly

Serranus hepatus, *Serranus cabrilla*, or *Epinephelus marginatus*, although further validation is required. Despite these associations, many detected sounds remain unidentified, which remains a significant challenge in PAM.

7.2. Temporal and spatial patterns (Chapter III)

PAM enables non-intrusive ecological observations by capturing marine soundscapes, including fish vocalisations (Duarte et al. 2021). Analysing these soundscapes aids in assessing species phenology and recognising temporal rhythms (Ruppé et al. 2015, Sueur et al. 2019). This study (**Chapter III**), submitted in Marine Biology, examines the temporal patterns of occurrence of the 33 fish sound types identified in **Chapter II.** Seasonality had no significant effect on overall soundscape composition, though certain sound types exhibited seasonal tendencies. Fish choruses are more common during the spring and summer months when fish are in their reproductive season (Gendriz & Padovese 2017, Vieira et al. 2022). Nevertheless, they were rarely recorded during this work, with only one observed chorusing event (associated with sound type #15 related to a nonnative species *Cynoscion regalis*). Non-chorusing vocalisations also showed some seasonal variations, with sound type #3 increasing in summer. This variability in activity could have been driven by reported sources of fish activity like migration, reproduction, and food availability (Rice et al. 2017). Species-specific responses to water temperature may further shape these patterns (Ladich 2018). Notably, time of day significantly influenced fish vocalisation behaviour, peaking at night and sunset, consistent with other studies (McCauley & Cato 2000, Parsons et al. 2016, Rice et al. 2017). For example, nocturnal activity, associated with feeding, aggression, and reproduction, is well-documented in species such as sciaenids, groupers, and toadfish (Mann et al. 2009, McIver et al. 2014). Increased sound production under low light conditions may function as a predator-avoidance and mate-attraction strategy (Wilson et al. 2020). The plainfin midshipman (*Porichthys notatus*) studied in California, exhibits pronounced diurnal and seasonal rhythms during the night of the breeding season (Rubow & Bass 2009). In Hawaiian coral reefs, high-frequency fish pulse-train sounds (0.5–1.5 kHz) increase markedly during moonlight hours, whereas low-frequency fish vocalisations (0.1–0.3 kHz) decrease, revealing a lunar-light influence on acoustic activity (Duane et al. 2024) in these environments.

Although there were no replicates per location, fish sound diversity increased with distance from the Sado estuary. Estuarine gradients and habitat features such as mangroves, seagrass beds, and reef complexity are known to significantly influence fish and invertebrate assemblages (Nagelkerken et al. 2015). Also, most sounds occurred in the rocky BA zone, while the rock/sandy interface with a shorter rocky reef in FPA had lower richness, highlighting the importance of habitat complexity for biodiversity (Gratwicke & Speight 2005). In fact, complex substrates are recognised for shaping fish community composition by promoting greater species richness (Sánchez-Caballero et al. 2017, Castaño et al. 2021). Rocky coastal habitats, characterised by their heterogeneous physical structures, are well documented to support diverse biological assemblages due to the range of microhabitats available. Classic ecological studies have shown that such environments host a high diversity of species, including molluscs, crustaceans, and algae, which may explain the elevated diversity recorded in these zones (Little & Kitching 1996).

7.3 Visual versus acoustic monitoring, a method comparison (Chapter IV)

Effective marine conservation relies on robust monitoring to inform management and detect ecological changes (Fortuna et al. 2024). In **Chapter IV**, we compare fish community diversity in the PLSMP using visual (UVC, BRUVS) and acoustic (PAM) methods. Our results reveal clear differences in biodiversity metrics among methods. UVC recorded the highest abundance, species richness, and diversity indices, detecting 43 species of the 150 fish species identified in the area, mainly benthic and reef-associated families such as Labridae and Gobiidae (Serrão et al. 2021). In contrast, BRUVS detected slightly fewer species (39), favouring mobile, bait-attracted taxa such as Sparidae and Carangidae (Whitmarsh et al. 2017, Langlois et al. 2010). PAM detected 25 of the 33 sound types described in the area, representing a high proportion of the acoustic richness, and provided complementary data on cryptic and nocturnal taxa often missed by visual methods (Radford et al. 2015). Species accumulation curves confirmed UVC's greater efficiency at low sample sizes, while BRUVS showed a moderate performance and required a greater sampling effort to match UVC's richness. Although a substantially lower asymptote in the accumulation curve, reflecting its focus on vocal species, PAM showed an early plateau, highlighting its effectiveness. These results support previous findings that active visual methods are effective for detecting mobile reef species and assessing protection effects (Colton & Swearer 2010, Murphy & Jenkins 2010), while PAM offers the ability to detect patterns that may be overlooked by traditional visual surveys, giving unique insights into the acoustic structure of fish communities. PAM is especially effective at capturing signals related to habitat complexity, revealing how structural features of the environment influence species presence, behaviour, and sound production (Velasco-Charpentier et al. 2021). The spatio-temporal differences among methods were driven more by species detectability inherent to each technique, together with seasonal and habitat preferences of the species and overall habitat complexity, than by protection areas itself. Interestingly, no single method sufficiently captured fish biodiversity within the study area as each monitoring technique has its own limitations. UVC can overestimate conspicuous or diver-attracted species while underestimating shy ones, and its effectiveness depends on visibility and diver endurance (Ward-Paige et al. 2010, Pais & Cabral 2018). BRUVS tends to favour bait-attracted species and is constrained by a limited field of view (Whitmarsh et al. 2017). PAM, on the other hand, cannot detect silent or low-vocalizing species or life stages, faces difficulties in identifying overlapping or similar sounds, is affected by environmental noise, and relies on acoustic propagation conditions (Browning et al. 2017)

These results align with previous studies showing complementary strengths of visual and acoustic methods across habitats and seasons (Bertucci et al. 2016, Harris et al. 2016). Overall, combining or interspersing these approaches offers a more comprehensive and reliable way to assess fish assemblages in relation to both protection status and environmental variability. Combining acoustic monitoring with complementary approaches such as visual surveys and environmental DNA (eDNA) provides a more comprehensive and robust assessment of fish biodiversity (Valdivia-Carrillo et al. 2021). For instance, integrating PAM and eDNA in Réunion Island coral reefs yielded more complete species inventories and improved understanding of assemblage structure (Elise et al. 2022). Embedding these tools within unified monitoring frameworks enhances biodiversity detection, reduces method-specific biases, and expands spatial–temporal coverage (Muenzel et al. 2024).

Chapter IV also compared traditional ecoacoustic indices (ACI, H, SPL) and provided additional perspectives on soundscape diversity. ACI correlated positively with traditional biodiversity metrics, though it may overemphasise dominant sound sources; H reflected acoustic balance but declined in uneven soundscapes; and SPL, though uncorrelated with biodiversity indices, captured seasonal or event-driven changes in acoustic intensity. These results were consistent with other studies (Bolgan et al. 2018, Pieretti et al. 2020, Halliday et al. 2020). The relationship between acoustic and visual diversity metrics remains inconsistent across studies (Bertucci et al. 2016, Desiderà et al. 2019), but combining acoustic indices with visual surveys, potentially enhanced by automated methods can improve the accuracy and efficiency of biodiversity monitoring (Buxton et al. 2018, Williams et al. 2022). Ecoacoustic measures such as ACI and H demonstrate moderate to strong correlations with species richness and habitat quality.

In temperate reef environments, ACI has proven sensitive to biological pulsed signals and habitat quality, although its performance varies with noise levels and signal dominance (Pieretti et al. 2011, Roca & Van Opzeeland 2020). A comparative study indicates that ecoacoustic indices overall maintain a moderate positive relationship with traditional diversity measures, but outcomes are highly context-dependent and warrant cautious interpretation (Bertucci et al. 2016, Raick et al. 2023). Acoustic indices can accurately predict avian vocal diversity in some contexts, providing insights into species richness and vocal activity (Buxton et al. 2018). They also capture functional processes such as insect herbivory, though their reliability may be reduced by non-biological noise or environmental factors (Martini et al. 2024). Since their performance is highly context-dependent (Sethi et al. 2023), integrating acoustic indices with established approaches reduces variability and strengthens biodiversity monitoring (Ross et al. 2017, Gaspar et al. 2023).

7.4 Behaviour patterns of bait-attracted blue shark (Chapter V)

7.4.1 Observation of occurrence and behavioural patterns

In marine conservation, besides assessing biodiversity and communities, it is important to monitor target species, namely top predators, as sharks, that maintain community structure and the balance of marine ecosystems (Heupel et al. 2014). Because elasmobranchs are generally considered silent species (for exceptions see Almagro & Barria 2024, Barroil et al. 2024, Nieder et al. 2025), PAM was not used to monitor this group. In **Chapter V**, BRUVS were employed to examine the occurrence and behavioural patterns of blue sharks outside the PLSMP, with particular attention to the effects of boat noise on their foraging behaviour. Blue shark distribution in the study area varied by life stage and sex. Juveniles were more common in spring and found in epipelagic and mesopelagic zones, while adults (mainly males) were more frequently observed in autumn and in deeper bathypelagic areas, particularly near the Lisbon and Setúbal canyons. These patterns align with North Atlantic studies showing that larger blue sharks prefer warmer and deeper waters, while smaller individuals are more abundant in colder waters (Nakano & Stevens 2008, Vandeperre et al. 2014, Coelho et al. 2017). Longline data indicate that juvenile females dominate catches along the Portuguese coast (Queiroz et al. 2005). Breeding occurs in spring, with nursery areas identified off Portugal, northern Spain, and the Azores (Aires-da-Silva et al. 2008). High shark density and prolonged presence suggest the study area may also serve as a nursery (Heupel et al. 2018), which has important implications for management and conservation efforts. Interestingly, behavioural patterns differed by life stage. Juveniles exhibited greater curiosity toward bait, frequently interacting with BRUVS using visual, tactile, and electroreceptive senses (Gardiner et al. 2014). This suggests that BRUVS may be especially useful to study the behaviour of juveniles. In summary, this study demonstrates the effectiveness of BRUVS in studying elusive species' movements and behaviour patterns, as well as evaluating the impact of human activities over these patterns, as discussed below.

7.4.2 Impact of boat noise over behaviour patterns

Boat noise falls within the hearing range of sharks (from 40 Hz to 1500 Hz) (Chapuis & Collin 2022, Nieder et al. 2023) and can cause behavioural changes in aquatic animals (Chapuis et al. 2019, Alves et al. 2021). In this study, sharks that were exposed to boat noise did not show a significant difference in foraging duration compared to control sharks despite these typically exhibiting 20% longer behaviours on average. However, the frequency of behaviours per minute decreased significantly during periods of boat noise exposure, suggesting that anthropogenic disturbances can potentially disrupt essential behaviours such as foraging. While some studies found no impact on feeding (Ryan et al. 2017), others reported escape responses in stingrays (Mickle et al. 2022) and reduced shark presence near BRUVS when exposed to continuous artificial sound below 1 kHz (Chapuis et al. 2019). Small-spotted catsharks also altered their swimming patterns in response to loud sounds (de

Vicenzi et al. 2021). Sharks may habituate to recurrent noise exposure, as seen in areas with high boat activity (Rider et al. 2021). This could lead to chronic stress (Amorim et al. 2022, Di Franco et al. 2023), attraction to boats, especially if associated with hooked fish (Mitchell et al. 2018), and bycatch risk. Additionally, anthropogenic noise has been shown to mask biologically relevant sounds, potentially interfering with the ability of sharks to detect prey (Simpson et al. 2016, Nedelec et al. 2017). This acoustic masking may disrupt foraging efficiency and influence habitat use, leading to broader ecological consequences. Given the reliance of many shark species on acoustic cues for foraging, further research is warranted to understand how chronic exposure to boat noise may affect their spatial distribution and behavioural ecology. Furthermore, anthropogenic noise has been increasingly recognised as a disruptive factor in fish communication, especially during critical behaviours such as courtship, spawning, and territory defence (Radford et al. 2014). These findings further support the urgent need to consider acoustic pollution in marine spatial planning, particularly in MPAS and in known spawning aggregation and nursery areas.

7.5 Challenges and perspectives of PAM

7.5.1 Identification of fish sound sources

Effective PAM monitoring requires identifying sound-producing species (Mouy et al. 2018). Field studies have reported a low number of identified fish sound sources, with Anderson and colleagues (2008), and Puebla-Aparicio and colleagues (2024) each identifying sources for only four fish sound types in different regions. In this thesis, besides comparing the detected sound types with the ones reported in the literature, identification of sound sources was also attempted by fitting autonomous acoustic data loggers together with BRUVS. Video analysis identified six distinct sound types but determining the exact source was challenging as sounds could not be directly linked to a fish in the videos. In general, challenges in identifying sound sources with BRUVS included noise from the bait device or other sources, the presence of multiple species in the frame, species located outside the frame, and difficulties in linking sounds to specific behaviours. Competitive feeding may also not be the best context to observe sound production in fishes as it is seldom reported in the literature (e.g. Amorim et al. 2004). Although video techniques are valuable for identifying fish sound sources in natural habitats, BRUVS may not be the most effective method. Alternative approaches include Remote Underwater Video (RUV) (Puebla-Aparicio et al. 2024), hydrophone arrays with cameras (Mouy et al. 2018), directional hydrophones, and 360º cameras with acoustic recorders (Maralunda et al. 2017, Dantzker et al. 2025) or Long-life Remote Underwater Video (LL-RUV) (Buschmann et al. 2024). In Italy, marine cabled observatories have also been utilised, integrating video and acoustic technologies to enable continuous, multiparametric monitoring of marine ecosystems (Buscaino et al. 2025). These approaches can help to address issues encountered with BRUVS, although they also have limitations.

Combining field research with captive studies, where sound production can be recorded from a single species, can help identifying sound-producing species. Researchers have identified fish sound sources both in their natural habitats, for example through combined audio and video recordings (Puebla-Aparicio et al. 2024), and in the laboratory (Bolgan et al. 2019, Amorim 2023). Both approaches combined provide a comprehensive view of fish acoustic communication and enhance our understanding of soniferous fish species. Furthermore, by integrating various research methods (video and acoustic), we can enhance the detection of sound sources.

The field of fish bioacoustics has long been constrained by the absence of a comprehensive inventory of soniferous fishes, limiting progress in the study of acoustic communication. A systematic database of fish species examined for sound production is necessary to avoid duplication of efforts and to reveal ecological and evolutionary trends. Comprehensive datasets are also essential to ensure that the identification of soniferous species is not biased towards certain taxa or geographic regions. To address this challenge, several initiatives have been established to centralise and share bioacoustic information. Examples include FishSounds.net and the *Global Library of Underwater Biological Sounds (GLUBS https://www.glubs.org/)*, developed in collaboration with the *World Register of Marine Species (WoRMS)* (Looby et al. 2023, Parsons et al. 2024). Beyond fish-focused libraries, open-access soundscape repositories are emerging as critical resources for ecological and conservation studies. As for example the site maintained by the *UK Acoustic Network* https://acoustics.ac.uk/open-access-underwater-acoustics-data. *The International Quiet Ocean Experiment* (IQOE) maintains an *Acoustic Data Portal* that aggregates passive acoustic datasets from national and regional monitoring systems worldwide (https://iqoe.org/acoustic-data-portal). The NOAA *National Centers for Environmental Information* (NCEI) maintains a Passive Acoustic Data Archive (https://www.ncei.noaa.gov/products/passive-acoustic-data), which houses raw and processed recordings submitted by NOAA programs and external collaborators. Meanwhile, NOAA's Pacific Marine Environmental Laboratory (PMEL) leads a global passive acoustics research program, deploying hydrophones across multiple ocean basins to characterize ambient and anthropogenic sound (Dziak et al. 2023). The *SanctSound* project (Wall et al. 2021) operates hydrophone stations across U.S. national marine sanctuaries, generating multi-year acoustic datasets archived at NCEI under standardized soundscape processing protocols. Finally, the *Open Portal to Underwater Soundscapes* **(**OPUS **https://opus.aq/)** aims to serve as a global hub for standardized, MANTA-processed passive acoustic data, enabling large-scale, long-term comparisons of ocean soundscapes and anthropogenic noise (Thomisch et al. 2021).While these initiatives represent essential steps forward, sustaining and expanding such portals requires considerable coordination, long-term funding, and standardisation of metadata and methodologies (Parsons et al. 2022).

Such databases are valuable not only for cataloguing known sounds but also for storing unknown ones. Maintaining collections of unidentified sounds is crucial—not only for immediate species identification but also for advancing bioacoustics, contributing to biodiversity research, and informing conservation efforts (Jarriel et al. 2024, Vieira et al. 2024). The prevalence of unidentified sound types underscores significant opportunities for future research (Carriço et al. 2020). Crucially, linking these sounds to their source species is essential both for advancing the field of bioacoustics (Wall et al. 2013) and for improving the effectiveness of PAM (Browning et al. 2017).

Moreover, standardized sound archives enable cross-regional comparisons and support biogeographic assessments (Vieira et al. 2024). Furthermore, the Worldwide Soundscapes project (Darras et al. 2025) provides an extensive, cross-realm repository of passive acoustic data, offering researchers a valuable resource to study biodiversity patterns, monitor anthropogenic impacts, and advance bioacoustic research globally. Expanding these databases and applying AI to compare acoustic features can greatly improve the efficiency of automated analyses, allowing researchers to extract insights more effectively. In this regard, world acoustic databases represent a major step forward in documenting global acoustic diversity, and a promising future for the use of bioacoustics.

7.5.2. Long term acoustic monitoring

Manual analysis of large acoustic datasets, like that conducted in **Chapter III**, is a major limitation in bioacoustics research and restricts detailed study of temporal patterns in fish acoustic communities. Acoustic monitoring also faces practical challenges, including power supply, data transfer, and managing large volumes of data (Sheng et al. 2019, Bradfer-Lawrence et al. 2023). Recent advances, such as audio source-separation models applied to seafloor observatory recordings, allow marine soundscapes to be separated into biophonic, geophonic, and anthropophonics components, enabling more precise ecoacoustic monitoring (Lin et al. 2021).

Manually reviewing recordings takes time and makes it hard to detect detailed patterns or long-term trends in sound production. Analysing large datasets from multiple sites is particularly demanding, often leading to inconsistent classifications and observer bias (Campos-Cerqueira & Aide 2016, Roch et al. 2016), a limitation similar to that observed in other methodologies. A promising solution lies in the automation of sound detection by an initial classification and posterior Artificial Intelligence (AI) implementation using machine learning techniques for data processing. Machine learning can provide a robust solution to detect and classify fish sounds (Barroso et al. 2023). By using machine learning, researchers can process extensive datasets, aiding in the identification and classification of these unidentified sounds. These approaches have shown great potential for streamlining the analysis of large bioacoustic datasets, improving efficiency, and reducing human error (Stowell et al. 2019, Priyadarshani et al. 2020). However, the main challenge of automated and recognized fish sounds is still the lack of validated sound data for individual species (Barroso et al. 2023). Nevertheless, advanced machine learning tools used to analyse acoustic data, like those used by Kahl et al. (2021) (Deep Artificial Neural Networks – applied for acoustic event detection and classification), are crucial for identifying changes in threatened populations or overall acoustic diversity to inform conservation efforts. Wall et al. (2021) highlight that the adoption of internationally recommended metrics for quantifying ambient noise, combined with publicly available, open-source processing scripts such as Manta https://bitbucket.org/CLO-BRP/manta-wiki/wiki/Home, Triton https://github.com/MarineBioAcousticsRC and PamGuide (Merchant et al. 2015), allows the scientific community to standardise analyses and implement these methods efficiently. Using cloud-based routines and interactive platforms like Jupyter notebooks, researchers can process raw acoustic data into calibrated bands, improving the scalability and effectiveness of global use of PAM (Wall et al. 2021).

With advances in technology, the resolution of key challenges, and the growing global interest in knowledge sharing, acoustic monitoring is becoming increasingly important for marine conservation and management, and the development and adoption of these tools hold great promise for advancing the field.

7.5.3 Ecoacoustic indices

Applying acoustic indices such as the Acoustic Complexity Index (ACI), Sound Pressure Level (SPL), and Acoustic Entropy (H) to assess fish marine biodiversity faces several constraints. Most indices were developed for terrestrial systems, and their suitability for marine environments is still under evaluation, requiring refinement to capture the unique acoustic properties of the ocean (Bohnenstiehl et al., 2018). For instance, ACI is sensitive to variations in both sound abundance and diversity, making it difficult to distinguish between these variables without careful calibration of parameters such as frequency and temporal resolution (Bolgan et al. 2018). Similarly, SPL, while useful for capturing overall acoustic intensity, may not reliably reflect biodiversity metrics, as it can be influenced by non-biological noise and environmental factors (Bertucci et al. 2016). Furthermore, H, which measures the balance of acoustic energy across frequencies, can be affected by the presence of dominant sound sources, potentially masking the contributions of less abundant species (Pieretti et al. 2020). These complexities highlight the need for context-specific calibration and the integration of multiple indices to enhance the accuracy of biodiversity assessments in marine environments.

Recent studies have further elucidated these challenges. For example, Raick et al. (2023) found that α-acoustic diversity indices, including ACI, did not consistently correlate with sound type richness or abundance in natural environments, suggesting their limited applicability for inferring marine fish sound diversity. Similarly, Jarriel et al. (2024) reported that unidentified fish sounds, while indicative of coral reef health, require careful interpretation when using indices like ACI and H, as these sounds can be influenced by various environmental factors. Indeed, to be effective, ecoacoustic indices must be validated and calibrated against traditional biodiversity measures, ensuring they reflect marine ecosystem health (Pieretti et al. 2017). More empirical studies tailored to marine dynamics are needed, but once these limitations are addressed, ecoacoustics could provide a powerful, non-invasive approach to monitoring biodiversity (Minello et al. 2021).

7.6 Future applications and conservation relevance

Acoustic monitoring is an emerging method that has undergone substantial advancements in efficiency and analytical capacity for the investigation of marine environments in recent years. Its strengths have increasingly demonstrated its applicability as a powerful tool for marine conservation and ecosystem management. Future applications are described below:

Monitoring rare, cryptic, or nocturnal species:

By capturing sounds from species that are difficult to detect visually, PAM enhances biodiversity inventories and informs conservation priorities. Kaplan et al. (2015) used PAM to detect vocalizing fish species in Caribbean coral reefs that were largely undetected by UVC surveys, including nocturnal or cryptic species. Acoustic monitoring effectively registers rare, cryptic, or nocturnal fish often missed by visual surveys. For example, PAM has recorded spawning sounds of cusk-eel (*Ophidion rochei*) in the Mediterranean (Bolgan et al. 2020), nocturnal choruses of Holocentridae and Priacanthidae (Radford et al. 2008, Banse et al. 2024), and vocalizations of invasive weakfish (*Cynoscion regalis*) in estuaries (Amorim et al. 2023), supporting species inventories and conservation monitoring. These applications expand knowledge of species distributions, inform management decisions for vulnerable taxa, and complement visual and video-based approaches.

Temporal biodiversity monitoring and spatial connectivity movement:

Continuous passive acoustic monitoring allows the detection of temporal trends in species presence and community composition, missed by traditional surveys. For example, PAM has been successfully used to monitor fish assemblages in temperate reefs, revealing seasonal and interannual variations that would not be captured by visual methods alone (Radford et al. 2014). In addition, knowing the temporal dynamics that take place in the marine soundscape is essential for proper management of MPAs. Sounds made by fish and other marine organisms are associated with significant life events, including spawning behaviour, territoriality, and social interactions (Lobel et al. 2010, Rountree et al. 2019). Enhancing our understanding of the temporal change in acoustic communities can therefore be an indicator of ecosystem state and offer essential insight into the reaction of fish communities to human pressures and environmental drivers (Pieretti et al. 2017). Long-term use of PAM through the utilization of autonomous recorders enables the identification of trends and changes in the production of biological sounds, which can be attributed to climate change, habitat loss, fisheries, or anthropogenic noise pollution (Warren et al. 2021). Importantly, this type of monitoring can direct contribute the management of MPAs by revealing where and when vocal animals are most active—e.g., spawning or in nursery habitats for sensitive or commercially important species—so that boat noise or other disturbance can be more precisely managed where it has most effect (Radford et al. 2014).

Hydrophone arrays allow the study of species movements, migrations, and connectivity between habitats. Although this is an example regarding another taxon, Andrews-Goff et al. (2018) tracked humpback whale migrations across Antarctic waters, providing insights into habitat use and connectivity patterns. These temporal and spatial insights can guide MPA design and habitat connectivity management. Such information supports the design of MPAs, ecological corridors and reproductive key areas, ensuring that spatial management measures maintain functional connectivity for marine species.

Detection of human impacts and anthropogenic pressures:

PAM is a powerful tool for tracking both marine life and human activity. By continuously recording underwater soundscapes, PAM detects noise from ships, fisheries, and recreational activities, providing detailed insights into human pressures and compliance with regulations (Merchant et al. 2015, La Manna et al. 2019). Beyond monitoring human activity, PAM assesses anthropogenic impacts on marine fauna as chronic noise exposure can mask biologically important sounds, reduce communication ranges, and displace species from critical habitats. Marine mammals often modify vocalizations or abandon areas under sustained noise (Erbe et al. 2019, Duarte et al. 2021), while shifts in fish acoustic activity indicate stress and behavioural changes in temperate reefs (Harris et al. 2016).

PAM has demonstrated practical management applications: it identified vessel noise in Canadian MPAs, supporting spatial planning and enforcement, and detected unauthorized vessel activity in Hawaii’s Marine National Park (Erbe et al. 2019, Lammers et al. 2020). Integration with machine learning, particularly convolutional neural networks (CNNs), enables accurate detection of anthropogenic sounds in complex soundscapes, distinguishing vessels from environmental or biological sources, and facilitates near real-time monitoring with minimal manual effort (Ji et al. 2024, Allen et al., 2021). Coupling PAM with complementary technologies, such as the Automatic Identification System (AIS), Vessel Monitoring System (VMS), and satellite remote sensing, further improves detection of unreported or non-cooperative vessels, enhancing enforcement and MPA management effectiveness (Kline et al. 2020, McKenna et al. 2024).

By quantifying anthropogenic noise and its ecological effects, PAM informs mitigation strategies, sustainable planning, and regulatory compliance, including EU Marine Strategy Framework Directive (MSFD) Descriptor 11 on underwater noise (European Commission, 2008; Farcas et al., 2020). This integrated approach provides actionable data for adaptive management, revealing human activity hotspots, evaluating protection measures, and guiding conservation decisions. Ultimately, PAM enhances ecological monitoring while delivering critical insights for evidence-based, effective MPA management.

Emerging questions and research opportunities:

During the development of this thesis, numerous questions were brought up that show the complexity and prospects of future research work. One of these questions concerns the identification of species that produced the recorded sounds—a step of primary importance in becoming aware of their ecological functions and how to improve detection. The integration of PAM with camera systems opens up new possibilities for behavioural studies, yet further studies are needed to determine how the integration may be used to push the frontiers of sound source recognition and behaviour analysis. Additionally, integrating the analysis with long-term acoustic recordings reveals patterns that have hitherto not been seen, especially those with a relationship to environmental or anthropogenic variables. Exploring different taxonomic levels - for example, the acoustic signature of invertebrates - remains mostly unexplored. Therefore, identifying long-term patterns in their vocalizations and observing them alongside other taxa could meaningfully supplement ecological monitoring. Recent studies have further suggested that some elasmobranch may be capable of creating sounds, suggesting that they could also be monitored with PAM. Future research should investigate a broader range of environments, with particular attention to the role of sound communication in deep-sea fishes. Equally important is advancing our understanding of how anthropogenic pressures—especially their timing and intensity—affect species of commercial and conservation value within the park. Addressing these open questions will not only advance knowledge in this dynamic field but also emphasize the need for sustained interdisciplinary research efforts.

7.7 Conclusions

Monitoring fish communities is vital for assessing ecosystem health and guiding effective management decisions. Traditional approaches such as UVC and BRUVS remain widely used, but both present limitations. UVC can lead to biased estimates of abundance due to behavioural responses of fish to divers, while BRUVS may struggle to provide reliable density estimates in turbulent environments. PAM offers a complementary, non-invasive, and cost-effective alternative that enables continuous observation under diverse conditions and over extended timescales. Crucially, it captures natural behaviours without disturbing the habitat. When integrated with visual methods, PAM can enhance biodiversity assessments and mitigate resource constraints. Yet, the full potential of this approach depends on efficient data processing, including automated techniques powered by machine learning and artificial intelligence, to manage the vast datasets generated.

The diversity of fish sound types recorded in the PLSMP underscores the importance of studying acoustic communities and their temporal dynamics. Such insights are valuable for conservation, as fluctuations in acoustic activity may reflect changes in population structure or ecosystem health. This research establishes a foundation for future monitoring programmes in the PLSMP and other Atlantic regions, advancing understanding of fish acoustic communities. Notably, it provides the first acoustic library for mainland Portugal, thereby enriching the national acoustic database, supporting further research, and increasing public awareness of fish sound production in Portuguese waters.

The work also contributes to regional comparisons (Vieira et al. 2024) and to global biodiversity monitoring initiatives (Darras et al. 2025). Through participation in the Worldwide Soundscapes project, the acoustic surveys undertaken here have added to a growing international database designed to expand ecosystem coverage and facilitate the development of cross-realm acoustic baselines. By enabling standardised, long-term data collection, this study demonstrates how PAM can advance macroecological research and conservation practice, even where spatial and taxonomic information remains incomplete.

Beyond ecological monitoring, PAM is particularly valuable for detecting anthropogenic activities and evaluating their impacts on marine species. By documenting both human presence and associated noise, PAM supports adaptive management strategies that reduce disturbance and strengthen conservation measures (Desjonquères et al. 2019, Dunham et al. 2020, McGeady et al. 2023). This aligns with the growing demand for effective MPA monitoring systems capable of assessing conservation outcomes and informing responsive management.

Finally, effective marine conservation depends not only on robust scientific research but also on meaningful public engagement. By combining academic dissemination with outreach initiatives this thesis has helped to bridge the gap between science and society, raising awareness of the ecological importance of underwater soundscapes and their role in conservation.

Ethical use of artificial intelligence: During the development of this thesis, artificial intelligence was utilized primarily for English language correction and structural improvement, bibliographic searches, statistical analyses, and the improvement of R scripts.

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