Permanent Reservoir Monitoring (PRM) and Distributed Acoustic Sensing (DAS) as Advanced Technologies for Maximizing the Acquisition of Acoustic Environmental Information

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Contents

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Abstract

This chapter explores new methods to enhance the use of existing data from established technologies, namely Permanent Reservoir Monitoring (PRM) and Distributed Acoustic Sensing (DAS), to maximize the acquisition of acoustic environmental information. Cetaceans, particularly baleen whales, rely heavily on sound and are vulnerable to human-made underwater noise, which can mask their vital low-frequency vocalizations. High operational costs and logistical challenges in deep, offshore environments often constrain Traditional Passive Acoustic Monitoring. This work presents the repurposing of PRM data from the Jubarte Field, and the implementation of a DAS field measurement campaign using an existing Petrobras submarine fiber-optic cable, both in the Campos Basin, Brazil. The PRM dataset, sampled at 500 Hz, confirmed the seasonal occurrence of multiple mysticete species, including humpback (*Megaptera novaeangliae*), sei (*Balaenoptera borealis*), and fin (*Balaenoptera physalus*) whales. Preliminary DAS laboratory and field tests established a robust technical basis, with the system demonstrating sensitivity to low-frequency biological sounds, thereby enabling the detection of periodic calls strongly resembling fin whale vocalizations (40–120 Hz). The results demonstrate the potential of integrating geophysical and bioacoustic data, leveraging existing infrastructure for sustainable, continuous, large-scale oceanic monitoring. This strategic approach significantly expands acoustic knowledge of baleen whales in the South Atlantic, supporting conservation and impact mitigation efforts.

Keywords

Baleen whales · Bioacoustics · Acoustic monitoring · Deep Sea · Geophysical instruments

Introduction

Cetaceans depend on sound for communication, navigation, and survival. As such, they are highly sensitive to underwater noise, which can mask their complex signals, resulting in behavioral disruption and ecosystem instability. International organizations such as the International Maritime Organization and the International Whaling

Commission emphasize research and mitigation to reduce the impacts of human-made noise. The IWC supports initiatives aligned with UN 2030 Agenda Goal 14 on ocean conservation. Cutting down underwater noise through ecosystem-based, precautionary strategies is crucial for protecting cetaceans. Sensory adaptations of marine life have evolved to thrive in an environment where limited visibility makes sound a vital tool for survival. Cetacean acoustic ecology offers essential insights for conserving threatened species and evaluating human impacts on marine ecosystems.

Brazil's extensive coastline and rich marine biodiversity, including endemic and vulnerable species, highlight the urgency of studying acoustic impacts and mitigation strategies. The acoustics of many species found in Brazilian waters remain understudied, making impact assessments difficult.

Mysticetes (baleen whales) are key marine predators essential to ocean health, supporting nutrient cycling, carbon storage, and nutrient enrichment through carcass decomposition. The Marine Mammal Taxonomy Committee lists 15 species worldwide, nine of which are found in Brazil, such as the humpback whale (*Megaptera novaeangliae*), sei whale (*Balaenoptera borealis*), and fin whale (*Balaenoptera physalus*) (Milmann et al. 2020). Their conservation status ranges from "Least Concern" (LC) to "Endangered" (EN). These whales produce low-frequency ($<$1000 Hz), high-energy sounds, linked to behaviors such as feeding, reproduction, and nurturing (e.g., Parks et al. 2014). Acoustic features of their calls—type, frequency, duration, and interval repetition—differ among species and regions (e.g., Širović et al. 2007). While calls of species like humpback, fin, and blue whales are well-studied, information on others, also found in Brazil, remains limited. Data on whale occurrence along the Brazilian coast mainly come from coastal visual surveys, opportunistic sightings, and stranding reports, especially for species inhabiting the continental shelf and slope (e.g., Milmann et al. 2020). Although bioacoustics studies provide valuable insights, they are mostly limited to coastal areas or short-term, opportunistic efforts (e.g., Širović et al. 2007), making it difficult to achieve comprehensive assessments of species' temporal and spatial distribution.

As collecting high-quality whale data, especially offshore or for cryptic species, is often expensive and challenging, developing efficient monitoring methods is essential. This chapter presents new analytical approaches, built upon existing technologies, that can enhance our capacity to understand and monitor the marine environment, ultimately supporting more effective ocean conservation efforts.

Acoustic Approaches

Passive acoustic monitoring (PAM) is crucial for cetacean research, helping study occurrence, distribution, habitat use, and density through vocalization analysis (Andriolo et al. 2017). However, deploying PAM recorders? in deep or offshore areas—habitats for many cetaceans—is costly and logistically challenging. Recently, alternative methods using datasets collected for other purposes—such as Ocean Bottom Seismometers (OBS) for permanent reservoir monitoring (PRM) and submarine fiber-optic cables for Distributed Acoustic Sensing (DAS)—are gaining scientific interest (Bouffaut et al. 2022).

Permanent Reservoir Monitoring (PRM)

Seabed seismic data acquisition systems have evolved considerably over the decades, transitioning from single-use research instruments to long-term hydrocarbon reservoir monitoring networks. These developments use multi-component (4C) sensors fixed to the seabed to simultaneously record sound pressure in the water and particle motion in the seafloor as a core principle. This shared technological foundation is what establishes their potential for application in Passive Acoustic Monitoring (PAM).

OBS are autonomous seafloor-based systems developed initially for geophysical investigations of sub-seafloor structures, employed in both earthquake and controlled-source seismology (Mànuel et al. 2012). The recording capability of these instruments lies in their 4C measurement, which simultaneously provides two types of received sound levels: pressure fluctuations in the water, recorded by the hydrophone, and seabed motion, captured by the directional three-component seismometer.

Ocean Bottom Nodes (OBN), developed after Ocean Bottom Seismometers and Cables, are autonomous systems used in the exploration and production industry for four-component seismic surveys, optimized for high-density surveys (Guimarães et al. 2017). OBNs enable real-time reservoir monitoring with 4D seismic and elastic data, providing wide-azimuth coverage and functioning in deep-water environments (Balabekov et al. 2017).

PRM systems, like Ocean Bottom Nodes, use seafloor four-component sensors for elastic acquisition, designed for long-term hydrocarbon reservoir monitoring to detect subtle changes via 4D seismic methods.

Besides providing data for subsurface surveys, multicomponent OBS are now used for passive acoustic monitoring (PAM) of low-frequency marine mammal sounds. This approach is valuable for studying calling whales, allowing localization, tracking, seasonal pattern detection, and source level estimation through sound pressure and particle velocity correlation, aiding conservation and impact mitigation (Dréo et al. 2019). OBN and PRM systems, with similar multicomponent capabilities, could also enhance PAM coverage.

Given limited acoustic data on baleen whales in Brazilian waters and few long-term PAM projects, using data from existing systems like PRM offers a strategic opportunity. These datasets can expand knowledge of species’ acoustic repertoires, ecology, diversity, occurrence, distribution, habitat use, and support environmental impact assessments and conservation strategies for marine biodiversity.

Distributed Acoustic Sensing (DAS)

Initially developed for geophysical data collection, DAS technology has increasingly been used to monitor underwater sound sources. Using an interrogator unit, DAS repurposes unused fiber-optic cables to detect nanodeformations along the cable length (Bouffaut et al. 2022). This approach has strong potential for real-time monitoring, covering tens or even hundreds of kilometers, with spatial resolution below a few meters (Bouffaut et al. 2022).

DAS technology is promising for bioacoustics because it records sound over large areas with many sensing channels. It simplifies sensor installation and maintenance in aquatic environments, though challenges include decreased sensitivity in some cable sections and the need for higher sampling rates for detecting high-frequency sounds (Bouffaut et al. 2022).

The effectiveness of DAS for cetacean detection depends on its ability to localize animals and differentiate their vocalizations from other sound sources. Recent studies have demonstrated its capacity to detect and analyze low-frequency sounds in controlled environments. However, its ability to capture high-frequency sounds, such as odontocete clicks, still requires improvement in sampling rates and cable configurations. As a recent innovation, the technology lacks standardized field protocols, and few empirical studies are currently available to guide its implementation (Bouffaut et al. 2022).

These technologies offer valuable tools for addressing research questions related to animal behavior, ecological dynamics, and the potential impacts of human activities on marine ecosystems.

Case Study Methods

Permanent Reservoir Monitoring

The Campos Basin is Brazil’s largest oil-producing region. The Jubarte Field, in the basin’s northern section between 1000 and 1500 meters isobaths, ranks among the top 20 in national oil production (ANP 2024). Data from Petrobras were mainly used to validate fiber-optic sensor technology for deepwater applications.

In 2012, a PRM system was installed in the Jubarte Field at depths of 1200 to 1300 meters (Fig. 1a). It includes a network of fiber-optic sensors on the seafloor, with 35 km of cable in 11 lines. With 712 sensors spaced 50 meters apart, it covers 9 km^2 (Thedy et al. 2013). About 60 days of continuous data from June to August 2013 are available. SGY files were recorded and converted to .wav files with 500 Hz sampling frequency and a response up to 250 Hz.

Distributed Acoustic Sensing

Before collecting field data, the Immer Messen team, together with the CENPES/Petrobras, Aqualie Institute and the Federal Institute of Juiz de Fora, conducted laboratory tests. These tests aimed to assess a DAS approach for detecting mysticete vocalizations under controlled conditions. Conducted in 2024, they validated the methodology and set the technical basis for field deployment.

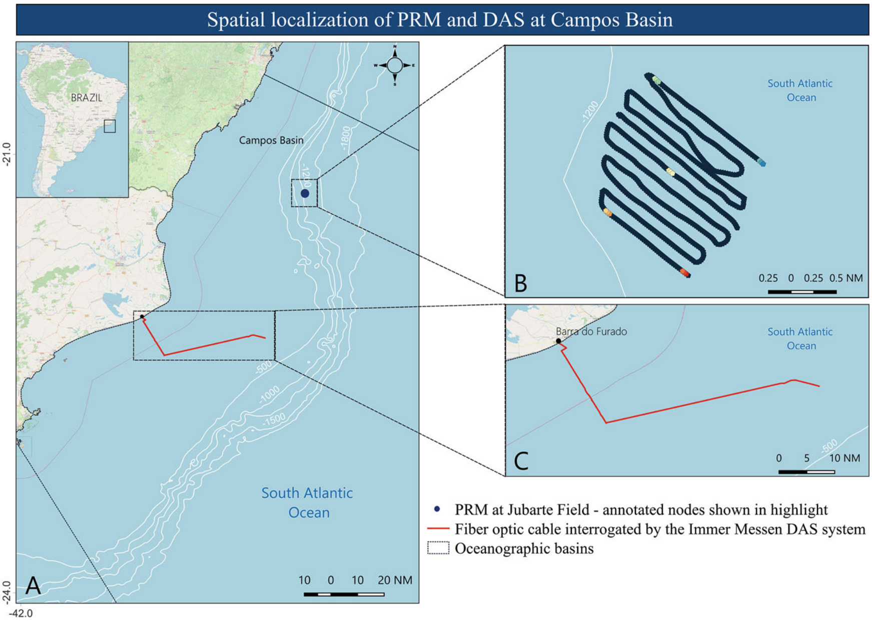

Fig. 1 Location of the Campos Basin, Brazil (**a**), with detailed positions of PRM system in Jubarte Field (**b**) and subsea optical cable interrogated by the Immer Messen DAS at Barra do Furado (**c**)

The initial testing phase focused on understanding the fundamental mechanisms governing DAS sensitivity to underwater acoustic waves. Particular attention was given to (i) the influence of acoustic wave incidence and coupling relative to the fiber's longitudinal axis, and (ii) the impact of the selected gauge length (fiber segment length, over which the vibration is averaged) on the demodulation of the backscattered phase signal, which forms the basis of DAS measurements.

The pool tests utilized a sensor setup comprising a submerged speaker, a hydrophone, and the optical fiber cable, enabling the DAS system to register data across different speaker positions and angles. Detection sensitivity was influenced by the acoustic field, with the strongest response to longitudinal waves; normal incidence resulted in weaker coupling. Each frequency generated a unique strain pattern that affected the backscattered phase and spectral selectivity. Cable tension was crucial—initially loose, but later anchored, which improved sensitivity and stability. A second test involved a 100 m optical fiber in a 1 m^3 tank, which increased acoustic-to-phase efficiency due to confined space and uniform tension from coiling, boosting sensitivity and consistent coupling. The DAS signal increased with larger fiber segments excited by waves of similar amplitude, as phase variations enhanced the signal. Another test in a larger lagoon used a fiber on the lagoon bed and another was tensioned with pulleys. Results showed that fiber tension impacts the spectral response: tensioned fibers had stronger responses at lower frequencies with higher signal intensity, while untensioned fibers captured richer harmonic content at higher frequencies, though with lower amplitude.

This study aimed to assess DAS data from submarine optical cables designed with multilayered structures for underwater applications. These cables include stainless-steel or gel-filled tubes that protect the optical fibers, surrounded by metallic shielding, moisture barriers, and two layers of galvanized steel armoring, all encased in high-density polyethylene (HDPE). Acoustic coupling depends on cable design, especially on the fiber-jacket connection. For our tests, commercially available loose-tube gel-filled telecommunication cables with an external polymer sheath, about 10 mm in diameter, reinforced with aramid, were used. Their diameter ranged from 25 to 35 mm, depending on armor and fiber count. While durable, this construction reduces acoustic coupling, potentially lowering DAS sensitivity underwater.

Petrobras provided a 13-m submarine optical cable for lab testing. A loudspeaker played mysticete calls near the coiled cable, and the DAS system successfully detected signals with a good signal-to-noise ratio and expected spectral content. Although the acoustic levels weren't typical of real underwater conditions, the experiments offered valuable insights into DAS coupling and reconstruction capabilities on submarine cables.

Field Measurements Campaign at Barra do Furado, Rio de Janeiro State, Brazil

A 110 km Petrobras submarine fiber-optic cable in the Campos Basin, Brazil (Fig. 1), was interrogated using Immer Messen's Distributed Acoustic Sensing (DAS) system. The phase-sensitive optical time-domain reflectometer (Φ-OTDR), based on heterodyne detection, was installed at the cable's terminus in a Barra do Furado telecommunications facility. The cable route, shown in Fig. 3, runs from the coast into deeper waters and is buried at depths up to two meters.

Data acquisition occurred over 4 days with the system running at 900 Hz pulse repetition rate, using optical pulses lasting 50 and 100 ns. The gauge length was set at 1.5 times the pulse length, at about 7.5 m and 15 m. Overall, 48 TB of raw data were collected.

Analytical Approach

The PRM system's data in .sgy format (SEG-Y, a geophysical data standard) were converted to .wav audio files for acoustic analysis using Python.

A unified framework utilizing converted signals is implemented through four distinct steps: (1) temporally and spatially subsampling data and manual inspection with Raven Pro 1.6 to identify signals at low frequencies; (2) extracting spectral and temporal acoustic parameters, such as signal type, frequency range, duration, and repetition interval, for comparison with baleen whale signals and species identification; (3) automated detection of signals across the dataset; and (4) triangulating detections from at least three sensors using Time Difference of Arrival to locate signals and track individuals.

The conversion, subsampling, and manual annotation steps have already been performed. Data were spatially (five triads at different depths) and temporally (from 1434 h to approximately 240 h, about 15% of the total) subsampled.

The automatic detection has been tested at subsamples of the dataset using detectors previously described in the literature (e.g., Ishmael's Spectrogram Correlation, Energy Sum, and Matched Filter), available in acoustic analysis software such as PAMGuard, as well as algorithms based on machine learning techniques, such as Deep Neural Networks implemented through Python libraries.

Consequently, this is an ongoing project and the overall aim is to optimize the use of geophysical instruments to access a valuable database, expand acoustic knowledge about baleen whales in Brazil, and contribute to the development of automated frameworks for monitoring these species.

In parallel, the DAS interrogator compiles vast data volumes, meaning manually inspecting all signals for bioacoustic events is inefficient. Detecting these events requires scanning data in spatial and temporal windows and analyzing each segment for acoustic patterns. Additionally, a window's visual output varies with frequency filter settings; certain signals may be visible or not depending on the cutoff frequencies.

The difficulty of manually inspecting all signals comes from the DAS system's broadband sensitivity, which detects mechanical and physical perturbations coupled to the optical fiber. As a result, the measured signal reflects the combined effects of multiple sources (temperature, pressure changes, etc.), each with distinct spectral content, spatial distribution, and strength. Clearly showing these specific features in the demodulated phase signal depends on predefined parameters, further complicating the manual identification of bioacoustic signatures.

To address this challenge, Immer Messen designed an algorithm for automatic detection of anomalous events. The term *anomalies* refers to the underlying detection principle relying on identifying changes in the power spectral density (PSD) characteristics of the signal at each spatial position over defined temporal windows. The algorithm performs a spatiotemporal scan of the dataset, detecting abrupt variations in PSD intensity across frequency bins. Processing the complete dataset from the campaigns yielded a catalog of anomalous events, each associated with its spatial position, timestamp, and spectral content. From this catalog, specialists at Instituto Aqualie conducted detailed analyses of the identified signals to assess their bioacoustic relevance.

This process begins with the analysis of the spatiotemporal signal corresponding to each selected matrix. The selected matrix is further processed using an appropriate band-pass filter with cutoff frequencies defined within the frequency bins previously identified by the detection algorithm. Median filtering is also applied to enhance signal clarity and suppress low-frequency noise. This processing stage is particularly critical, as it enables the visualization of acoustic events within the DAS data. The used configuration of the band-pass and median filters was guided by insights and best practices reported in the literature, especially in the studies conducted by Bouffaut and collaborators (Bouffaut et al. 2022; Landrø et al. 2022). A thorough, repeated review of these works was instrumental in outlining the methodological steps for effectively extracting acoustic features from DAS measurements. The publicly available processing functions in the open-source DAS4Whales Python library (Bouffaut 2023) were fundamental in both understanding and developing customized, optimized data processing codes for the Immer Messen DAS interrogator.

A developed real-time display shows all acoustic events, including potential vocalizations, as "V-shaped" patterns, which are space–time hyperbolic signals indicating acoustic wave propagation along the cable at different times. The apex of this pattern marks where sound was first detected in the fiber. A point near the apex is chosen as a reference for reconstructing local acoustic time series. With a gauge length between 7.5 and 15 m, data resolution is improved using a finer spatial sampling of about 25 cm. The temporal signal is reconstructed by averaging signals from adjacent spatial samples within a window matching the gauge length, around 30 to 60 points, enhancing the local SNR. The effective spatial resolution depends on the gauge length rather than sampling density. After reconstruction, spectrograms are analyzed by specialists at Instituto Aqualie to confirm their similarity to baleen whale vocalizations.

Results

Permanent Reservoir Monitoring

The dataset contained vocalizations attributable to multiple mysticete species, confirming the seasonal presence of humpback, fin, and sei whales. The spectrogram of fin doublets in 20 Hz and partial notes of humpback whales can be seen in Fig. 2. Manual annotation, though time-consuming, will be essential for training and validating detection algorithms.

Distributed Acoustic Sensing

As an initial example of DAS detection capability, Fig. 3 shows a representative signal acquired near the cable's shoreline entry segment. The data corresponds to the spatial range between 500 m and 2000 m along the cable (y-axis) over the first 200 s of measurement (x-axis). Signal intensity is represented in radians, corresponding to the spatiotemporal phase differences of the backscattered signal. The data were high-pass filtered at 0.01 Hz, revealing slow, periodic patterns originating offshore and propagating toward the coastline beyond approximately 1150 m, with no similar behavior observed landward. These patterns are attributed to surface wave motion approaching the shore and breaking near the surf zone. The slope of these features

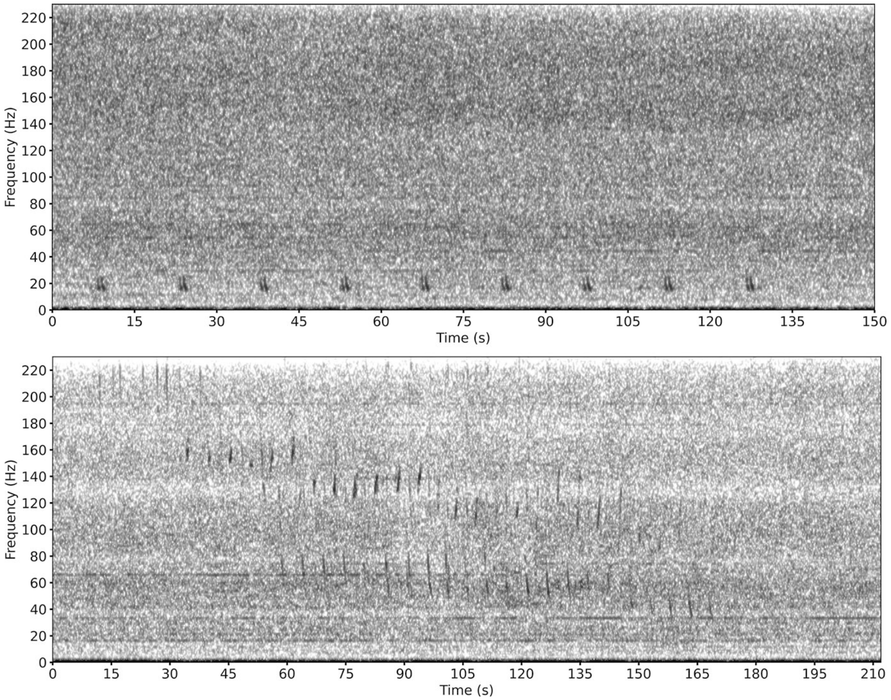

Fig. 2 Spectrogram of fin doublets at 20 Hz and partial humpback signals

reflects the apparent velocity associated with the coupling between the ocean waves and the geometry of the fiber-optic cable on the seabed.

This interpretation aligns with recent findings that DAS-interrogated submarine fiber-optic cables are sensitive to both local acoustic sources and large-scale oceanic processes. Surface gravity waves modulate the hydrostatic pressure at the seafloor, causing measurable strain variations along buried fibers. DAS detects these strain fluctuations, enabling reconstruction of nearshore wave heights and seabed pressure (Meulé et al. 2024). This sensitivity shows DAS's broad spectral and spatial responsiveness, capturing signals from low-frequency oceanic processes to higher-frequency acoustic emissions like whale vocalizations.

When applying spatial and temporal zooming to the same region with a higher high-pass cutoff frequency of 5 Hz, the slow, shoreward-propagating patterns terminate abruptly at a specific spatial transition. At this interface, higher-frequency mechanical oscillations emerge and propagate across neighboring positions, corresponding to mechanical waves generated by surf-zone impacts as ocean waves break upon reaching the shore–sand boundary.

Further offshore, the submarine cable lies directly on the seafloor and is continuously exposed to the underwater acoustic soundscape. Vessel engines produce characteristic acoustic signatures that propagate through the water and can be readily detected by DAS. Figure 4 presents an example of a DAS signal acquired 33.5 km

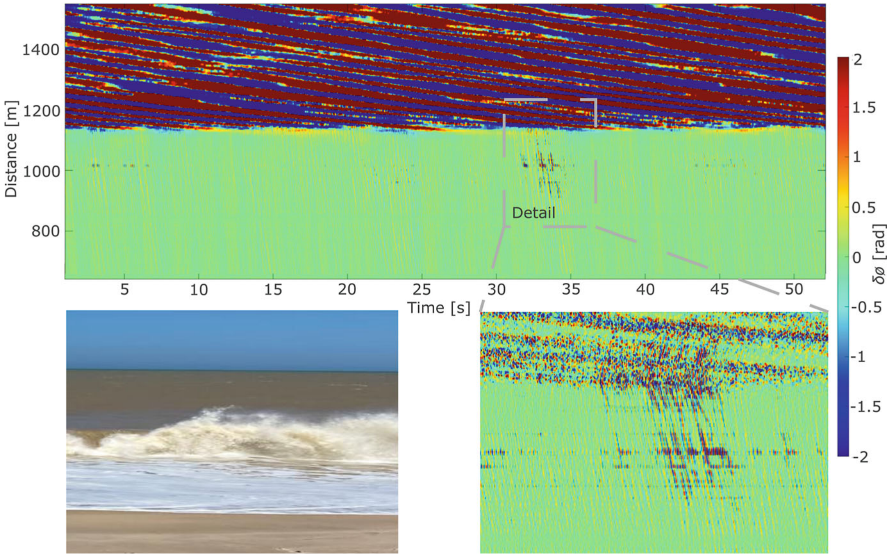

Fig. 3 DAS signal measured through the submarine optical cable at the nearshore section where the cable enters the sea. The y-axis represents the cable length, the x-axis shows time over a 50-second window, and the signal intensity—expressed in radians—is displayed using a color scale. The transition between sand and sea is clearly revealed by periodic patterns propagating from the ocean toward the shore, resembling surface wave motions. The inset highlights mechanical patterns observed at the transition zone, 5 Hz high-pass filtered, indicative of shorebreak dynamics

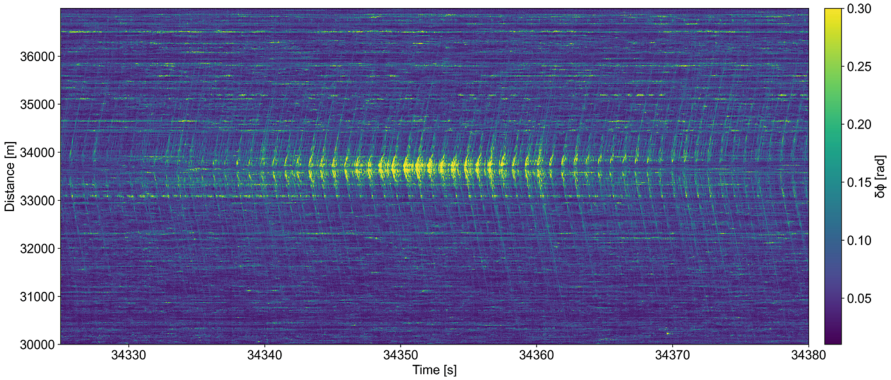

Fig. 4 DAS signal recorded on October 23, 2024, at 01:28:00 UTC, covering the 30–37 km section of the submarine fiber-optic cable. The spatio-temporal representation displays distance along the cable (y-axis, in meters) and time from the start of acquisition (x-axis, in seconds), with signal intensity shown in radians and color-scaled. The observed acoustic patterns exhibit periodic hyperbolic signatures characteristic of vessel-generated sounds, with a marked increase in intensity at a specific moment, suggesting a potential crossing or close approach of the vessel over the submarine cable

offshore, showing acoustic patterns consistent with ship noise previously reported by Wilcock, Abadi and Lipovsky (2023). The spatiotemporal signal was processed using a 20 Hz high-pass filter and a 2D-median filter of 12 m/4.65 ms. The detected ship-related pattern consists of a series of sequential hyperbolic traces. The attenuation observed near the apex of each pattern is related to the vessel movement versus cable geometry. Signal intensity peaks increase as the ship approaches the fiber and diminish as it moves away. The temporal evolution of these acoustic arrivals can be used to infer the vessel's direction of motion (Wilcock et al. 2023) and, through integration with AIS data, to estimate or track its trajectory (Landrø et al. 2022).

DAS technology detected biological sounds of large marine mammals, shown in Fig. 5a, with 17 vocalizations over 90 s recorded on October 22, 2024. The "V-shaped" patterns originate near 20.87 km, spanning roughly 6.5 km from 17.5 to 24 km. The DAS signal was band-pass filtered (43–85 Hz) with a Butterworth filter, then processed with a 12 m/4.65 ms median filter to reduce noise. Phase change data were plotted for contrast, revealing alternating low- and high-frequency components confirmed by the analysis of the local signal reconstructed at the apex (Fig. 5b) through the spectrogram in Fig. 5c, which used a 0.2 s Hann window with 98% overlap. Low-frequency vocalizations were more intense and persistent, aligning with their longer wavelengths and lower propagation loss. Periodic calls

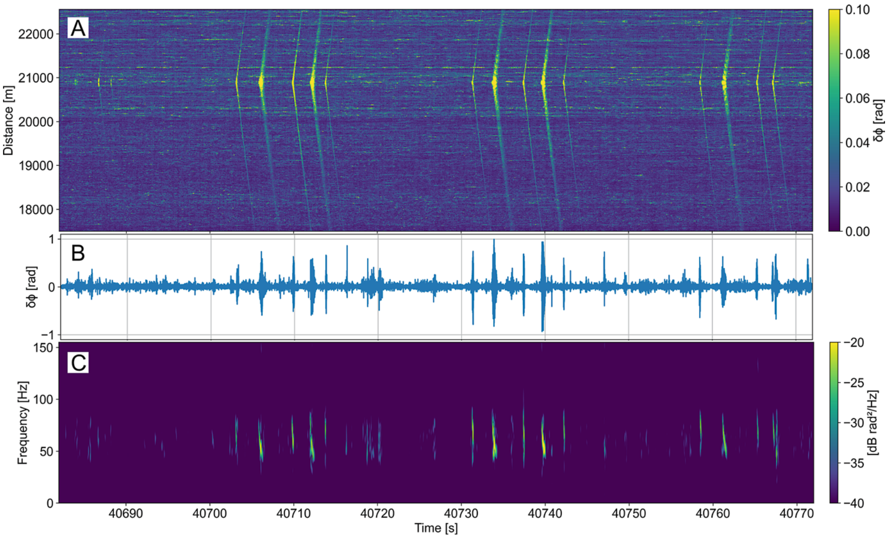

Fig. 5 DAS signal recorded on October 22, 2024, at 09:32:18 UTC, spanning the 17.5–24 km section of the submarine fiber-optic cable. (**a**) Spatio-temporal representation showing distance (y-axis, in meters) and time (x-axis, in seconds from acquisition start), with signal intensity expressed in radian units and color-mapped. Seventeen distinct vocalizations are identifiable, forming characteristic "V-shaped" patterns with apexes near 20.87 km (**b**), marking the point of initial detection. (**c**) The spectrogram of the channel at 20.9 km shows the temporal evolution of frequencies, revealing periodic signals in the [40–120] Hz range, potentially fin whale calls

within the low frequency range of 40–120 Hz exhibited features consistent with frequency-modulated, patterned baleen whale vocalizations.

Automatic Detection

Given the large datasets generated by long-term PRM and DAS, automated methods are essential to efficiently detecting and classifying signals. However, to ensure consistent and effective analysis, it is recommended to first establish a standardized analytical protocol, starting with the annotation of a representative subset (e.g., Miller et al. 2021).

Several sound detection methods are currently being tested for PRM. The Whistle and Moan detector worked well for tracing sound shapes but often missed short or low-pitched calls. Matched filtering, which uses sound templates, performed effectively when call patterns were clear. Spectrogram correlation was accurate when call patterns were easy to identify, although it required significant computer power. The Energy sum method was useful for cleaning and preparing recordings but tended to generate false alarms in noisy environments. Additionally, machine–learning–based detectors will be trained and tested using key sound features extracted from the recordings.

Discussion

Our findings indicate that PRM data can be repurposed for cetacean acoustic monitoring, thereby expanding data-collection opportunities without requiring new infrastructure. This represents a significant methodological advance, particularly in oil and gas exploration regions, where anthropogenic pressures on marine mammals are high.

The DAS method provides significant advantages over traditional PAM by reducing vessel and flow noise interference and enabling continuous, large-scale oceanic monitoring. Results show DAS as a practical, expandable option, particularly in regions where traditional PAM is logistically difficult. Unlike towed hydrophone arrays or bottom-mounted sensors, DAS avoids cavitation noise and offers long-term, cost-effective surveillance through existing fiber optic networks. It allows pre- and post-processing filters, enabling data sampling in noisy environments. This is the first DAS whale monitoring in the South Atlantic, laying a foundation for species identification and ecological research. Future work will improve signal processing, incorporate machine learning for classification, and expand DAS monitoring globally. DAS, leveraging telecom infrastructure, transforms marine mammal conservation by enhancing real-time acoustic monitoring and understanding whale distribution. Challenges such as background noise, overlapping calls, storage, and computation remain, but machine learning promises to improve detection and classification.

Conclusions

This work demonstrates the potential of combining geophysical and bioacoustic data to monitor cetaceans in offshore environments. PETROBRAS's PRM and DAS technologies proved feasible and valuable for investigating remote underwater environments. Utilizing existing infrastructure offers a sustainable and strategic approach to enhancing monitoring, aiding whale conservation in the South Atlantic, and reducing impacts from seismic activities. Future efforts should focus on developing species-specific detectors and more robust automated frameworks.

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Competing Interest Declaration The author(s) has no competing interests to declare that are relevant to the content of this manuscript.

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