FEDERAL UNIVERSITY OF JUIZ DE FORA
BIOLOGICAL SCIENCES INSTITUTE - ICB
POST-GRADUATE PROGRAM IN BIODIVERSITY AND NATURE CONSERVATION

João Pedro Motta Mura

Estimation of distribution, density, and abundance of franciscana dolphins (*Pontoporia blainvillei*) in FMA-Ia (Espírito Santo, Brazil) using passive acoustic monitoring (PAM)

Juiz de Fora
2025

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Dissertation submitted to the Post Graduate Program in Biodiversity and Nature Conservation at the Federal University of Juiz de Fora as a partial requirement for obtaining the degree of Master in Biodiversity and Nature Conservation. Concentration area: Zoology.

Supervisor: Prof. Dr. Alexandre N. Zerbini
Co-supervisor: Prof. Dr. Artur Andriolo

Juiz de Fora
2025

Ficha catalográfica elaborada através do programa de geração
automática da Biblioteca Universitária da UFJF,
com os dados fornecidos pelo(a) autor(a)

Mura, Joao Pedro M..
Estimation of distribution, density, and abundance of franciscana dolphins (Pontoporia blainvillei) in FMA-Ia (Espírito Santo, Brazil) using passive acoustic monitoring (PAM) / Joao Pedro M. Mura. -- 2025.
80 p. : il.

Orientador: Alexandre N. Zerbini
Coorientador: Artur Andriolo
Dissertação (mestrado acadêmico) - Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Ciências Biológicas: Comportamento Animal, 2025.

1. Passive Acoustic Monitoring. 2. Density Estimation. 3. Pontoporia blainvillei. 4. Conservation. I. Zerbini, Alexandre N., orient. II. Andriolo, Artur , coorient. III. Título.

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Approved on October 31, 2025

Examination Committee

____
Prof. Dr. Alexandre N. Zerbini – Supervisor
Federal University of Juiz de Fora

____
Prof. Dr. Tiago Marques
University of St Andrews

____
Prof. Dr. Renan Paitach
Univille

I dedicate this work to my grandmother Dalva and to my mother, for all the effort and sacrifices they made throughout their lives to ensure that I could pursue my studies

“He always thought of the sea as feminine and as something that gave or withheld great favors, and if she did wild or wicked things it was because she could not help them.”

“Every day is a new day. It is better to be lucky. But I would rather be exact. Then when luck comes you are ready.”

— Ernest Hemingway, *The Old Man and the Sea* (1952)

ABSTRACT

The franciscana dolphin (*Pontoporia blainvillei*), recently subdivided into the distinct subspecies *P. b. pukusi* in Espírito Santo, Brazil, is one of the most threatened cetaceans in South America. Conventional visual surveys are limited in its turbid, nearshore habitat, highlighting the need for alternative approaches. This dissertation develops a stepwise framework for passive acoustic density estimation (PADE). Chapter 1 defines biologically meaningful acoustic groups using clustering of echolocation click trains. Chapter 2 calibrates acoustic footprints by pairing passive acoustic detections with drone counts, enabling group-size estimation. Chapter 3 integrates these advances to provide the first abundance and trend estimates for *P. b. pukusi* in FMA-1a (2019–2022). Surveys covered 4,562 km, yielding 224 click trains aggregated into 71 clusters. Nearly all detections were concentrated near the Doce River mouth, while the northern stratum showed minimal occurrence. Annual abundance estimates ranged from ~1,150 to ~1,300 individuals, indicating a small, spatially restricted, and possibly declining population. This work demonstrates the feasibility of PADE for small coastal dolphins and provides critical baseline data for the conservation of *P. b. pukusi*.

Keywords: Passive Acoustic Monitoring; Density Estimation; *Pontoporia blainvillei;* Conservation.

PORTUGUESE ABSTRACT

A toninha (*Pontoporia blainvillei*), recentemente subdividida na distinta subespécie *P. b. pukusi* no Espírito Santo, é um dos cetáceos mais ameaçados da América do Sul. Levantamentos visuais convencionais apresentam fortes limitações em seu habitat costeiro turvo, ressaltando a necessidade de abordagens alternativas. Esta dissertação desenvolve um arcabouço progressivo para a estimação acústica passiva de densidade (PADE). O Capítulo 1 define agrupamentos acústicos biologicamente significativos a partir da clusterização de cadeias de cliques de ecolocalização. O Capítulo 2 calibra taxas de emissão por meio da integração entre detecções acústicas e contagens com drones, possibilitando a estimação do tamanho de grupo. O Capítulo 3 integra esses avanços para fornecer as primeiras estimativas de abundância e tendência para *P. b. pukusi* na FMA-Ia (2019–2022). As campanhas totalizaram 4.562 km de esforço, resultando em 224 cadeias de cliques agregados em 71 clusters. Quase todas as detecções se concentraram na foz do rio Doce, enquanto o estrato norte apresentou ocorrência mínima. As estimativas anuais de abundância variaram entre ~1.150 e ~1.300 indivíduos, indicando uma população pequena, espacialmente restrita e possivelmente em declínio. Este trabalho demonstra a viabilidade do PADE para pequenos golfinhos costeiros e fornece dados críticos de base para a conservação de *P. b. pukusi*.

Palavras-chave: Monitoramento Acústico Passivo; Estimativa de densidade; *Pontoporia blainvillei;* Conservação.

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1 GENERAL INTRODUCTION

The franciscana dolphin (*Pontoporia blainvillei*) is a small coastal odontocete endemic to the western South Atlantic, ranging from Espírito Santo, Brazil, to Golfo San Matías, Argentina (Secchi et al., 2003; Cunha et al., 2014). It is widely regarded as one of the most threatened cetaceans in South America, currently listed as Vulnerable on the IUCN Red List (Zerbini et al., 2017) and Critically Endangered in Brazil (Brazil, 2003; MMA, 2014; MMA et al., 2018). Bycatch in artisanal and industrial gillnet fisheries represents the most severe threat to the species, causing unsustainable mortality levels across its distribution (Prado et al., 2013; Secchi et al., 2022). Its shallow nearshore distribution, low potential for population growth, and occurrence in small, inconspicuous groups further exacerbate extinction risk (Secchi, 2010; Simões-Lopes & Cremer, 2022).

Recent evidence has highlighted the geographical structuring of *P. blainvillei*. Franciscana Management Areas (FMAs) were established based on genetic, morphological, and ecological data to support regional conservation (Secchi et al., 2003; Crespo et al., 2010). Among them, the northernmost unit (FMA-Ia, Espírito Santo) was recently recognized as a distinct subspecies, *Pontoporia blainvillei pukusi* (Nara et al., 2024). This lineage is highly isolated and concentrated along a restricted stretch of coastline, reinforcing the urgency for robust monitoring and protection.

Monitoring franciscana populations is particularly challenging. Traditional visual survey methods are strongly limited in nearshore waters, where turbidity and sea-state frequently compromise detectability (Sucunza et al., 2022). Franciscana dolphins also surface unobtrusively in small groups, usually of two or three individuals, rarely exceeding six (Crespo et al., 1998; Bordino et al., 1999), further reducing the reliability of visual estimates. These constraints highlight the urgent need for alternative approaches capable of overcoming the shortcomings of traditional sighting methods.

Passive acoustic monitoring (PAM) has in recent years emerged as a powerful tool for cryptic and elusive species. Cetaceans produce diverse species-specific vocalizations (clicks, whistles, and pulsed calls) that can be continuously recorded to infer occurrence, behavior, and, increasingly, density and abundance (Thomas & Marques, 2012; Marques et al., 2013; Andriolo et al., 2018; Barlow et al., 2022). For franciscanas, the stereotyped narrow-band high-frequency (NBHF) echolocation clicks, peaking at 130–140 kHz, provide an unambiguous acoustic signature (Melcón et al., 2012; Amorim et al., 2022; Paitach et al., 2022), making the species an ideal candidate for PAM-based monitoring.

A central challenge in acoustic-based abundance estimation is translating acoustic cues into biologically meaningful units, such as the number of animals. Depending on the approach taken, this might require defining social groups from acoustic detections and calibrating cue production rates (Thomas & Marques, 2012; Marques et al., 2013). Recent methodological advances have opened new opportunities to address these gaps. Unsupervised spatiotemporal clustering of click trains allows objective identification of group-level acoustic units (Campello et al., 2013; McInnes et al., 2017; Mura et al., 2025), while the integration of PAM with drone-based surveys enables independent group-size estimation (Frouin-Mouy et al., 2020; Martin et al., 2021). Drones are increasingly recognized as powerful tools for cetacean research, providing cost-effective and accurate counts, photogrammetry, and behavioral data under conditions where boat-based surveys are limited (Hodgson & Koh, 2016; Burnett et al., 2019; de Oliveira et al., 2023).

This dissertation integrates these advances into a stepwise framework for passive acoustic density estimation (PADE) tailored to the franciscana dolphin. It is organized into three chapters:

- Chapter 1 applies hierarchical density-based clustering (HDBSCAN) to define spatiotemporal groupings of echolocation click trains, establishing biologically meaningful acoustic units (Mura et al., 2025 - DOI).
- Chapter 2 calibrates acoustic footprints by combining PAM detections with synchronous drone counts, enabling the translation of click-train clusters into group-size estimates (Submitted and under peer-revision at the Endangered Species Research Journal, Manuscript ID ENSR-2023-10-007/R2).

- Chapter 3 applies the complete framework to large-scale passive acoustic data collected in FMA-Ia, generating the first integrated distribution and abundance estimates for this subspecies.

Together, these chapters build a methodological and empirical foundation for PADE in *P. blainvillei*, advancing ecological understanding and delivering critical insights for the conservation of one of the most threatened dolphins in South America.

2 CHAPTER 1 - SPATIOTEMPORAL GROUP DEFINITION OF FRANCISCANA DOLPHINS FROM PASSIVE ACOUSTIC DATA

2.1 INTRODUCTION

Defining what constitutes an animal group remains a central challenge in behavioral and ecological studies. Variability in social behavior both across and within species, along with the fluid spatiotemporal nature of animal aggregations, complicates the establishment of consistent grouping criteria (Sumpter et al., 2008; Syme et al., 2022). Environmental drivers such as resource distribution and predation pressure, as well as inter-individual interaction dynamics and observation limitations, add further complexity (Kasozi and Montgomery, 2020; Shapiro, 1988). These challenges underscore the necessity for flexible and context-specific definitions when studying animal groups. This is particularly the case for species like *Pontoporia blainvillei*, whose social organization remains poorly understood due to limited visual detectability and elusive behavior in coastal, turbid environments (Cremer et al., 2022; Crespo et al., 2010; Sucunza et al., 2022; Zerbini et al., 2022; Wells et al., 2013).

The concept of a group is fundamental in ecological and behavioral research because it defines the basic sampling unit for studies of abundance, space use, and social structure (Couzin et al., 2002). However, operational definitions of groups are often ambiguous, which hinders the comparability and reproducibility of findings across studies (Viscido and Shrestha, 2015). While theoretical frameworks provide valuable perspectives - such as Whitehead's (2008) definition of groups as "sets of animals that actively achieve or maintain spatiotemporal proximity and within which most interactions occur", or Wilson's (2000) definition based on sustained cohesion and frequent interaction - these remain difficult to translate into field-based, data-driven criteria. As Syme et al. (2022) argue, there is a growing need for empirical definitions based on spatial proximity and interaction rates to enable biologically meaningful and reproducible analyses.

Among small odontocetes, group definition poses additional challenges due to their cryptic surface behavior, high mobility, and limited detectability in visual surveys. In this context, acoustic communication becomes a key proxy for understanding social organization. Dolphins rely heavily on echolocation clicks and other vocalizations to coordinate movement, forage, and maintain social bonds (see Simões Amorim et al., 2019; Andriolo et al., 2018; Lopez-Marulanda et al., 2020; Lubis et al., 2016; Papale et al., 2021; Viana et al., 2022). The development of passive acoustic monitoring (PAM) techniques has greatly advanced the study of cetacean behavior and ecology, enabling continuous, non-invasive data collection in otherwise inaccessible conditions (Andriolo et al., 2018; Gibb et al., 2019; Usman et al., 2020). These acoustic signals, when georeferenced, can serve not only as behavioral indicators but also as proxies for social and spatial structure (Gibb et al., 2019; Gerrodette et al., 2011). The size of a group is closely linked to the acoustic footprint it generates (DiMarzio et al., 2008).

The franciscana dolphin (*Pontoporia blainvillei*) is one of South America's most threatened cetaceans, classified as Vulnerable by the IUCN due to high levels of bycatch and ongoing coastal degradation (Secchi et al., 2003; Secchi and Wang, 2002; Zerbini et al., 2017). This species produces stereotyped, high-frequency narrow-band echolocation clicks, with peak frequencies around 139 kHz (Amorim et al., 2022; Melcón, et al., 2012; Paitach et al., 2021, 2022), which allows clear acoustic discrimination from other sympatric species (Amorim et al., 2022). However, its social behavior is still poorly understood due to the logistical difficulties in observing individuals at the surface (Cremer et al., 2022; Simoes-Lopes and Cremer, 2022; Sucunza et al., 2022). As such, high-resolution acoustic tracking represents a valuable alternative for revealing their group structure.

Traditional analyses of dolphin echolocation clicks often rely on manual or semi-automated segmentation using tools such as PAMGuard (Gillespie et al., 2009), where click trains are identified based on temporal patterns, amplitude thresholds, and visual inspection of spectrograms. Advances in machine learning and data science now offer tools to automate this process and minimize user bias. In this study, we applied the *Hierarchical Density-Based Spatial Clustering of Applications with Noise* (HDBSCAN; Campello et al., 2013; McInnes et al., 2017) algorithm to identify spatiotemporal aggregations of localized click trains. HDBSCAN is particularly well suited to ecological acoustic data because it does not require prior knowledge of the number of clusters, is robust to statistical noise, and can detect variable-density structures, like acoustics footprints.

Given that echolocation clicks are produced in sequences during movement and interaction, and that proximity in time and space may reflect social cohesion, we propose that clusters identified by HDBSCAN – termed Click Train Clusters (CTCs) – serve as biologically meaningful proxies for franciscana dolphin groupings. This approach contributes not only to the characterization of group-level structure in a data-limited species but also establishes a foundation for integrating spatiotemporal clustering into passive acoustic density estimation frameworks, thereby enhancing conservation and monitoring strategies.

2.2 MATERIALS

2.2.1 Data collection

The data used in this study comprised vocalizations of franciscana dolphins (*Pontoporia blainvillei*) recorded in their natural habitats in Ubatuba, São Paulo, Brazil (−23.530287, −45.037574) in December 2021 over 7 days (13 h and 49 min of total acoustic data). An AUSET Technology ® recording system was employed to record the data, including a SailDaq (Sea Mammal Research Unit – University of St Andrews) recording module with a sampling frequency of 500 kHz and a 50-meter array consisting of four omnidirectional hydrophones, positioned at intervals of 0.4 m, 3 m, and 5 m. A 12 V stationary battery powered all the equipment. Acoustic and GPS data were recorded using the PAMGuard software (v2.01.03, Gillespie et al. 2009). Additionally, a Phantom 4 Pro drone flying at an altitude of 80 m was used to locate groups of animals and identify optimal spots for acoustic acquisition.

2.2.2 Data processing and analysis

Acoustic data were processed using the Click Detector Module (CDM) from PAMGuard software (v2.01.03; Gillespie et al., 2009), configured to detect narrowband high-frequency (NBHF) clicks through a high-pass trigger filter set at 100 kHz. To optimize NBHF signal detection, the first and the second hydrophones – spaced 0.4 meters apart – were selected for their short baseline, and a 10 dB detection threshold was applied to reduce background noise interference. A click classification tool was then used, with energy and frequency parameters tailored to the known characteristics of franciscana dolphin clicks (Melcón et al., 2012; Amorim et al., 2022). These parameters were iteratively adjusted using a subset of the dataset to ensure optimal calibration and species-specific sensitivity (Frasier et al., 2016).

To detect temporally structured sequences of clicks (click trains), PAMGuard's automatic click train identification tool was applied, following established methodologies for odontocete signal analysis (Gillespie et al., 2013). The tool's parameters—such as maximum inter-click interval, minimum train duration, and number of clicks per train—were refined using a representative subset of the data to consistently identify biologically meaningful click trains across varying signal-to-noise conditions. Once classified and grouped into trains, the detections were spatially localized using Target Motion Analysis (TMA), which computes the Time Difference of Arrival (TDOA) between hydrophones H1 and H2 and applies hyperbolic triangulation to estimate the emitter's position relative to the recording platform (Barlow and Taylor, 2005; Lewis et al., 2007). Specifically, the perpendicular distance of each train to the transect line was derived from these spatial estimates. Both the least-squares and 2D simplex localization algorithms available in PAMGuard were tested. Model selection was guided by Akaike Information Criterion (AIC) and chi-square goodness-of-fit tests, following established practices in passive acoustic localization for cetaceans (Gillespie et al., 2020; Houégnigan et al., 2010; Mellinger et al., 2007; Zimmer, 2011).

The resulting dataset comprised timestamped, georeferenced acoustic events representing distinct vocalizations or vocal groups. Geographic coordinates were projected into a metric coordinate system using the Universal Transverse Mercator (UTM) projection to ensure consistency in spatial measurements across datasets (Iliffe, 2000; Snyder, 1987). Timestamps were converted into continuous numeric variables, expressed as the number of seconds elapsed since the beginning of each recording session, to represent the temporal dimension of detections. All features were then standardized using z-score normalization to balance the influence of spatial and temporal variables during clustering, following best practices in density-based clustering of multidimensional ecological data (Schubert et al., 2017).

The localized events, using geographical coordination and timestamp as input jointly, were submitted for a spatio-temporal clustering analysis using the Hierarchical Density-Based Spatial Clustering of Applications with Noise (HDBSCAN) algorithm (Campello et al., 2013; McInnes et al., 2017), which is particularly suited to ecological acoustic data due to its ability to detect clusters of varying density and handle statistical noise explicitly without requiring a priori specification of the number of clusters.

The Euclidean distance metric was employed to jointly evaluate proximity in both 2D space and time (Ester et al., 1996). Two key parameters were explored: *min_cluster_size*, defining the minimum number of points (click trains) to form a cluster, and *min_samples*, which determines the stringency for core point designation. These parameters were tuned via exploratory analyses aimed at optimizing resolution and minimizing over-segmentation and noise (McInnes et al., 2017).

A grid search across a range of *min_cluster_size* and *min_samples* combinations was conducted. For each parameter set, we calculated a suite of internal clustering metrics: total number of clusters, proportion of noise, average cluster persistence (a measure of hierarchical stability), number of robust clusters (persistence ≥ 0.7), and average outlier score. These indicators were normalized and combined into a composite robustness index to identify the most stable and biologically interpretable solution, penalizing configurations with excessive fragmentation or noise (Wang et al., 2023; Campello et al., 2013. The parameter set with the highest robustness index was selected as optimal.

Clusters resulting from HDBSCAN were interpreted as biologically meaningful aggregations of vocal activity, termed Click Train Clusters (CTCs), and considered potential social groups. Detections classified as noise were retained and treated as valid solitary events, potentially representing individual dolphins or small groups with low vocal activity – an important consideration for species exhibiting context-dependent acoustic behavior (Frasier et al., 2016; Giorli et al., 2016; Syme et al., 2022).

Following the initial clustering, a post-hoc refinement step was applied to improve cluster cohesion. For each CTC, we computed internal metrics including spatial and temporal distances between consecutive click trains. Clusters exhibiting exceptionally high spatial or temporal dispersion relative to the overall distribution were classified as weakly cohesive based on Tukey's IQR method (Tukey, 1977). These clusters were segmented at the point of deviation, and new CTCs were initiated to ensure spatiotemporal consistency. This refinement step aimed to reduce the influence of loosely structured or artifact-driven sequences.

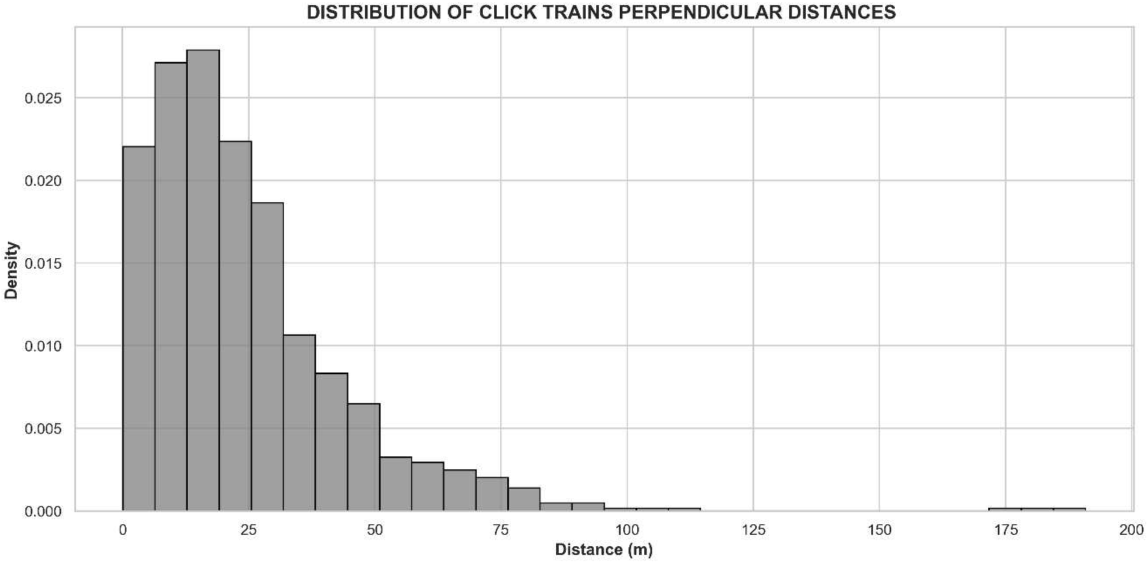
Once clustering and refinement were complete, we quantified CTC structure through a set of intra- and inter-cluster metrics. Intra-CTC metrics included the mean, minimum, and maximum spatial and temporal distances between successive click trains, providing insight into internal cluster cohesion. Inter-CTC metrics – quantifying the distances between adjacent clusters – were used to assess external distinctiveness. Together, these metrics offered a detailed perspective on the spatial continuity and temporal dynamics of franciscana dolphin acoustic groupings (Caruso et al., 2020; Gowans et al., 2007; Jacoby et al., 2016).

2.3 RESULTS

A total of 1,022 localized click trains were recorded over seven days of acoustic surveys. The perpendicular distance of click trains from the survey track ranged from 0 to 190 meters, with a mean of 24.32 meters, indicating the spatial extent of franciscana dolphin acoustic activity relative to the recording platform (Figure 2.1).

Figure 2.1 – Histogram of perpendicular distances from the trackline to the localized franciscana dolphin click trains. Distances were computed based on time-

difference-of-arrival (TDOA) localization estimates. The distribution reflects the spatial dispersion of detections relative to the survey axis.



The application of the HDBSCAN clustering algorithm identified 300 Click Train Clusters (CTCs), with sizes ranging from 1 to 45 click trains (mean = 3.4 ± 5.36). Temporal and spatial metrics were extracted to evaluate internal cohesion (intra-CTC) and separation between clusters (inter-CTC) (Table 2.1, Figure 2.2).

Within clusters, temporal intervals between consecutive click trains averaged 5.65 ± 5.15 seconds (range: 0–22.95 s), and spatial distances averaged 46.84 ± 28.94 meters (range: 1.7–141.2 m).

In contrast, inter-cluster intervals were substantially larger, with temporal gaps averaging 45.46 ± 35.78 seconds (range: 0.11–167.22 s) and spatial distances averaging 108.31 ± 59.12 meters (range: 7.25–323.87 m).

Non-parametric Mann–Whitney U tests confirmed that both temporal and spatial differences between clusters were significantly greater than within clusters ($p < 0.001$ for both comparisons), supporting the validity of the clustering approach in separating acoustically and spatially cohesive click train groups.

The spatial distribution of clusters along the survey track is illustrated in Figure 2.3, highlighting the clear distinction between cohesive vocal activity within clusters and broader spatial-temporal separation between them.

Only CTCs containing two or more click trains were included in the intra-CTC visualisations. Significance levels (*p*-values) from Mann–Whitney U tests comparing intra- vs. inter-CTC distributions are shown in the right-hand plots.

Table 2.1 - Summary metrics of intra- and inter-CTC levels, characterizing the spatiotemporal structure of localized franciscana dolphin click trains. Intra-CTC metrics describe the temporal and spatial distances between consecutive click trains within each click train cluster (CTC), while inter-CTC metrics represent the distances between distinct CTCs.

Metrics	**Intra-CTC [mean ± std (min – max)]**	**Inter-CTC [mean ± std (min – max)]**
⊗ Time (seconds)	5.65 ± 5.15 (0 – 22.95)	45.46 ± 35.78 (0.11 – 167.22)
⊗ Spatial (meters)	46.84 ± 28.94 (1.7 – 141.2)	108.31 ± 59.12 (7.25 – 323.87)

Figure 2.2 - Temporal and spatial differences within and between clusters (CTCs) of franciscana dolphin click trains. Top panel: boxplots of temporal differences (Δ Time, in seconds), illustrating intra-CTC variation across individual clusters (left) and aggregated comparisons between intra- and inter-CTC values (right). Bottom panel: equivalent representation for spatial differences (Δ Space, in meters).

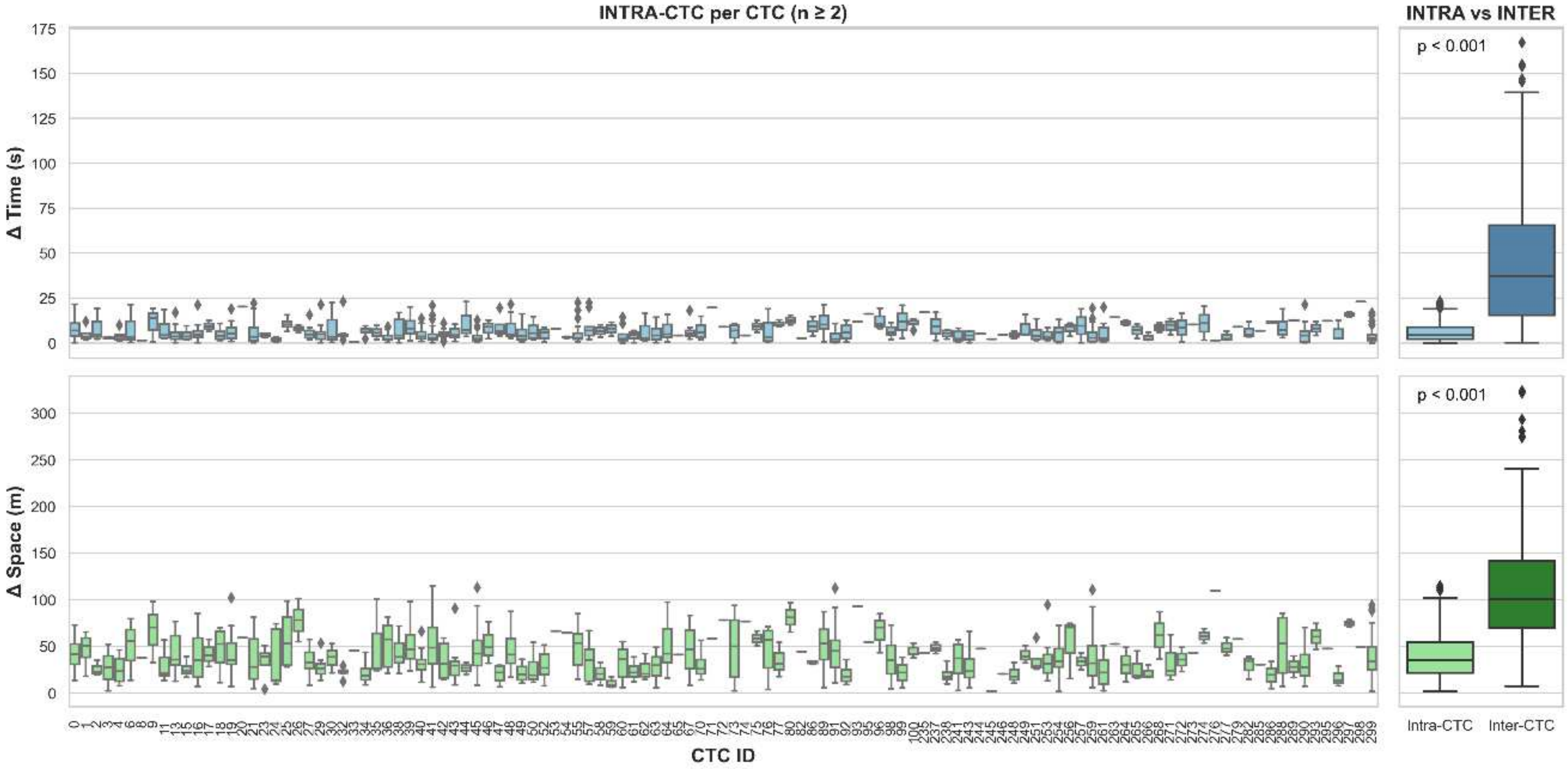
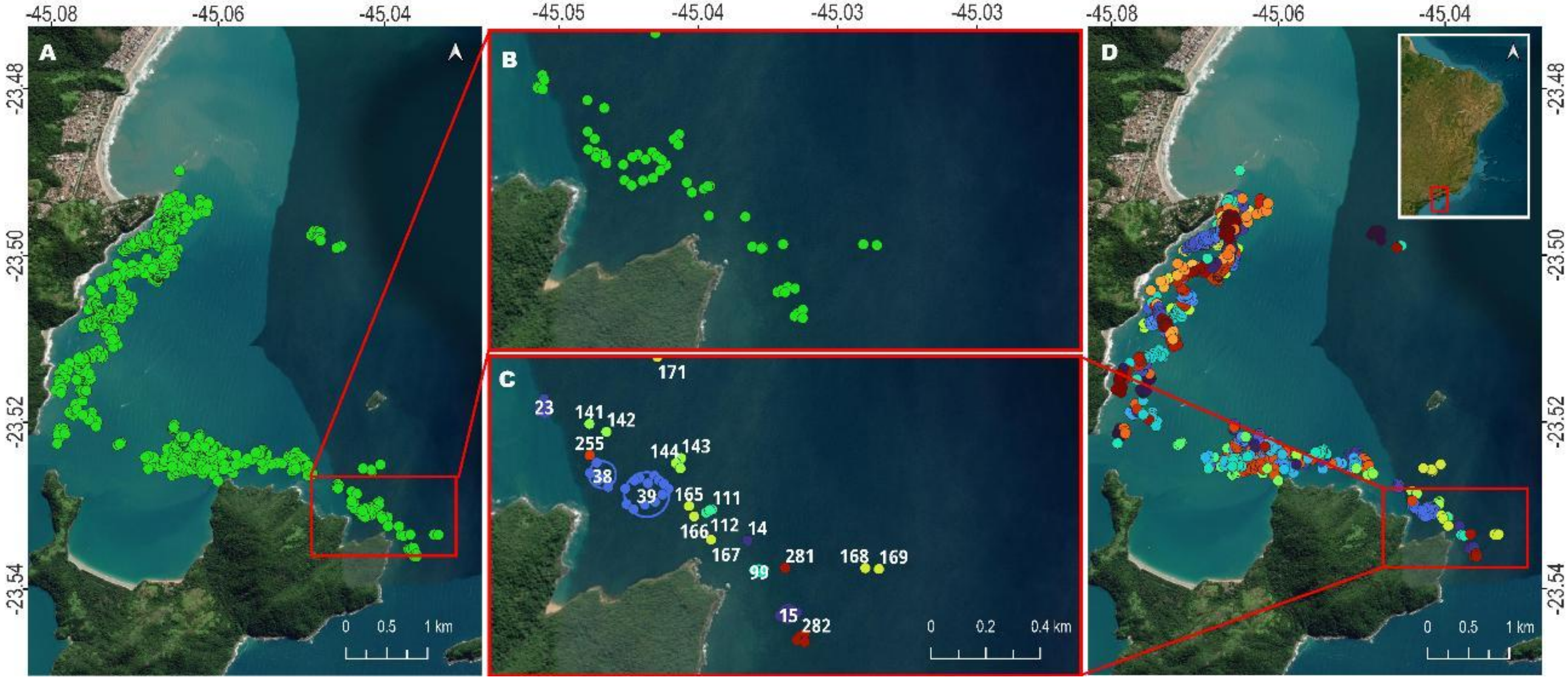


Figure 2.3 - Spatial visualisation of franciscana dolphin click train data and clustering results. (A) All localized click trains used in the analysis, providing a general spatial overview. (B) Zoomed-in view of a specific region within the dataset, allowing inspection of individual detections. (C) Clustering output, with each cluster represented by a distinct colour and number (CTC Id). Clusters with more than one click train are delimited by its minimum circle. (D) Same region as in (A), now showing clustered data, enabling direct comparison between raw and post-clustering patterns.



2.4 DISCUSSION

Given the franciscana dolphin’s elusive behavior, coastal distribution, and typically small group sizes (Cremer et al., 2022; Crespo et al., 2010; Danilewicz et al., 2010; Zerbini et al., 2010), passive acoustic monitoring (PAM) using a towed hydrophone array proved to be an effective strategy for sampling their acoustic activity. The observed distribution of perpendicular distances from the survey track—ranging from 0 to 190 m, with a mean of 24.32 m—demonstrates the adequacy of the data collection and localization framework. Most detections occurred within an effective range for accurate localization, minimizing spatial uncertainty. Conversely, fewer detections beyond 100 m suggest a limitation in system sensitivity, likely due to the high-frequency, narrowband nature of franciscana dolphin echolocation clicks, which attenuate rapidly in the water column (Au, 1993). This variability in detection range highlights the importance of incorporating spatial metrics into group definition and density estimation frameworks to account for detectability constraints in ecological inference.

This study demonstrates that clustering methods based on spatiotemporal proximity can provide objective insights into cetacean group structure. We applied HDBSCAN to automatically classify localized click trains into Click Train Clusters (CTCs), defined by consistent temporal and spatial proximity. As emphasized by Syme et al. (2022a), grouping based on spatial criteria facilitates comparability across taxa and enhances the ecological relevance of sampling units. The CTCs identified by HDBSCAN exhibited shorter intervals within clusters than between them, both in space and time, indicating that the algorithm effectively differentiated detections likely originating from the same social unit from those representing separate individuals or groups.

This approach is particularly relevant for the franciscana dolphin (*Pontoporia blainvillei*), a cryptic and poorly studied species whose surface behavior limits the effectiveness of visual observations. Although franciscana dolphins are most frequently seen in small groups of two to three individuals—and occasionally up to six during feeding or breeding (Bordino et al., 1999; Crespo et al., 1998) – their social cohesion and stability are not well understood. Genetic analyses suggest the existence of small matrilineal units, often involving mothers and calves, indicating kin-based associations and natal philopatry (Costa-Urrutia et al., 2012; Valsecchi and Zanelatto, 2003). Some individuals may travel or forage within these stable family groups (Cremer and Simões-Lopes, 2005), but the lack of long-term behavioral data limits our ability to characterize these associations.

Given these limitations, the application of passive acoustic monitoring provides a rare window into franciscana dolphin social behavior. The internal consistency of CTCs—marked by closely spaced click trains in both time and space—suggests coordinated vocal behavior and potentially cohesive movement. Conversely, larger inter-cluster gaps reinforce the likelihood of distinct social units. Thus, our clustering approach, grounded in high-resolution acoustic data, offers an indirect but powerful proxy for inferring group cohesion and structure. Importantly, the unsupervised, data-driven nature of HDBSCAN reduces user bias and supports replicable, scalable applications to study small, elusive cetaceans like the franciscana dolphin.

Moreover, the structure revealed by HDBSCAN provides a foundation for future integration into passive acoustic density estimation (PADE) frameworks (Marques et al., 2013). A major challenge in PADE is linking acoustic events to the number of animals producing them, which typically requires assumptions about cue production rates or external calibration. Although this study did not estimate absolute abundance, it establishes a protocol for grouping click trains into cohesive acoustic entities. To progress toward density estimates, future work should aim to empirically determine the rate of localized click trains per animal—ideally through simultaneous drone-based observations. This would enable the calibration of acoustic footprints (e.g., click trains per animal), a key parameter for converting acoustic detections into animal densities. Additional efforts should explore behavioral variability in click productions – such as differences during foraging or traveling – and address detectability biases related to distance, angle of approach, or group structure (Baldachini et al., 2025; Lewis et al., 2018; Warren et al., 2017). These steps would allow for more accurate density estimation in future applications of acoustic monitoring for cryptic species like the franciscana dolphin, where visual-based approaches remain challenging (Booth et al., 2017; Frasier et al., 2021; Küsel et al., 2011; Moretti et al., 2010).

2.5 CONCLUSION

This study demonstrates that passive acoustic monitoring combined with clustering algorithms like HDBSCAN offers a powerful, objective framework for defining social groups in *Pontoporia blainvillei*, a species with elusive behavior and limited visual detectability. By clustering localized echolocation click trains into cohesive units based on spatiotemporal proximity, we provide novel insights into the species' social organization that were previously inaccessible through conventional observation methods. The clear differentiation between intra- and inter-cluster metrics supports the biological relevance of these groupings.

Moreover, this approach establishes a methodological foundation for future integration into density estimation protocols, an essential step for effective conservation management of franciscana dolphins. While further work is required to calibrate acoustic acoustic footprints and account for behavioral and environmental variability, the automated and data-driven nature of HDBSCAN clustering facilitates reproducible, scalable analyses. Ultimately, combining passive acoustics with emerging technologies such as drone surveys could enhance our capacity to monitor and understand this vulnerable species, contributing critical information for its conservation.

3 CHAPTER 2 – DRONE-CALIBRATED ACOUSTIC FOOTPRINTS INFER GROUP SIZE IN FRANCISCANA DOLPHINS (*PONTOPORIA BLAINVILLEI*)

3.1 INTRODUCTION

Franciscana dolphins (Pontoporia blainvillei) are the sole representative of the Pontoporiidae genus (Secchi 2010). Their distribution is concentrated between the north of Espírito Santo in Southeast Brazil to the southern regions of Argentina, divided into four major franciscana management areas (FMA I, II, III, and IV) (Secchi et al. 2003, Cunha et al. 2014a). Currently, franciscanas are the most vulnerable of the South American dolphins due to high mortality caused by bycatch, resulting in a significant population decline (Di Beneditto et al. 2010, Prado et al. 2013, MMA 2014, MMA et al. 2018, Mayorga et al. 2020). Franciscanas are currently considered “Vulnerable” by the IUCN (Zerbini et al. 2017) and “Critically Endangered” in the Brazilian Book of Endangered Species (Brasil 2003). Their conservation outlook is particularly severe due to high bycatch mortality (Secchi et al. 2003, Prado et al. 2013), low potential population growth rates, and their restricted coastal distribution (Simoes-Lopes & Cremer 2022). Importantly, no empirical updates of population growth rates exist for the species, highlighting the need for routine demographic monitoring to inform conservation policies.

Franciscanas exhibit unique adaptations to coastal habitats. Melcón et al. (2012a) recorded the echolocation behaviour of franciscanas and found that they emitted short bursts and click trains of high-frequency clicks. These echolocation clicks, combined with their highly agile swimming styles, play a crucial role in the dolphins' ability to hunt in murky waters (Cremer et al. 2022, Paitach et al. 2022). Studies have also indicated variability in the acoustic behaviour between estuarine and open-sea environments, with dolphins adapting their click rates and intensities based on the habitat, as noted by Paitach et al. (2021a). Social sounds such as whistles have also been recorded although less frequently than in other dolphin species. A study by Cremer et al. (2017) highlighted the production of social sounds during interactions, suggesting that these signals are essential for maintaining group cohesion and coordinating activities within small groups.

Passive acoustic monitoring (PAM) has gained widespread use in the long-term and real-time monitoring of marine environments, particularly in remote areas. It is a valuable tool for studying the behaviour and ecology of cetaceans, which are characterised by intense vocal activity and being unavailable for surface observation for extended periods. PAM enables acoustic monitoring of the marine environment under challenging collection conditions not requiring daylight, leading to significant advancements in the understanding of the biology, behaviour, and ecology or marine mammals (Andriolo et al. 2018a).

Accurate estimation of distribution and abundance is crucial for a species' conservation. Traditional methods rely on visual surveys, in which animals must be observed (Sucunza et al., 2022; Zerbini et al., 2010). However, PAM offers an alternative technique for accurate density estimation. (Marques et al. 2012, 2013a b, Thomas & Marques 2012, Ward et al. 2012, Paitach et al. 2021, Barlow et al. 2022). Vocalisations such as calls, songs, clicks, and whistles are distinctive signatures that can be detected, recorded, and analysed to estimate population abundance (Thomas & Marques 2012, Marques et al. 2013a b). This may involve estimating vocalisation rates to correlate sound detections with population density. Detected vocalisation serves as the sampling unit and must be converted into numbers of individuals.

Drones have proven valuable for cetacean monitoring, offering cost-effective and logistically feasible data collection methods (Hodgson & Koh 2016, Burnett et al. 2019a, de Oliveira et al. 2023). The increasing affordability of drones, along with the wide range of models and ease of control, has contributed to their widespread adoption in scientific research. Drones can be equipped with various sensors, allowing for diverse analyses depending on the research objectives. (Whitehead & Hugenholtz 2014, Johnston 2019, Frouin-Mouy et al. 2020). Drones have multiple applications in marine mammal monitoring, including aerial photogrammetry for estimating morphometric measurements and assessing body conditions in various species (Christiansen et al. 2016, Durban et al. 2016, de Oliveira et al. 2023), abundance estimation (Barreto et al. 2021), behavioural studies (Fettermann et al. 2019), and aiding in cetacean photo-identification (Fiori et al. 2017). The synchronous use of drones and PAM has previously only been used in two published studies, the first by Frouin-Mouy et al. (2020) on grey whales (Eschrichtius robustus) and the second on Commerson's dolphins (Cephalorhynchus commersonii) by Martin et al. (2021).

We developed a statistical framework combining passive acoustic monitoring (PAM) and drone-based visual surveys. Acoustic detections were used to quantify echolocation activity, and a subset of detections paired with drone observations provided ground-truth group sizes. These paired data were used to calibrate andetection model, reflecting the expected number of click trains detected as a function of time available for detection and distance from the trackline, which was then applied to unpaired acoustic clusters to estimate group size probabilistically, aiming to contribute to distance sampling analysis for estimating density and abundance using PAM.

3.2 METHODOLOGY

3.2.1 Data collection

The acoustic dataset analysed in this study was originally collected and described in detail by Mura et al. (2025). In brief, we recorded narrowband high-frequency (NBHF) vocalizations of franciscana dolphins in Ubatuba, São Paulo, Brazil, during December 2021. The study area comprised shallow coastal waters typically ≤10 m deep. In this region, franciscanas are the only species producing this type of NBHF click, ensuring species-specific attribution of detections. Recordings were obtained over seven days, totalling 13 h and 49 min of acoustic data, using an AUSET Technology® recording system equipped with a SailDaq module (Sea Mammal Research Unit, University of St Andrews, UK) and a 50-m linear hydrophone array. According to the manufacturer's specifications, the hydrophone system presented an approximately flat response (±4 dB) within the 50 – 180 kHz bandwidth of interest. Hydrophone spacing, sampling frequency, and all other acoustic data acquisition procedures followed exactly those described in Mura et al. (2025).

To complement the acoustic sampling, synchronous drone-based surveys were conducted along approximately systematically transects. A DJI Phantom 4 Pro drone was flown at an altitude of 80-100 m above a reference buoy while the research vessel navigated the transects, enabling the simultaneous acquisition of aerial imagery and underwater acoustic data. Visual data analysis was conducted considering only periods when the drone maintained a stable and consistently straight trajectory above the buoy, referred to as sections.

3.2.2 Data processing

The acoustic preprocessing steps, including click detection, classification, localization, and spatiotemporal clustering using the HDBSCAN algorithm, were performed as described in Mura et al. (2025). Briefly, narrowband clicks were detected using the PAMGuard Click Detector Module, classified based on species-specific parameters, and aggregated into click trains. Spatiotemporal clustering was then applied to identify cohesive groups of acoustic activity, referred to as click train clusters (CTCs), which were considered potential social groups. All methodological details, parameter tuning, and validation procedures are provided in Mura et al. (2025).

Complementary bioacoustics analysis was conducted using the R (R Core Team, 2024) package PAMpal (Sakai 2021). Acoustic parameters were extracted from individual clicks within click trains. A bandpass filter with a range of 90 to 170 kHz was applied to extract acoustic parameters and employed a window size of 2.5 milliseconds (winlen_sec). We retrieved the parameters peak frequency (PF), 3 dB bandwidth (BW3), and 10 dB bandwidth (BW10).

Drone footage was calibrated across the nine sections of the field of view using a photogrammetric model described in de Oliveira et al. (2023), with the survey boat serving as the reference object. The calibration aimed to correct lens distortion and ensure accurate spatial measurements from drone footage. Ground sample distance (GSD) was then computed to standardize coverage and convert pixel dimensions into precise real-world measurements (Burnett et al. 2019b, Hillcoat et al. 2021, de Oliveira et al. 2023). The maximum field of view covered by the drone footage was calculated, and all video segments were manually inspected to identify franciscana dolphin groups. Once a group was detected, we counted the total number of individuals (corresponded to the maximum number of dolphins observed surfacing consecutively during the drone pass), recorded the group's geographic position, defined as the centroid of the group at the midpoint of its visual exposure and calculated the perpendicular distance from this centroid to the vessel track line.

To ensure that visual and acoustic detections referred to the same franciscana group, we applied a two-step decision tree adapted from Ollier et al. (2023). In the first step, we defined a temporal threshold based on the geometry of our survey setup and vessel average speed. Since the hydrophone array and drone were vertically aligned (0 m separation in both x and y axis), and the maximum detection ranges were 97.5 m for acoustic detections and 90 m for visual detections, the effective range for synchronous monitoring between platforms was therefore limited to 90 m. Considering the average vessel speed of 4.18 knots (≈2.15 m/s), the maximum time window within which a visual and other acoustic detection could originate from the same group is 90 s (-45 s before or 45 s after). Any pair of events occurring within this time interval was classified as a potential duplicate In the second step, we applied a spatial threshold to account for the potential movement of the animals between the two detections. We used the maximum reported swimming speed of franciscana dolphins (3.49 knots; ≈1.80 m/s; Crespo 2018) to define a mobility buffer around the first detection. If the position of the second detection fell within this buffer, the potential duplicate was confirmed as a true duplicate event.

For detection, what matters is the number of animals present in the group rather than their instantaneous visibility on the surface. Dolphins frequently dive and resurface, causing fluctuations in the number of individuals visible at any given moment, but these oscillations do not alter the true group size N. Thus, we assumed that the full group size remained constant throughout each visual encounter. When more than one acoustic cluster met the temporal and spatial matching criteria for a single visual sighting, that sighting was subdivided into independent, non-overlapping segments, each matching the start and end time of one synchronous acoustic cluster. This ensured that each acoustic cluster was paired with a corresponding visual segment of identical duration, preventing overlap between calibration units.

For clusters with more than one detected click train ($C \geq 2$), the cluster duration Δt_i (defined as the time elapsed between the first and last click train within each cluster) was directly measured, and animal-seconds were calculated as $\mathcal{A}_i^{sec} = N \cdot \Delta t_i$. For clusters containing a single detected click train (C = 1), Δt could not be computed because it represents an interval between multiple click trains. Using the duration of an isolated click train as a proxy would markedly underestimate the time during which an animal was acoustically detectable, as individual trains typically last from fractions of a second to only a few seconds. Therefore, we assigned a standardized duration W equal to the global median Δt_i of paired clusters (C ≥ 2). In these cases, animal-seconds were computed as $\mathcal{A}_i^{sec} = N \cdot W$. To evaluate the sensitivity of this assumption, we repeated the analyses using W defined as the 25th and 75th percentiles of the empirical distribution of Δt. This procedure ensured that all acoustic clusters could be retained for model calibration, while effort was consistently allocated in a way that reflected the true group size rather than momentary fluctuations in visibility.

3.2.3 Statistical modelling framework

3.2.3.1 False-positive correction

In the context of automated acoustic analysis, it was necessary to account for the presence of false positives in click-train detection. To quantify this effect, an independent acoustician manually examined the database blindly, without access to the automated outputs. For comparability, the expert applied a few basic parameters consistent with those used in the automated detector – such as the minimum number of clicks per cluster and minimum/maximum inter-click interval (ICI) – while making manual selections. This procedure allowed a consistent benchmark to evaluate the reliability of the automated detection.

The comparison between manual and automated detections yielded a false-positive rate, denoted here as p_{FP}. To correct for this bias, the raw number of detected click trains (C) in acoustic cluster i was adjusted as:

$$C_i^* = C \cdot (1 - p_{FP}),$$

where C_i^* represents the corrected number of click trains (Thomas & Marques 2012).

3.2.3.2 Acoustic footprint detection model (paired clusters)

For clusters synchronized with drone observations, we modeled the number of echolocation click trains detected acoustically within each cluster as a stochastic count process. The objective of this analysis was to estimate the baseline acoustic footprint of a typical animal at zero distance from the trackline (r_0), while explicitly accounting for distance-dependent detectability — i.e., the probability that a click train emitted by an animal is recorded by the hydrophone given its perpendicular distance from the array.

Because detection probability decreases with distance due to geometric spreading, absorption, and signal-to-noise constraints, this effect must be incorporated into the model to avoid biasing the inferred acoustic footprint downward for distant clusters. Here, the detection function $f(d)$ describes that decline and enters the GLM as an additive offset on the linear predictor scale, in the same way that exposure or effort terms are included in count models.

Let C_i^*denote the total number of click trains detected acoustically for cluster i, which corresponds to a drone-measured group of $N_i$animals observed during a time interval Δt_i. Assuming independent emissions among individuals and a stationary rate process, the expected number of detected click trains is:

$$E[C_i^*] = N_i \cdot \Delta t_i \cdot r_0 \cdot f(d_i),$$

Where

- r_0= baseline acoustic footprint ($\text{click trains} \cdot \text{animal}^{-1} \cdot \text{s}^{-1}$) at zero distance,
- $f(d_i)$= detection function ($0 \leq f(d) \leq 1$) describing the relative detection probability at distance d_i,
- $N_i \cdot \Delta t_i$= "effective exposure", i.e. the total potential number of acoustic footprints detected by all animals in the cluster over its duration.

Because $C_i^*$are counts, they were modeled under the Poisson or, when over dispersed, Negative Binomial distribution:

$$C_i^* \sim NegBin\ (\mu_i, k)\ \ or\ \ C_i^* \sim Poisson\ (\mu_i),$$

where $\mu_i = E[C_i^*]$ and k is the dispersion parameter.

Taking the logarithm of the mean gives the familiar log-link formulation of a GLM:

$$\log \mu_i = \log(A_i^{sec}) + \alpha + \log(f(d_i)),$$

With

- $A_i^{sec} = N_i \cdot \Delta t_i$(the offset term accounting for exposure effort),
- $\alpha = \log(r_0)$, the intercept corresponding to the expected log acoustic footprint at zero distance,
- and $\log(f(d_i))$, an additive term representing the decline in detectability with increasing distance.

In practice, $f(d)$ is a parametric function of distance characterized by one or two shape parameters that control how steeply detectability decreases. Thus, the

GLM estimates both the baseline acoustic footprint $r_0$and the parameters of the detection function, simultaneously linking acoustic detections to drone-observed animal groups through shared exposure terms.

For each acoustic cluster i, we summarized detection geometry by two metrics:

- the effective squared distance,

$$d_{eff,i}^2 = \frac{1}{C_i}\sum_{k=1}^{C_i}(\mu_{ik}^2 + s_{ik}^2),$$

used for the half-normal model (where detectability declines as a Gaussian function of d^2 from the trackline), and

- the mean perpendicular distance,

$$\bar{d}_i = \frac{1}{C_i}\sum_{k=1}^{C_i}\mu_{ik},$$

used for the linear and hazard-rate forms (which depends directly on d).

These definitions ensure that each acoustic cluster's representative distance incorporates localization error and the spatial distribution of detected click trains within the cluster. Here, μ_{ik} is the perpendicular distance of click train k from the trackline and s_{ik} its localization error. Localization error for click trains was characterized internally within PAMGuard's Time Difference of Arrival (TDOA) localization module. The software computes the uncertainty of each position estimate based on the variance in arrival-time differences across hydrophone pairs, incorporating array geometry, sampling rate, and cross-correlation confidence.

We evaluated three competing functional forms for the distance-dependent detection function $f(d_i)$, harzard-rate, half-normal and linear detection function, commonly used in acoustic distance sampling (Buckland et al. 2015). Each detection function was fitted under both Poisson and Negative Binomial assumptions, resulting in six candidate models in total.

Model parameters $(\alpha, \sigma, b, \beta, k)$ were estimated by maximum likelihood using *glm* and *glm.nb* families in R. Model selection was based on Akaike's

Information Criterion (AIC), and Pearson's dispersion statistic was used to assess overdispersion and justify the Negative Binomial formulation when necessary. Uncertainty in r_0 and in the detection parameters was quantified using the delta method applied to the log-scale intercept and functional form parameters, respectively. Confidence intervals for r_0 were converted back to the natural scale ($\mathrm{trains} \cdot \mathrm{animal}^{-1} \cdot \mathrm{s}^{-1}$).

Model explanatory power was summarized by pseudo-R^2 values: McFadden's statistic (comparing the log-likelihoods of fitted and null models) and Efron's statistic (measuring the proportion of variance explained in observed counts). These diagnostics provided complementary perspectives on model fit and predictive adequacy.

3.2.3.3 *Conceptual interpretation*

In ecological terms, this GLM framework extends the counting approach used in distance sampling to a context where individual detections are temporally clustered and spatially referenced to drone observations. The model treats the number of click trains as a product of three components:

$C_i^* \propto (group\ size) \times (\mathrm{time\ observed}) \times (\mathrm{acoustic\ footprint}) \times (\ detectability\ at\ d_i).$

By explicitly including $f(d_i)$ in the model, we separate biological variation in vocalization detection (the true r_0) from physical and geometric variation in detectability. This is crucial for extrapolating acoustic detections to animal abundance in Passive Acoustic Density Estimation (PADE) frameworks.

3.2.4 Estimation of Group Size for Unpaired Clusters

To test the model, we applied it to clusters recorded without drone footage, with maximum perpendicular distance of 100 m. Group size is inferred by inverting the calibrated relationship. With corrected counts C_j^* and observed duration Δt_j:

$$\hat{N}_j = \frac{C_j^*}{r_0 \cdot f(d_i) \cdot \Delta t_j}.$$

For clusters where duration could not be reliably defined — either single-train clusters or multi-train clusters with extremely short durations ($\Delta t_j < W$) — we assigned a standardized duration of $\Delta t_j = W$. Very short clusters (lasting only fractions of a second) would otherwise lead to implausibly high group-size estimates within the model, as the number of individuals per unit time would be inflated. Using W as a lower threshold (floor') for cluster duration provides a conservative and biologically reasonable estimate of the period over which animals were acoustically detectable. Sensitivity analyses used alternative values of W set to the 25th and 75th percentiles of the paired-cluster distribution.

Because multiple clusters could originate from the same visual group, we computed cluster-robust standard errors (sandwich estimator) with groups defined by the visual detection ID. To propagate parameter uncertainty into group size estimates, we implemented a parametric bootstrap (B=9999) based on the covariance structure of the fitted GLM parameters. Uncertainty was summarized using the total coefficient of variation (CV):

$$CV_{total} = \frac{SD(\hat{N}_{total})}{\mathbb{E}\,[\hat{N}_{total}]}.$$

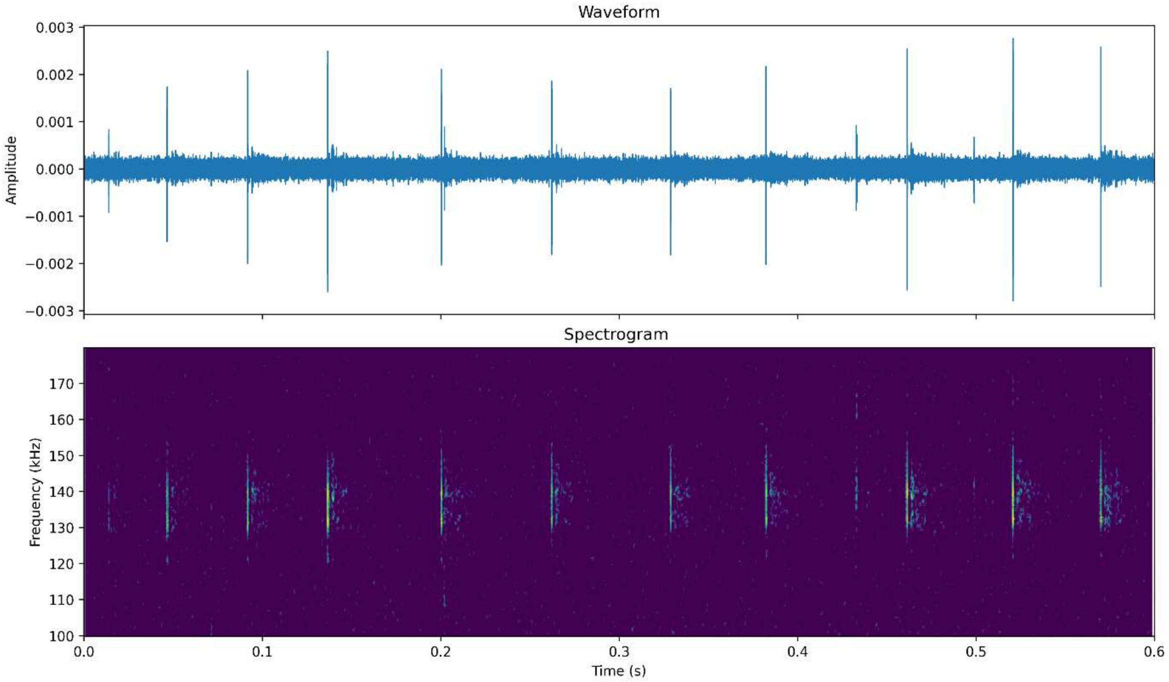
All analyses were performed in Python (version 3.11). Data handling and preprocessing were conducted using pandas (McKinney 2010), while generalized linear models (GLMs) were fitted with statsmodels (Seabold & Perktold 2010). Numerical simulations and bootstrapping procedures were implemented with NumPy (Harris et al. 2020). Visualization and graphical outputs were generated using matplotlib (Hunter 2007) and seaborn (Waskom 2021).

3.3 RESULTS

3.3.1 Acoustic data

A total of 1,012 click trains (10,433 clicks) were detected during the eight surveys, averaging 10.3 clicks per train (±8.1 SD). Their spatial distribution showed a mean slant range of 23.37m (±17.68 SD). Acoustic parameters averaged 129.34 kHz (±10.43 SD) for peak frequency, 3.86 kHz (±2.56 SD) for 3 dB bandwidth, and 11.22 kHz (±8.16 SD) for 10 dB bandwidth (Figure 3.1).

Figure 3.1 - Identified franciscana click trains example. Above: waveform visualization. Below: spectrogram visualization (Hann window, 1024 pts).



Cluster analysis yielded 300 click-train clusters (Mura et al. 2025), of which 183 (61%) were singletons containing only one click train. Among clusters with $C \geq 2$, durations ranged from a few seconds to nearly a minute, with a median of 36.45 s (interquartile range: 23.02–73.53 s). These distributions were subsequently used to define the standardized exposure window W applied in the calibration of singleton clusters.

3.3.2 Visual data

Environmental and logistical constraints restricted drone-based monitoring to two surveys, during which 14 sections were synchronously sampled (total effort: 54 min; 7.04 km). During the same time windows, the acoustic analysis identified 37 clusters comprising 119 click trains. Within five of these sections, eight franciscana groups were visually detected, comprising a total of 25 individuals. Table 1 summarizes these observations.

Table 3.1 - Summary of synchronized drone and acoustic monitoring during two surveys, including the number of acoustic click train clusters (CTC), click trains and visual detections.

Survey	Section	Length (m)	Duration (mm:ss)	Visual detections	CTC	Click trains
4	A1	670.71	04:57	0	0	0
4	B1	465.75	03:32	0	0	0
4	C1	166.72	01:15	0	0	0
4	D1	470.43	02:26	2	13	42
4	E1	495.97	03:55	2	10	33
4	F1	536.61	04:05	0	0	0
4	G1	445.46	04:31	0	0	0
7	A2	777.6	06:37	0	0	0
7	B2	65.17	00:34	1	1	12
7	C2	886.01	06:09	2	8	24
7	D2	277.07	02:07	1	3	3
7	E2	799.29	06:01	0	2	5
7	F2	553.85	04:07	0	0	0
7	G2	433.02	03:45	0	0	0
Total		**7043.66**	**54:01**	**8**	**37**	**119**

3.3.3 Matched data

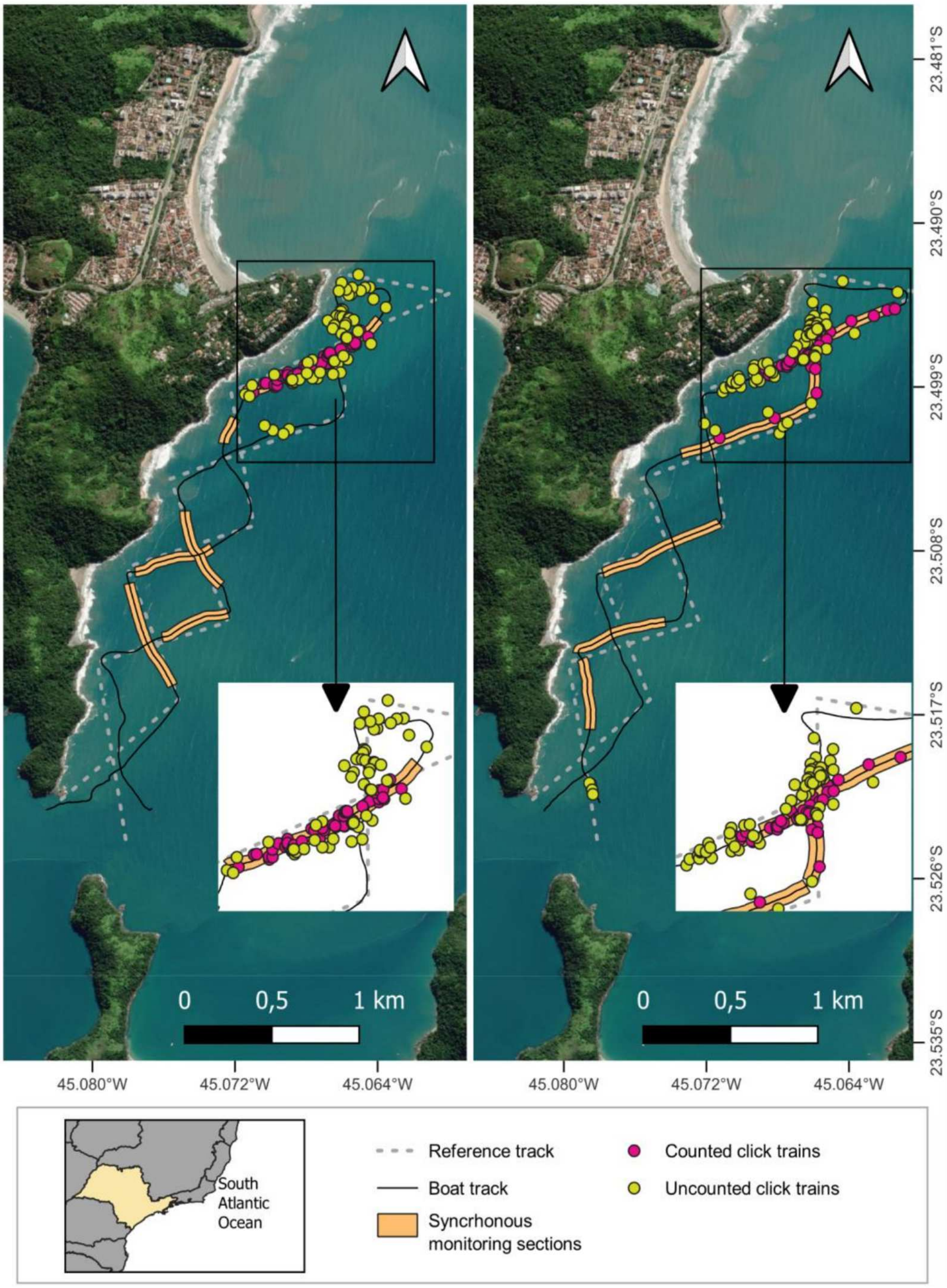
Applying the two-step matching decision tree, we identified instances where acoustic and visual detections we considered to refer to the same franciscana group. The temporal threshold yielded 30 potential matches across the synchronized dataset. After applying the spatial mobility buffer, 28 of these were confirmed as true acoustic–visual matches corresponding to eight visual sightings (Table 2). In several cases, a single visual group was associated with more than one acoustic cluster within the defined thresholds. To accommodate this, visual detections were subdivided into non-overlapping segments matching the duration of the corresponding acoustic clusters, yielding 28 paired cluster-level records in total (Figure 2). Animal-seconds were allocated to each subdivision, ensuring that acoustic footprint detection model reflected the true group size rather than short-term variation in visibility.

Table 3.2 – Subdivided confirmed matches between acoustic clusters and drone sightings of franciscana dolphins after applying temporal and spatial thresholds (28 matches across eight visual sightings).

Section	Visual ID	Number of animals $[N_i]$	Number of Sync Acoustic Clusters	Click trains $[C_i]$	Cluster duration $[\Delta t_i]$
D1	D1_1	1	5	1	W
D1	D1_1	1	5	1	W
D1	D1_1	1	5	1	W
D1	D1_1	1	5	1	W
D1	D1_1	1	5	1	W
D1	D1_2	7	8	20	89.281
D1	D1_2	7	8	4	23.428
D1	D1_2	7	8	5	16.016
D1	D1_2	7	8	4	20.273
D1	D1_2	7	8	1	W
D1	D1_2	7	8	1	W
D1	D1_2	7	8	1	W
D1	D1_2	7	8	1	W
E1	E1_1	3	2	10	39.494
E1	E1_1	3	2	12	73.534
E1	E1_2	3	5	1	W
E1	E1_2	3	5	1	W
E1	E1_2	3	5	1	W
E1	E1_2	3	5	1	W
E1	E1_2	3	5	1	W
B2	B2_1	4	1	12	90.105
C2	C2_1	2	2	5	36.45
C2	C2_1	2	2	2	23.028
C2	C2_2	2	2	1	W
C2	C2_2	2	2	1	W
D2	D2_1	3	3	1	W
D2	D2_1	3	3	1	W
D2	D2_1	3	3	1	W

$*W = 36.45\ s$ (interquartile range: 23.02 – 73.53 s) is the standardized exposure

Figure 3.2 - Map for the two synchronous surveys, presenting the boat track during PAM and drone monitoring, *Pontoporia blainvillei* click trains detected, and the area covered for each section monitored.



3.3.4 False Positive Rate

Across the eight survey campaigns, a manual inspection of the acoustic database identified 1,098 click trains (10,018 clicks in total), with a mean slant range of 24.32 ± 18.21 m. Table 3 summarizes the comparison between manual and automated detections. On average, the automated procedure slightly overestimated detections relative to manual analysis, with a ratio of 0.935. This corresponds to a false-positive rate (p) of 6.42% (Thomas & Marques 2012).

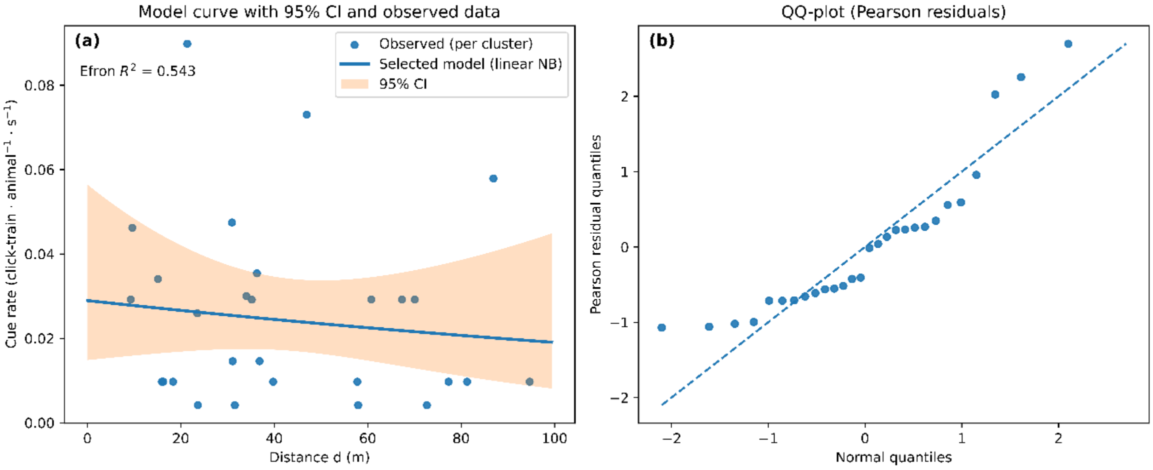
Table 3.3 - Summary of results for automated and manual click-train analyses analysis.

Survey	Automated analysis		Manual analysis		Automated/ manual rate
	Click trains	**Clicks**	**Number of click trains**	**Total number of clicks**	
1	129	1224	112	1136	1.151
2	277	2969	233	2038	1.188
3	225	2476	282	2475	0.797
4	124	1363	153	1301	0.810
5	81	1015	87	1018	0.931
6	73	796	85	786	0.858
7	119	1387	146	1264	0.815
8	0	0	0	0	NA
				Mean ratio	**0.935**

3.3.5 Acoustic footprint detection model

Six candidate cue-rate models were fitted to the subset of acoustic clusters paired with drone observations. Akaike's Information Criterion (AIC) identified the linear Negative Binomial (Figure 3.3) model as the most parsimonious, consistent with evidence of overdispersion in the data (Pearson dispersion ratio = 2.78). The estimated baseline emission rate at zero distance was $r_0 = 0.028\, clicktrain \cdot animal^{-1} \cdot sec^{-1}$ (95% CI: 0.014 – 0.056; CV=0.34), equivalent to $1.68\, clicktrain \cdot animal^{-1} \cdot min^{-1}$.

Figure 3.3 - Model fitting. Left panel: Observed acoustic footprints per cluster (blue dots) plotted against distance and the fitted detection function from the selected linear Negative Binomial model (blue line), with orange areas representing 95% confidence intervals. The model explained a substantial portion of the variation (Efron pseudo-R² = 0.543). Right panel: QQ-plot of Pearson residuals against theoretical normal quantiles, indicating the adequacy of residual distribution and overall model fit.

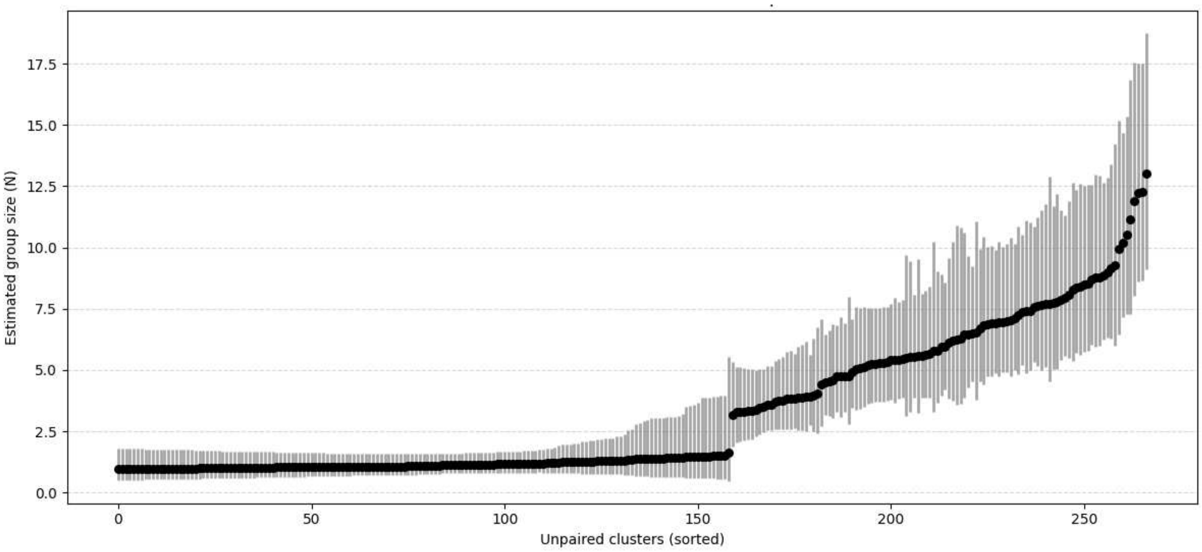


Model fit diagnostics indicated contrasting values for different $pseudoR^2$ measures. The McFadden $pseudoR^2$ was very low ($R^2_M = 0.003$), reflecting the modest improvement in log-likelihood relative to a null model with no distance effect, a pattern often expected in generalized linear models for overdispersed count data. In contrast, the Efron $pseudoR^2$ was high ($R^2_E = 0.543$), indicating that the selected linear Negative Binomial model explained approximately 54% of the variability in observed click-train counts. Together, these results suggest that while the inclusion of distance only marginally improved model likelihood compared to the null, the fitted model nonetheless provided a good fit to the data and captured the main ecological trend of declining detection rates with increasing distance.

3.3.6 Estimation of group size in unpaired clusters

The model was applied to predict group size for 267 unpaired acoustic clusters. To reduce the influence of extreme values, group size was summarized by the median of the bootstrap distribution, providing robust and standardized estimates. Predicted group sizes ranged from 0.95 to 13.03 individuals, with a median of 1.34 (95% CI: 0.79 - 2.57; Figure 4). When aggregated across all clusters, the bootstrap procedure yielded a total coefficient of variation (CV) of 0.15, indicating high overall precision.

Figure 3.4 - Estimated group sizes for 267 unpaired acoustic clusters. Black dots represent the bootstrap median for each cluster, sorted in ascending order. Gray bars indicate the corresponding 95% confidence intervals, illustrating the uncertainty of each estimate.



3.4 DISCUSSION

This study presents a practical framework for linking acoustic detections of franciscana dolphins (*P. blainvillei*) to group size through acoustic footprint detections model, combining passive acoustic monitoring (PAM) with drone-based visual counts. Beyond the methodological advances, our results also provide ecological insights into the species' social structure and echolocation behavior.

3.4.1 Group size and social organization

Visual studies consistently describe franciscanas in small groups of two to three individuals, with larger aggregations (>6) observed only occasionally during feeding events (Crespo et al. 1998, Bordino et al. 1999). Genetic analyses corroborate this pattern, indicating matrilineal units and kin-based associations (Valsecchi & Zanelatto 2003, Costa-Urrutia et al. 2012). Our calibrated estimates, dominated by small groups, align with this evidence and reinforce the view that *P. blainvillei* exhibits fine-scale social cohesion and limited aggregation tendencies (Secchi et al. 2001, Cremer & Simões-Lopes 2005, Denuncio et al. 2013).

This study establishes a practical pathway to translate echolocation detections into biologically meaningful group-size estimates. By pairing passive acoustic monitoring with short drone overflights, we calibrated a detection model at the click-train level, correcting for false positives and standardizing exposure by animal-seconds. Although our paired dataset was limited (eight groups across two drone surveys), it demonstrated the feasibility of acoustic footprint models and allowed predictions to be extended to 267 unpaired acoustic clusters with higher precision for lower group sizes than larger group sizes (CV ≈ 0.15).

Counting individual animals using acoustic techniques has been demonstrated previously for odontocete species (e.g., sperm whales: Barlow & Taylor 2005.; beaked whales: Moretti et al. 2010). More recently, approaches that infer group size from acoustic metrics – such as the acoustic footprint method for Blainville's beaked whales (Marques et al. 2019) – have shown that integrating additional information can substantially improve abundance estimates derived from passive acoustics. By calibrating acoustic footprints with drone-based counts, our framework incorporates both vocal and silent animals within each group, providing a more reliable basis for franciscana abundance estimation.

3.4.2 Integrating drones and PAM for acoustic footprint detection model

Detection models are critical because density estimates are highly sensitive to the number of acoustic footprints detected per animal per unit time (Marques et al. 2009, Thomas & Marques 2012). By pairing PAM with drone-based counts, we obtained a grounded detection rate, minimizing biases from counting only vocally active animals. This dual-platform approach reflects broader advances in Passive Acoustic Density Estimation (PADE), where combining observation modalities reduces uncertainty associated with behavioral variability (e.g.,Warren et al. 2017).

Drone–PAM integration for franciscanas is novel but echoes similar applications in other cetaceans, such as synchronized acoustic–visual sampling in gray whales and Commerson’s dolphins (Frouin-Mouy et al. 2020; Martin et al. 2021). These studies demonstrate that drones improve group-size counts, behavioral classification, and geometric context, all of which enhanced our calibration as well. Limitations remain – short flight endurance, weather constraints, and potential behavioral responses – but impacts are generally minor above 30–40 m altitude (Christiansen et al. 2016). Incorporating brief drone flights into larger PAM campaigns thus provides a realistic balance between accuracy and logistics.

The decrease in acoustic footprint detection with increasing distance may reflect a combination of factors, including signal attenuation, orientation effects (animals facing away from the recorder), and behavioral changes such as increased echolocation when individuals are closer and possible interacting with the hydrophone. A key implication is that acoustic footprint detections are context-dependent, influenced by behavioral state, group composition, and environmental conditions. Current PADE best practice emphasizes that such variability must be explicitly quantified (Thomas & Marques 2012, Marques et al. 2013b). Our bootstrap procedures and false-positive correction partially address this requirement, but broader calibration across seasons, habitats, and behavioral states remains essential to ensure the generality and transferability of estimates (Warren et al. 2017).

3.4.3 Bioacoustic parameters

Our acoustic measurements (median peak frequency ~129 kHz) fall within the known NBHF profile of franciscana clicks, typically peaking around 139 kHz (Melcón, et al. 2012a, Amorim et al. 2022). Habitat-related variability in echolocation has been documented, with estuarine versus open-sea habitats influencing click production patterns (Paitach et al. 2021). Social signals such as whistles and burst pulses are rare but occur in specific contexts (Cremer et al. 2017).

3.5 CONCLUSION

This study demonstrates the feasibility of calibrating acoustic footprints to estimate franciscana dolphins group sizes by combining PAM with drone-based counts. The approach addresses a central challenge in passive acoustic density estimation: translating click-train detections into biologically meaningful group-size estimates. Our calibration confirmed that franciscanas predominantly occur in small, cohesive groups, consistent with previous visual and genetic studies, and provided robust detection-rate estimates that incorporate both vocal and silent individuals.

By explicitly correcting false positives, standardizing exposure with animal-seconds, and applying the model to unpaired clusters, we produced group-size predictions. Although our paired dataset was limited, the framework offers a scalable template for future monitoring, where brief drone overflights embedded in longer PAM surveys can strengthen calibration.

The broader implication is that acoustic footprints may vary with behavior, group composition, and environment. Expanding calibration across seasons, habitats, and management areas is essential to increase transferability. Nevertheless, the integration of drones and PAM provides a promising pathway to reduce uncertainty in PADE, extend monitoring to cryptic species, and support conservation strategies for *Pontoporia blainvillei*.

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4 CHAPTER 3 – POPULATION ASSESSMENT OF FRANCISCANA DOLPHINS AT THE NORTHERN EDGE OF THEIR DISTRIBUTION (FMA-1A, BRAZIL) USING PASSIVE ACOUSTIC METHODS

4.1 INTRODUCTION

The franciscana dolphin (*Pontoporia blainvillei*) is a small, endemic odontocete inhabiting coastal waters of the western South Atlantic, ranging discontinuously from Espírito Santo, Brazil (~18°S), to Golfo San Matías, Argentina (~41°S). It is widely regarded as the most threatened cetacean in South America due to high, and possibly unsustainable, levels of bycatch combined with ongoing habitat degradation across its range (Ott et al., 2002; Secchi et al., 2003, 2021; Secchi, 2010; Pinheiro et al., 2019; Cremer et al., 2022). Accordingly, the species is currently listed as Vulnerable by the IUCN Red List (Zerbini et al., 2017) and Critically Endangered at the national level in Brazil (MMA, 2022). Its shallow, nearshore distribution further exacerbates vulnerability, as this habitat overlaps almost entirely with areas of intense human activity, including fisheries, coastal development, and port operations.

To guide research and conservation, the species' range has been subdivided into Franciscana Management Areas (FMAs), originally defined based on genetic, morphological, and ecological evidence (Secchi et al., 2003; Crespo et al., 2010). Morphological divergence across populations has also been documented, reinforcing the existence of geographical structuring and supporting the delineation of FMAs (Crespo et al., 2018; Secchi et al., 2003). Among them, FMA-1a, located along the northern coast of Espírito Santo, represents both the northernmost limit of the species' distribution and an isolated population unit (de Freitas Neto, 2007; Moreno et al., 2003; Cunha et al., 2014; do Amaral et al., 2018). Its distribution spans approximately 170 km of coastline, mostly within waters shallower than 20 m, between Itaúnas (18°25′S) and Santa Cruz (19°57′S) (do Amaral et al., 2018). Metapopulation theory predicts that peripheral and isolated stocks face disproportionately high extinction risks due to demographic stochasticity, reduced genetic variability, and the lack of recolonization potential. These concerns are particularly acute for FMA-1a, where no evidence of connectivity with adjacent FMAs has been found (Sucunza et al., 2023).

Recent genetic and morphological analyses have further reinforced this isolation**.** Nara et al. (2024) formally recognized the franciscana population of Espírito Santo (FMA-1a) as a distinct subspecies, *Pontoporia blainvillei pukusi*. This taxonomic revision highlights the deep evolutionary divergence and diagnosability of this unit relative to other populations, underscoring its singular conservation importance. As a result, FMA-1a is no longer only a management unit but now represents a unique subspecific lineage at the northern fringe of the species' range, further elevating the urgency for robust monitoring and protection.

Empirical information on abundance and trends for this stock is yet limited. Aerial visual surveys conducted in 2018 provided the only available estimates, suggesting that franciscanas in Espírito Santo are concentrated in a narrow coastal strip near the Doce River estuary (Sucunza et al., 2023). The 2018 effort covered 2,986 km of transects, yielding an abundance estimate of ~1,183 individuals after correcting for visibility and group-size biases (Sucunza et al., 2022, 2023).

Passive acoustic monitoring (PAM) has emerged as a powerful alternative for studying cryptic odontocetes, enabling continuous sampling regardless of turbidity, weather, or daylight (Boisseau et al., 2007; Risch et al., 2014; Andriolo et al., 2018). For franciscanas, PAM is facilitated by their stereotyped narrow-band high-frequency (NBHF) echolocation clicks, with peak frequencies around 130–140 kHz (Melcón et al., 2012; Amorim et al., 2022), which can be reliably distinguished from other sympatric cetaceans. Advances in passive acoustic density estimation (PADE) discussed the conversion of acoustic detections into abundance estimates by integrating cue-production rates, distance-dependent detection functions, and corrections for false positives (Marques et al., 2009, 2013; Thomas & Marques, 2012). While empirical applications have focused on harbor porpoises (*Phocoena phocoena*) in the Northern Hemisphere (Kyhn et al., 2012; Nuuttila et al., 2018), methodological developments – including detection, clustering, and calibration with paired visual–acoustic datasets – are increasingly being adapted to other cetaceans (Frasier et al., 2017; Ollier et al., 2023; Bopardikar et al., 2025). Notably, the VIII

Workshop for the Research and Conservation of the Franciscana (2015) highlighted the priority to evaluate alternative methods to assess abundance and monitor long-term population trends.

In this dissertation, we advanced two critical steps of a broader framework for applying PAM to abundance estimation in franciscanas using towed arrays: (i) defining biologically meaningful acoustic groupings through spatiotemporal clustering of click trains (Chapter 1 - Mura et al., 2025), and (ii) calibrating acoustic footprints by pairing PAM detections with drone-based visual counts (Chapter 2 - Mura et al., in review). Together, these contributions established the methodological foundation required to scale from raw acoustic detections to population-level inference.

In this chapter, we apply this framework to provide the first abundance estimates for the newly described subspecies *P. b. pukusi* in FMA-1a, based on passive acoustic data collected between 2019 and 2022. Specifically, we (i) combine automated detection of echolocation click trains with calibrated cue-rate models to estimate local abundance, (ii) delineate the effective area of occurrence from spatial detection patterns, and (iii) evaluate temporal population trends across four consecutive years. Given the demographic isolation, subspecific status, peripheral distribution, and persistent anthropogenic pressures on this unit, we hypothesize that *P. b. pukusi* supports a relatively small and spatially restricted population that may have remained stable at low levels or declined further in recent years. By addressing this gap, our results provide both a methodological proof-of-concept for PADE in small coastal cetaceans and critical empirical insights for the conservation of the franciscana dolphin.

4.2 METHODOLOGY

4.2.1 Study area

Data were collected in the Franciscana Management Area Ia (FMA-1a), located along the northern coast of Espírito Santo, Brazil, between approximately 20°18’S and 18°19’S, spanning 218 km of coastline and mostly restricted to depths shallower than 50 m. Surveys were conducted between 2019 and 2022, comprising 33 surveys and a total of 4562 km of raw effort. Surveys were carried out aboard a 40-foot sailboat research vessel following a zigzag transect design, except in cases where environmental conditions did not permit adherence to this scheme. The survey region was divided into two strata, north (2765 km²) and south (1353 km²), to account for potential heterogeneity in distribution. The latitudinal limit separating strata was defined either by the 40 m isobath or by the maximum offshore distance of franciscana records in the regional database plus 1 km, whichever occurred first.

Not all track effort was considered on-effort for abundance estimation. To ensure standardization and adherence to distance sampling assumptions, a custom Python script was developed to automatically identify and retain only linear transects. Track segments were segmented based on heading stability, and those with deviations greater than 20° were excluded, a tolerance threshold chosen to accommodate the maneuverability limitations of a sailing vessel. This procedure allowed for the objective validation of trackline effort while retaining the maximum amount of usable data for density estimation.

4.2.2 Acoustic data collection

Passive acoustic monitoring was conducted using a 50-m towed hydrophone array composed of four omnidirectional hydrophones (AUSET Technology®), spaced at 0.4, 3, and 5 m intervals. Each hydrophone operated with a 0.499 Hz high-pass filter, a sensitivity of –205 dB re 1 V/µPa, and a preamplifier gain of 20 dB. Frequency responses were 265 kHz for the first pair and 160 kHz for the second pair of hydrophones. The array was connected to a SAIL DAQ acquisition board, and recordings were made at a 500 kHz sampling rate. Acoustic and GPS data were continuously acquired using PAMGuard software (version 2.1; Gillespie et al., 2009). Acoustic effort was interrupted whenever environmental conditions compromised data quality or vessel safety, namely Beaufort sea state ≥ 6 (Andriolo et al., 2017), wave height > 3 m, or insufficient wind for safe sailing.

The franciscana dolphin is a cryptic species known to react to human presence (Secchi & Ott, 2003). Evidence indicates that its acoustic activity is distributed evenly across diel periods, with no marked preference for daylight or nighttime (Silva et al., 2022). For this reason, surveys were conducted with engines off whenever possible. This approach served two main purposes: (i) to minimize avoidance behavior by reducing acoustic and visual disturbance to dolphins, and (ii) to limit anthropogenic noise input, ensuring an environmentally friendly monitoring platform.

4.2.3 Acoustic data processing

Acoustic recordings were processed following Mura et al., 2025, using PAMGuard (version 2.1; Gillespie et al., 2009). NBHF clicks were detected with the Click Detector Module, configured with a 100 kHz high-pass filter and a 9 dB trigger threshold. To ensure species-specific attribution, classification parameters were tuned to the known frequency and bandwidth characteristics of franciscana dolphin clicks (Melcón et al., 2012; Amorim et al., 2022). Detected clicks were automatically grouped into click trains according to temporal and bearing cohesion. Click trains were then localized using time-difference-of-arrival (TDOA) across hydrophones, with positions estimated by hyperbolic triangulation following Barlow & Taylor (2005) and Lewis et al. (2007). Perpendicular distance to the trackline was calculated for each localized train.

All click-train detections were subsequently reviewed by hand to remove false positives, which were common due to electronic interference in the audio recordings. This interference was traced to the vessel’s power system and, despite multiple mitigation attempts during fieldwork, could not be completely eliminated. Although this manual curation was time-consuming, it was essential to ensure the reliability of the dataset.

Finally, to aggregate detections into biologically meaningful acoustic events, we applied the Hierarchical Density-Based Spatial Clustering of Applications with Noise (HDBSCAN) algorithm (Campello et al., 2013; McInnes et al., 2017). Input variables included both spatial (latitude and longitude) and temporal (datetime) metrics. Clusters of click trains were defined as click-train clusters (CTCs), representing cohesive acoustic activity potentially corresponding to social groups

(Mura et al., 2025). The CTCs were assigned temporally to their equivalent track segment.

4.2.4 Group size estimation

For each click-train cluster j, the number of click trains (C_j') was combined with the cue-rate calibration obtained in previous studies (Mura et al., in review) to estimate group size. The baseline cue rate (r_0, expressed in trains·animal^{-1}·s^{-1}) was applied together with cluster duration (Δt_j) and the detection function at distance d_j:

$$\widehat{N}_j = \frac{C_j'}{r_0 \cdot \Delta t_j \cdot f(d_j)}$$

where $f(d_j)$ is the distance-dependent detection probability for cluster j.

For clusters containing more than one click train, duration (Δt_j) was calculated directly. However, when the observed duration was shorter than the standardized median value derived from paired datasets (Mura et al., in review), the standardized median (Δt_j^*) was applied instead. This ensured that unrealistically short durations did not bias group size estimation. For singleton clusters, where duration cannot be reliably estimated, the same standardized median value was also used. Group size estimates were obtained as the bootstrap median of the distribution derived from parametric resampling of the fitted acoustic footprint model, and 95% confidence intervals were derived from the 2.5th and 97.5th percentiles of this distribution.

4.2.5 Environmental variables

Two environmental covariates were evaluated as potential predictors of detection probability: sea surface temperature (SST) and turbidity (Kd490). Daily SST fields were obtained from the Multiscale Ultrahigh Resolution (MUR) Level 4 Global Foundation SST Analysis (JPL, GHRSST project; Chin et al., 2017), accessed via NASA PO.DAAC OPeNDAP services. Values were extracted at clusters positions by selecting the nearest pixel in space and time.

Turbidity was represented by the diffuse attenuation coefficient at 490 nm, derived from the NOAA CoastWatch global daily kdMergedDINEOF dataset (Werdell et al., 2013). Data were accessed through OPeNDAP and extracted in the same way as SST. Both covariates were incorporated into multiple-covariate distance sampling (MCDS) models and tested individually and jointly with the years to assess their contribution to explaining variation in detection probability.

4.2.6 Abundance estimation

Abundance estimation was conducted using conventional distance sampling methods in the R package mrds (Laake et al., 2020). Perpendicular distances of click-train clusters were pooled across survey years to increase sample size and fit a single robust detection function. Hazard-rate and half-normal key functions were evaluated, and model selection was based on Akaike's Information Criterion (AIC). A truncation distance of 120 m was applied, retaining most biologically plausible observations.

Density and abundance were calculated separately for the northern and southern strata and subsequently combined for a total estimate in FMA-Ia. The analysis proceeded in two stages. First, cluster density ($\widehat{D}_{cl}$) was first estimated from line-transect distance sampling:

$$\widehat{D}_{cl} = \frac{n}{2\, w\, L\, \widehat{P}_a},$$

where n is the number of clusters detected, w is the truncation distance, L is the total validated trackline length and $\widehat{P}_a$ is the average probability of detection within w, estimated by the fitted detection function. Second, cluster-level group sizes derived from the cue-rate calibration were adjusted for size-related detection bias using the size-bias regression approach (Buckland et al. 2001). This regression yielded the expected group size ($\widehat{E}[s]$) at zero distance, which was then used to scale cluster abundance into an estimate of individual abundance per stratum and in total area ($\widehat{N}_{ind} = \widehat{D} \cdot A \cdot \widehat{E}[s]$).

To ensure proper uncertainty propagation, coefficients of variation (CVs) from both the detection model and the group-size regression were combined using the delta method (Buckland et al., 2001; Seber, 1982). For the product of two independent estimators, cluster density and expected group size $\widehat{E}[s]$ (which already incorporates uncertainty from cue rate, cluster duration, and distance-dependent detectability), the variance of individual abundance is approximated as:

$$CV^2(\widehat{N}_{ind}) = CV^2(\widehat{D}_{cl}) + CV^2(\widehat{E}[s])$$

This formulation ensures that both sources of uncertainty, detection probability and group-size estimation, are reflected in the final variance and confidence intervals.

4.2.7 Occurrence and trend analysis

To estimate the effective area of occurrence, we mapped all localized acoustic detections and defined the kernel density estimator enclosing click trains detections. Temporal trends were assessed by comparing annual abundance estimates from 2019 to 2022. A weighted log-linear regression model was fitted to the estimated abundances:

$$log\ log\ (\widehat{N}_{ind}) = \alpha + \beta \cdot t\ ,$$

where $\widehat{N}_t$ is the abundance estimate for year t. The model was fitted using Gaussian errors, with weights equal to the inverse of the variance of each estimate, so that more precise estimates contributed more strongly to the trend. The coefficient β was used to test for evidence of an increasing or decreasing trend, and was back-transformed to provide an annual percentage rate of change with 95% confidence intervals.

4.3 RESULTS

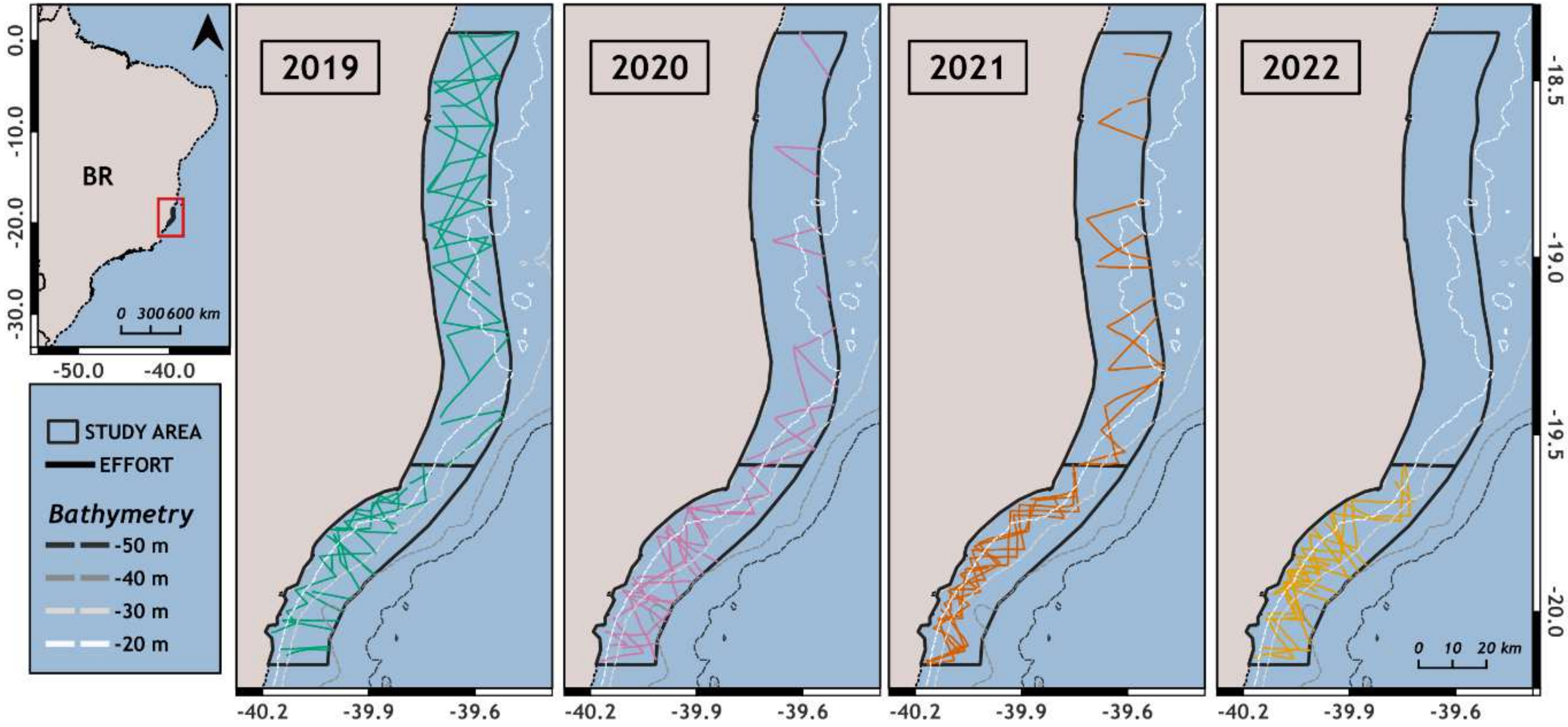
4.3.1 Survey effort

After applying the automated filtering to retain only linear transects, 3185.99 km (69.83% of the raw effort) were validated for abundance estimation, distributed across 220 transects (Table 4.1, Figure 4.1). In 2022, effort was restricted to the southern stratum due to logistical constraints and adverse oceanographic conditions. As a result, abundance estimates for this year should be interpreted with caution.

Table 4.1 - Summary of survey effort conducted in the Franciscana Management Area Ia (FMA-1a) between 2019 and 2022. Values are stratified by year and by subarea (north and south). For each stratum, the total kilometers surveyed and the validated kilometers and transects retained after automatic filtering. Only validated effort was considered in subsequent abundance estimation analyses.

Year	**Total effort (km)**	**Stratum**	**Validated effort (km)**	**Validated transects**
2019	1323.34	North	662.70	35
		South	401.78	39
2020	1454.56	North	216.92	15
		South	473.62	40
2021	1042.79	North	296.99	20
		South	552.70	50
2022	741.34	North	0	0
		South	581.28	21
Total	**4562.03**	**-**	**3185.99**	**220**

Figure 4.1 - Vessel survey effort conducted in FMA-Ia between 2019 and 2022 after automated filtering to retain only linear transects. A total of 3185.99 km of validated effort (69.8% of raw tracks) was distributed across 220 transects. In 2022, effort was restricted to the southern stratum due to logistical constraints and adverse oceanographic conditions, and abundance estimates for that year should be interpreted with caution.



4.3.2 Acoustic detections and clustering

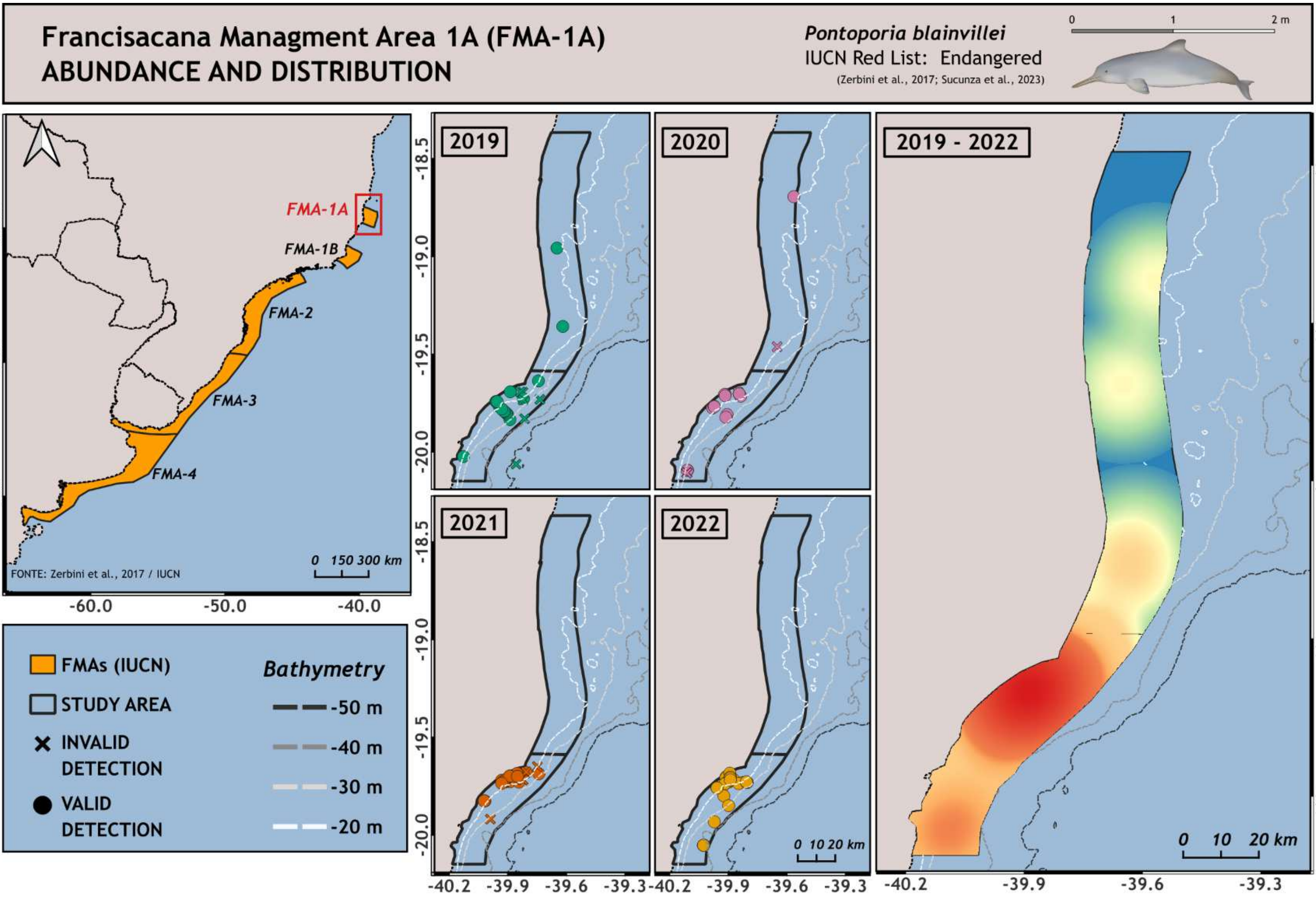
A total of 224 click trains were detected by PAMGuard and validated for abundance analysis after manual curation to remove false positives. These detections were aggregated into 71 click-train clusters (CTCs) using the HDBSCAN algorithm, with a mean of 3.15 click trains per cluster and mean estimated group size of 2.37 individuals per cluster (Table 4.2).

Table 4.2 - Summary of acoustic detections and click-train clustering per year in FMA-Ia (2019–2022).

Year	**Click-trains**	**Clusters**	**Mean CTC size (click trains)**	**Mean Group size (individuals)**
2019	69	16	4.31	1.86
2020	46	14	3.28	2.35
2021	54	20	2.7	2.91
2022	55	21	2.61	2.27
Total	**224**	**71**	**3.15**	**2.37**

Acoustic detections were highly concentrated in the southern stratum, with 95.78% (n = 68) of click-train clusters localized south of the latitudinal division and only 4.22% (n = 3) in the northern stratum (Figure 4.2). Of these northern detections, two occurred in 2019 and one in 2020, with none recorded in 2021. The kernel density analysis further indicated that the effective area of occurrence in FMA-Ia is strongly concentrated around the Doce river mouth.

Figure 4.2 - Distribution of acoustic detections of franciscana dolphins (*Pontoporia blainvillei*) in FMA-Ia between 2019 and 2022. Panels show annual detections (2019–2022) and kernel density estimates for the pooled period. Valid detections are represented by filled circles and off-effort detections by crosses. The kernel density surface highlights that the effective area of occurrence is strongly concentrated around the Doce river mouth.



4.3.3 Detection functions

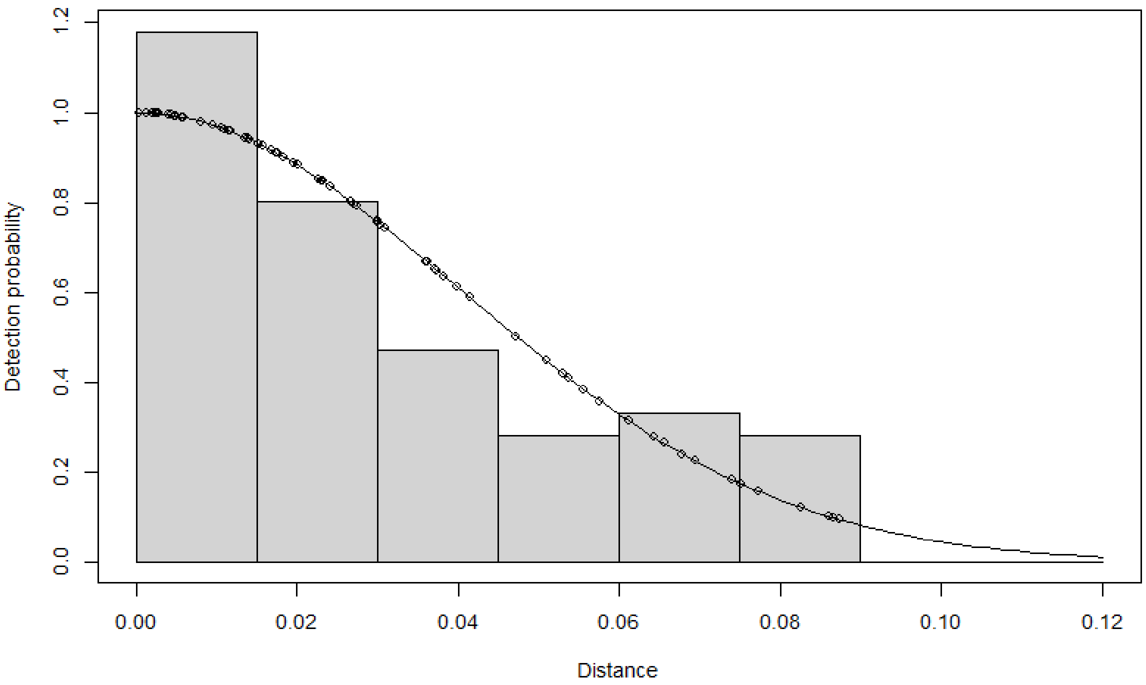
Hazard-rate and half-normal detection functions were fitted to the perpendicular distances of CTCs across survey years. Models including turbidity and SST as covariates did not show significant improvement in explaining detection probability compared to the null model. The half-normal model (Figure 4.3) provided the best fit based on AIC (Table 4.3). The estimated average probability of detection within this truncation distance was $\hat{P}$ = 0.418 (SE = 0.036, CV = 0.087). Group sizes estimated from size-bias regression yielded a median expected size of $\hat{E}[s]$= 2.375 individuals per cluster (CV = 0.071).

Table 4.3 - Model selection results for hazard-rate and half-normal detection functions fitted to perpendicular distances of click-train clusters (CTCs), with and without environmental covariates (turbidity, SST). The half-normal null model provided the best fit based on AIC.

Model	$\hat{P}$	$CV(\hat{P})$	ΔAIC
Half-normal (Hn)	0.418	0.087	0
Hazard-rate (Hr)	0.347	0.172	3.047
Hn + tubidity	0.307	0.208	4.342
Hr + tubidity	0.417	0.088	1.606
Hn + tubidity + year	0.3	0.198	5.555
Hr + tubidity + year	0.41	0.087	5.437
Hn + SST	0.238	0.265	0.815
Hr + SST	0.999	0.128	56.54
Hn + SST + year	0.33	0.166	4.487
Hr + SST + year	0.407	0.09	4.723

Figure 4.3 - Detection function fitted using a half-normal key to perpendicular distances of CTCs across survey years. The solid line shows the fitted detection probability. The

estimated average probability of detection within the truncation distance was $\hat{P}$ = 0.418 (SE = 0.036, CV = 0.087).



4.3.4 Abundance and density estimates

Abundance and density estimates were calculated annually for the northern and southern strata, as well as for the combined study area (Table 4.4). Results are presented as cluster density ($\widehat{D}_{cl}$, clusters·km⁻²) and individual abundance ($\widehat{N}_{ind}$, number of individuals). Estimates for 2022 should be interpreted with caution, as no survey effort was conducted in the northern stratum that year.

Table 4.4 - Annual density and abundance estimates of franciscana dolphins (*Pontoporia blainvillei*) in FMA-Ia between 2019 and 2022. Results are shown for the northern and southern strata and for the total study area. Estimates include the number of detected clusters (N), encounter rate (ER, clusters·km⁻¹ with coefficient of variation), cluster density ($\widehat{D}_{cl}$, clusters·km⁻²), individual abundance ($\widehat{N}_{ind}$), coefficient of variation

(CV), and 95% confidence intervals. No detections were recorded in the northern stratum in 2021, and survey effort in 2022 was restricted to the southern stratum only.

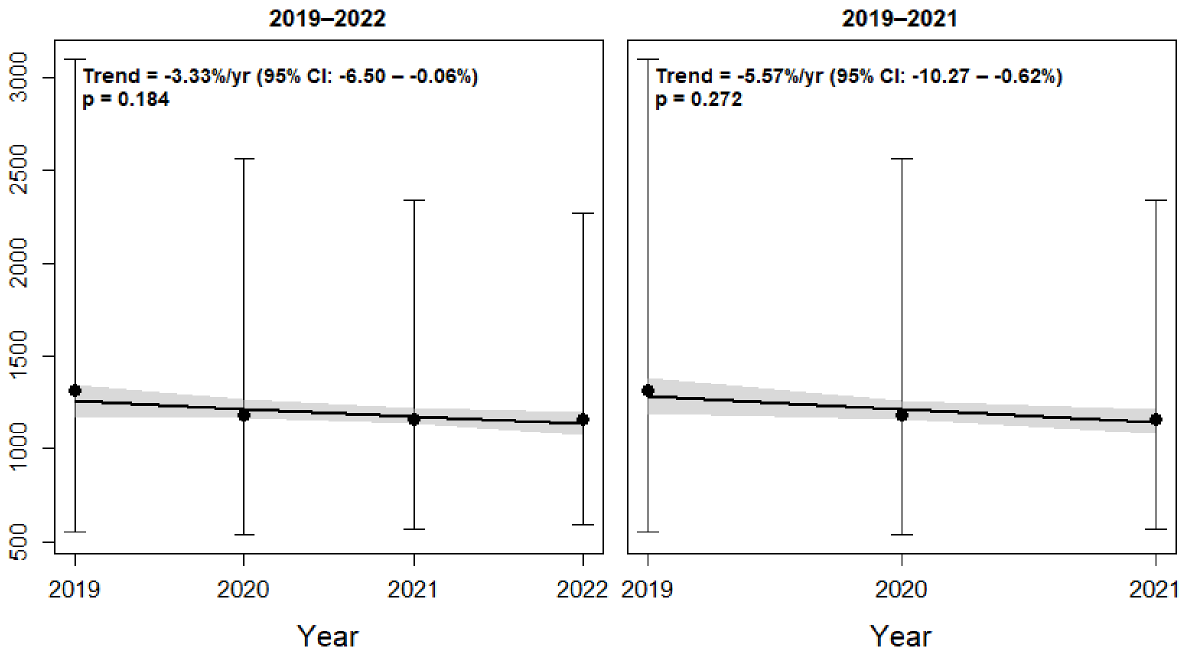
Year	Stratum	N	ER (CV)	Density $\widehat{D}_{cl}$	Abundance $\widehat{N}_{ind}$	CV	95% CI
2019	North	2	0.003 (0.68)	0.030	197	0.696	57.5–676
	South	14	0.034 (0.51)	0.346	1114	0.524	424.4–2923
	Total	**16**	**0.013 (0.44)**	**0.134**	**1311**	**0.461**	**554.9-3097**
2020	North	1	0.004 (1.0)	0.045	301	1.015	57.9–1567
	South	13	0.027 (0.4)	0.273	877	0.421	397.8–1935
	Total	**14**	**0.012 (0.39)**	**0.120**	**1179**	**0.413**	**541.8–2563**
2021	North	0	0 (0)	0	0	0	0
	South	20	0.036 (0.35)	0.359	1157	0.371	572–2338
	Total	**20**	**0.011 (0.35)**	**0.118**	**1157**	**0.371**	**572–2338**
2022	**Total (South)**	**21**	**0.036 (0.33)**	**0.359**	**1154**	**0.354**	**588.5–2266**

4.3.5 Temporal trend

Annual abundance estimates suggested a declining tendency (Figure 4.4). Considering the full period (2019-2022), the estimated trend was -3.33% per year (95% CI: -6.50 to -0.06; p = 0.184). When restricting the analysis to 2019-2021, the trend was slightly steeper at -5.57% per year (95% CI: -10.27 to -0.62; p = 0.272).

Neither of these trends was statistically significant, indicating that the population trajectory is best described as broadly stable or slowly decreasing. It should be emphasized, however, that the 2022 survey did not include sampling in the northern stratum, which may have biased abundance estimates downward. For this reason, the apparent decline when including 2022 should be interpreted with caution, and the shorter 2019-2021 series may provide a more reliable indication of population trajectory.

Figure 4.4 - Annual abundance estimates of franciscana dolphins (*Pontoporia blainvillei*) in FMA-Ia. Black lines show fitted log-linear trends with 95% confidence intervals (gray shading). Both 2019–2022 and 2019–2021 series suggested slight declines, but neither was statistically significant. Estimates for 2022 should be interpreted with caution due to lack of sampling in the northern stratum.



4.4 DISCUSSION

4.4.1 Integrating a methodological framework for PADE

This study represents the third step in a progressive framework for passive acoustic density estimation (PADE) applied to franciscana dolphins. In Mura et al. (2025), spatiotemporal clustering of echolocation click trains provided an objective basis for defining biologically meaningful acoustic groups, moving beyond arbitrary thresholds that have often limited acoustic surveys (e.g. Frasier et al., 2017; Ollier et al., 2023). In *Mura et al. (in review)*, acoustic footprints were calibrated by pairing PAM detections with drone-based visual counts, overcoming a central bottleneck in PADE: the translation of acoustic activity into individuals (Marques et al., 2013; Thomas & Marques, 2012). The present work integrates these components into a complete pipeline, extending from raw click-train detections to population-level inference. Together, these studies provide the first empirical proof-of-concept that PADE can be operationalized through towed arrays in small coastal odontocetes, a group historically considered difficult to monitor due to turbid habitats and cryptic behavior (Boisseau et al., 2007; Risch et al., 2014).

By applying clustering, acoustic footprint calibration, and size-bias regression within the distance-sampling framework, we demonstrate how PAM can generate abundance estimates with uncertainty propagation from multiple sources. This integrated approach represents a scalable monitoring tool not only for franciscana dolphins, but potentially for other coastal and riverine dolphins, such as the Guiana dolphin (*Sotalia guianensis*), Irrawaddy dolphin (*Orcaella brevirostris*), and river dolphins in the genera *Inia*, *Sotalia* and *Platanista* (Jensen et al., 2013; Kyhn et al., 2012; Bopardikar et al., 2025).

This framework, while innovative, also has important limitations that should be addressed in future work. Although NBHF clicks of franciscana dolphins can be classified with high confidence, the application of PADE to other odontocetes will be more challenging when echolocation signals are broadband and overlap among sympatric species (Kyhn et al., 2009; Jensen et al., 2013). Classifying such signals with sufficient accuracy will require both region-specific training datasets and the refinement of machine-learning detectors to minimize misclassification errors (Roch et al., 2011; Klinck & Mellinger, 2012). Another key limitation lies in the acoustic footprint calibration: the existing model is derived from a limited behavioral and geographic context. Echolocation rates are known to vary with behavioral state, prey availability, and environmental conditions (Akamatsu et al., 2013; Linnenschmidt et al., 2013). Expanding calibration efforts to cover multiple FMAs, seasonal conditions, and a wider spectrum of behaviors will be essential to capture the variability necessary for robust inference. Additionally, high-quality acoustic acquisition systems are fundamental to ensure reliability of PADE applications. In this study, electrical interference from the vessel's power system required extensive manual filtering, underscoring how noise contamination can inflate false-positive detections. Future monitoring should prioritize increasingly “clean” systems with optimized shielding, grounding, and hydrophone sensitivity, to reduce background noise and secure audio fidelity (Au & Hastings, 2008; Zimmer, 2011). Finally, integrating this framework with spatially explicit models such as density surface modeling (Hedley &

Buckland, 2004; Miller et al., 2013) could improve the ecological interpretability of abundance estimates by linking them to dynamic habitat features.

4.4.2 Ecological insights: distribution and occurrence

A central ecological finding is the strong spatial concentration of detections in the southern stratum, particularly around the Doce River mouth. Nearly 96% of click-train clusters occurred in this region, with only three clusters recorded in the north across four years. This spatial restriction echoes results from aerial surveys (Sucunza et al., 2023) and emphasizes the importance of estuarine and river plume systems for franciscanas. River plumes can enhance productivity and prey availability, creating localized foraging hotspots. Similar estuarine associations have been documented for other coastal cetaceans, such as Guiana dolphins in Brazil (Rossi-Santos et al., 2010) and Indo-Pacific humpback dolphins (*Sousa chinensis*) in China (Hung, 2008). The persistent concentration around the Doce River suggests that *P. b. pukusi* may rely heavily on this estuarine system, rendering the subspecies particularly vulnerable to local disturbances.

In contrast, the near absence of detections in the northern stratum highlights its role as marginal habitat. Peripheral areas often sustain fewer individuals due to lower productivity, increased predation risk, or limited habitat suitability (Vincent and Brown, 1984; Hanski, 1998). For *P. b. pukusi*, the lack of detections after 2020 may indicate a contraction of range, a worrying signal given the absence of connectivity with adjacent FMAs (Cunha et al., 2014; Sucunza et al., 2023). This pattern aligns with metapopulation theory, which predicts higher extinction risks at the edges of species' ranges due to demographic stochasticity and absence of rescue effects (Frankham, 2005).

4.4.3 Abundance and temporal dynamics

Estimated abundances ranged from 1,311 individuals in 2019 to 1,154 in 2022, with intermediate values in 2020 (1,179) and 2021 (1,157). While confidence intervals were wide, point estimates consistently indicate a small population. Weighted log-linear regression suggested a decline of –3.3% per year across 2019–2022, or –5.6% when excluding 2022. Although neither slope was statistically significant, the trajectory is concerning given bycatch pressure (Di Beneditto et al., 2001; Siciliano et al., 2002; Secchi et al., 2022). Even modest declines can have serious consequences for small, isolated populations, particularly when they exhibit reduced genetic diversity as reported for FMA-1a (Cunha et al., 2014; Nara et al., 2024).

The 2022 estimate must be interpreted cautiously, as effort was restricted to the southern stratum. However, the complete absence of detections in 2021, following decreasing detections in 2019 and 2020, may reflect a genuine contraction in occurrence. If so, the decline signal could be ecologically real rather than an artifact of sampling design.

4.4.4 Conservation implications

The recognition of *P. b. pukusi* as a distinct subspecies (Nara et al., 2024) raises the stakes for conservation. Our findings confirm that this lineage is demographically small, spatially restricted, and dependent on a single estuarine hotspot. Combined with intense artisanal gillnet fisheries, port expansion, and the legacy of the 2015 Doce River mining disaster (Gabriel et al., 2021), this creates a scenario of extreme vulnerability. Bycatch mortality alone has been estimated to exceed sustainable thresholds for franciscana populations elsewhere (Secchi et al., 2003, 2021; Franco-Trecu et al., 2009) and is likely unsustainable for a population of ~1,100 individuals.

Our methodological framework provides a replicable monitoring tool that can inform management. PADE with towed arrays can be implemented in long-term monitoring programs, offering seasonal and annual resolution unattainable with aerial surveys. For FMA-1a, establishing routine acoustic monitoring near the Doce River, coupled with independent bycatch assessments, would provide robust indicators of population trajectory. More broadly, this approach can be extended to other FMAs, filling possible data gaps.

Finally, conservation measures for *P. b. pukusi* must be urgent and multifaceted. Priority actions include the enforcement of gillnet restrictions in core habitats, creation of protected areas encompassing the Doce River estuary, and strengthening of artisanal fishery co-management. At an international level, recognition of this subspecies as Critically Endangered under the IUCN framework may catalyze additional protective measures. Without immediate action, the combination of small population size, spatial restriction, and anthropogenic pressures could drive this unique evolutionary lineage toward extinction.

4.5 CONCLUSIONS

This study provided the first abundance estimates for franciscana dolphins using an integrated passive acoustic density estimation framework. By combining click-train clustering, cue-rate calibration with drones, and size-bias correction, we moved from raw acoustic detections to population-level inference.

Results show that the population in FMA-1a is small, spatially restricted, and strongly concentrated near the Doce River mouth, with almost no detections in the northern stratum. Although annual estimates remained close to 1,100–1,300 individuals, there are indications of a declining trend and contraction of the occupied area.

Methodologically, this work demonstrates the feasibility of PADE for small coastal dolphins, while highlighting the need for broader cue-rate calibrations, improved classifiers, and cleaner acquisition systems to ensure data quality.

From a conservation perspective, the findings emphasize the critical status of *P. b. pukusi*, whose persistence will depend on effective actions to reduce bycatch, safeguard core habitats, and maintain long-term monitoring programs.

5 GENERAL CONCLUSIONS

This dissertation integrates three complementary advances to improve the monitoring and conservation of the franciscana dolphin (*Pontoporia blainvillei*). The first chapter introduced an objective method to identify biologically meaningful acoustic groups through spatiotemporal clustering of echolocation click trains. The second chapter translated these acoustic units into group-size estimates by calibrating acoustic footprints with drone-based observations, overcoming one of the major limitations of passive acoustic density estimation. The third chapter applied this complete framework to data collected in FMA-Ia, providing the first integrated abundance and distribution estimates for the newly recognized subspecies *P. b. pukusi*.

The results demonstrate that combining passive acoustic monitoring with drones is both feasible and effective for studying cryptic odontocetes. This integrated framework not only yields robust estimates of density and abundance but also allows explicit propagation of uncertainty across analytical steps. The approach provides a solid foundation for long-term monitoring programs and can be replicated across management areas, seasons, and behavioral contexts.

Beyond the case of franciscanas, the methodology has wider applicability. The same principles can be adapted to other coastal and riverine dolphins surveyed with towed arrays, provided that species-specific acoustic footprints are calibrated and classification challenges are addressed. As such, this work contributes both to the conservation of one of the most threatened dolphins in South America and to the development of a transferable tool for acoustic-based population assessments.

In summary, this dissertation advances ecological understanding and conservation practice for *P. blainvillei* by delivering a novel framework that connects acoustic detections to population-level inference. It highlights the potential of passive acoustics, when combined with emerging technologies such as drones, to transform how small and elusive cetaceans are monitored worldwide.

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