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Following the fishing trail

Detecting fishing discards in seabirds through DNA metabarcoding

Faculdade de Ciências e Tecnologias

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Dr. Jacob González-Solís

Dra. Rita Castilho

Faculdade de Ciências e Tecnologias

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Declaração de autoria Marta Nadal Pla

Declaro ser a autor/a deste trabalho, que é original e inédito. Autores e trabalhos consultados estão debidamente citados no texto e constam da listagem de referencias incluida.

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ABSTRACT

Upwelling regions along the northwest coast of Africa are known for their high productivity, which attracts both marine predators and intensive fishing activities. Understanding the impact of fishery discards on seabird populations is essential for effective conservation, as changes in fisheries management could significantly affect food availability for these species. This study investigates the dietary habits of Cape Verde shearwaters (*Calonectris edwardsii*) breeding in Cabo Verde, focusing on their reliance on fishery discards along the northwest coast of Africa. By using DNA metabarcoding on faeces combined with Global Positioning System tracking of seabirds and vessels, we analysed the diet of shearwaters across three breeding seasons (2018, 2019, and 2021) in relation to the association with the fishing activity. Results show that roughly 68% of their diet comprises fishery discards, with Actinopterygii accounting for 36.8% and arthropods 17% of the diet according to the RRA (relative read abundance). The presence/absence analysis highlighted a similar trend, with Actinopterygii (19.3%) and other Arthropoda (19.6%) being the most frequently detected taxa, indicating their consistent presence in the diet. Furthermore, discard consumption varied across breeding stages, with greater use during the incubation phase compared to the chick-rearing stage. No significant differences were found based on sex or type of vessel interaction. These findings highlight the potential vulnerability of Cape Verde shearwaters to changes in fisheries management in Western Africa and call for future studies to improve analytical methods, particularly in the application of DNA metabarcoding for dietary analysis.

RESUMO

Este estudo examina os hábitos alimentares da cagarra de Cabo Verde (*Calonectris edwardsii*), com ênfase na dependência de rejeições pesqueiros ao longo da costa noroeste da África, uma região com intensa atividade pesqueira e grande biodiversidade marinha. A cagarra de Cabo Verde, uma espécie endémica do arquipélago de Cabo Verde, está listada como "quase ameaçada" pela IUCN, devido às pressões ambientais e à degradação dos seus habitats naturais. Um dos principais fatores de risco para esta espécie é a sua interacção com frotas pesqueiras, onde frequentemente se alimenta de descartes, ou seja, restos de pesca que não são aproveitados pelos pescadores. Esta prática, embora ofereça uma fonte abundante de alimento, pode causar problemas de longo prazo, como o fornecimento de presas de baixa qualidade nutricional ou a dependência excessiva de fontes alimentares antropogénicas.

Com o objectivo de investigar a extensão desta dependência, utilizámos metabarcoding de DNA (informação genética de vários organismos presentes em uma determinada amostra é extraída e analisada) em amostras fecais de cagarras, juntamente com dispositivos de posicionamento global (GPS – Global Posotioning System) para rastrear as interações com embarcações pesqueiras. O metabarcoding de DNA permite a identificação precisa das presas consumidas pelas cagarras, enquanto os dispositivos GPS fornecem dados detalhados sobre os movimentos das aves e suas interações com os barcos de pesca. O estudo foi conduzido ao longo de três temporadas de reprodução (2018, 2019 e 2021) na colónia de aves de Curral Velho, em Cabo Verde.

Os resultados mostram que 67,6% da dieta das cagarras é composta por rejeições pesqueiros, evidenciando uma forte dependência de fontes alimentares de origem humana. Esta dependência representa uma vulnerabilidade significativa, pois qualquer mudança nas políticas de gestão pesqueira, como a redução dos descartes, pode impactar gravemente a disponibilidade de alimentos para esta espécie. A redução dos rejeições pode forçar as cagarras a viajar distâncias maiores em busca de alimento ou a competir de forma mais intensa por presas naturais, o que pode ter consequências negativas tanto para a sobrevivência dos adultos quanto para o sucesso reprodutivo.

Outro aspecto importante observado no estudo foi a variação na composição da dieta de acordo com o estado reprodutivo. Durante a incubação, as cagarras mostraram uma maior dependência dos rejeições em comparação com a época de criação dos filhotes. Esse comportamento pode ser explicado pela necessidade de economizar energia durante o período de incubação, quando os adultos precisam manter reservas corporais para sustentar o processo de reprodução. Durante a fase de criação dos filhotes, as

cagarras parecem priorizar presas naturais, possivelmente devido à maior demanda nutricional para alimentar os filhotes.

Apesar da forte dependência dos rejeições, não foram encontradas diferenças estatisticamente significativas no consumo de rejeições entre machos e fêmeas. Além disso, o estudo não identificou uma correlação significativa entre a proximidade dos barcos pesqueiros e o consumo de rejeições, o que sugere que as cagarras podem aceder a rejeições de embarcações mesmo sem se aproximar diretamente delas. Isso pode ocorrer porque os rejeições permanecem flutuando na superfície do mar por um período prolongado, tornando-se acessíveis às aves mesmo depois que os barcos já se terem ausentado da área.

Embora o metabarcoding de DNA se tenha mostrado uma ferramenta eficaz para identificar a composição da dieta, o estudo revelou algumas limitações metodológicas. Uma delas é a incapacidade do metabarcoding de distinguir diferentes estágios de vida das presas. Isso pode levar a erros na classificação de algumas espécies como descartes, quando na verdade poderiam fazer parte da dieta natural das rejeições. Outra limitação foi a redução do tamanho da amostra à medida que novos conjuntos de dados foram incorporados à análise, o que diminuiu o poder estatístico de algumas comparações. No entanto, o uso combinado de metabarcoding de DNA e rastreamento por GPS permitiu uma compreensão abrangente das interações das cagarras com as frotas pesqueiras e forneceu insights valiosos sobre seu comportamento alimentar.

Além disso, a análise das interações entre as cagarras e os barcos de pesca indicou dois principais tipos de eventos: "encontros" e "assistências". Nos eventos de encontros, as aves estavam a uma distância de até 30 km de uma embarcação, enquanto nos eventos de assistência, as cagarras se aproximaram a menos de 1,5 km e permaneceram nessa proximidade por pelo menos 10 minutos. Embora o estudo não tenha encontrado diferenças significativas no tipo de presa consumida com base nesses dois tipos de interacção, observou-se uma tendência para o consumo de rejeições em amostras recolhidas logo após eventos de assistência. Especificamente, amostras recolhidas menos de dois dias após um evento de assistência mostraram um maior consumo de descartes e um menor consumo de presas naturais, embora essa tendência não sido estatisticamente significativa.

Além disso, o estudo levanta a possibilidade de que algumas das interações das cagarras com os barcos de pesca possam não ter sido registadas com absoluta precisão, devido à ausência de dispositivos de monitorização em embarcações menores, que muitas vezes não são equipadas com sistemas de monitorização como o AIS (Automatic Identification System) ou VMS (Vessel Monitoring System). Esse facto pode explicar que algumas amostras continham cagarras, mesmo sem uma interacção directa registrda com embarcações.

Em termos de implicações para a conservação, os resultados destacam a importância de considerar as fases do ciclo reprodutivo das cagarras ao planear estratégias de gestão pesqueira. A alta dependência de rejeições durante a fase de incubação sugere que a redução dos rejeições pode afectar a capacidade dos adultos de manter suas reservas energéticas e, consequentemente, prejudicar seu sucesso reprodutivo. Além disso, a dependência de fontes alimentares antropogénicas pode aumentar o risco de ingestão de presas de baixa qualidade nutricional, como observado em outros estudos que relatam o impacto da teoria "junk food", onde presas provenientes de rejeições têm menor valor energético.

Conclui-se que, embora o estudo tenha encontrado evidências claras da dependência das cagarras de Cabo Verde em relação aos rejeições pesqueiros, vários aspectos do planejamento experimental poderiam ser aprimorados em estudos futuros para obter resultados mais robustos e estatisticamente significativos. A combinação de métodos tradicionais de análise morfológica de presas com o metabarcoding de DNA pode ajudar a corrigir alguns dos problemas observados na classificação das presas. Além disso, a coleta de dados adicionais sobre o comportamento das aves e o monitoramento mais preciso das interações com embarcações menores pode fornecer uma visão mais completa das interações entre as cagarras e a actividade pesqueira.

No geral, este estudo fornece uma base importante para trabalhos futuros sobre a ecologia alimentar das cagarras de Cabo Verde e suas interações com as frotas pesqueiras, contribuindo para a elaboração de

estratégias de conservação mais eficazes que considerem tanto os impactos directos quanto os indirectos das práticas pesqueiras sobre as populações de aves marinhas.

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1. INTRODUCTION

Upwelling regions are habitats with very high productivity, where large numbers of marine predators and industrial fishing fleets are concentrated (Grecian et al., 2016). These areas account for less than 1% of the world's oceans, yet they contribute approximately 20% of the global fish catch (Pauly & Christensen, 1995). Advances in industrial fishing developed over the last decades have allowed for harvesting over half of the world's oceans while introducing new and abundant resources for a variety of marine species, altering the global marine food web and significantly impacting predator-prey relationships. Among marine species, seabirds have one of the most impacted predators by the increase in industrial fishing and fishery waste.

The coexistence of seabirds and fisheries in the same habitat has been inevitable over centuries, however, nowadays with the extensive industrial fleet the interactions at sea have rapidly increased, even in the high seas (Weimerskirch et al., 2020). Approximately 60% of marine bird species are known to interact with fishing activities, which heightens concerns about the impacts on their populations (Pott & Wiedenfeld, 2017).

Figure 1.1 Aggregation of seabirds around a fishing vessel can be observed. Author: Marta Nadal.

Traditionally, fisherman relied on seabird aggregations to locate schools of pelagic fish, benefiting both parties: seabirds found food with minimal effort, and fisherman identified fish schools to optimize their catch (Figure 1.1) (Arcos & Oro, 2002; Crawford & Shelton, 1978; Tasker et al., 2000). However, with the increase of the fishing industry, this mutual benefit has

evolved into a threat to seabird populations, making the study of this interaction a priority in seabird conservation (Arcos & Oro, 2002; Lewison et al., 2012). The introduction of an abundant and predictable resource by industrial fishing led seabirds to feed on fishery discards. The impact of fishing discards on seabird populations is an increasing concern in marine conservation (Cianchetti-Benedetti et al., 2018; Votier et al., 2023), several studies suggesting that some species obtain 75% of their energy needs from discards (Soriano-Redondo et al., 2016). While seemingly beneficial, discards often results in the seabirds consuming low-calorie prey or prey with high concentrations in heavy metals, known as the "junk food" hypothesis (Grémillet et al., 2008). Specifically, Cape Gannets consuming fishery discards had a low-energy diet insufficient for chick survival, as the discarded prey had lower lipid concentrations compared to natural prey. Furthermore, seabirds become accustomated to feeding on discards, posing a challenge when policies change to prohibit discards, making it difficult for the birds to find adequate food for survival (Grémillet et al., 2008) or increasing predation rates on protected species (Votter et al., 2004).

Moreover, approaching fishing vessels carries different risks such as, accidental collisions with cables, entanglement with nets and being hooked in the attempt to catch bait known as by-catch (Le Bot et al., 2018). Seabird mortality due to by-catch is an enormous impact for seabird populations that occurs on all fishing fleets around the world (Anderson et al., 2011; Calado et al., 2021). Many seabirds die each year due to by-catch (Votier et al., 2023). It is estimated that annually, between 160,000 and 320,000 seabirds perish due to long-liners (Anderson et al., 2011), tens of thousands due to trawlers (Žydelis et al., 2013), and thousands due to purse seiners (Carle et al., 2019). Seabirds exhibit high adult survival rates (often over 90%), delayed maturity, and low fecundity, and therefore any factor increasing adult mortality can severely impact population dynamics (Furness, 2003).

One of the most affected regions by intensive fishing worldwide is the Canary Current Large Marine Ecosystem (CCLME), given the increasing industrialization of fisheries (Worm et al., 2009) and the pervasive threat from bycatch, understanding how marine predators interact with fishing activity in the CCLME and beyond is a key conservation goal (Eckert, 2006; Grecian et al., 2016). In this part of the Atlantic, the relationship between seabirds and fisheries has not been extensively studied (Lewison et al., 2012; Votier et al., 2023). However, it is expected that these interactions are quite frequent due to the high productivity of the area and the intensive fishing efforts, highlighting the necessity to understand their interactions with fishing activities (Cianchetti-Benedetti et al., 2018; Depestele et al., 2018; Le Bot et al., 2018; Votier et al., 2023).

Traditionally, interactions between seabirds and fisheries have been studied through direct observation aboard fishing vessels. While direct observation provides valuable real-time data on seabird behaviour, it has significant limitations. The observer's viewpoint is restricted to a few vessels, offering only a limited perspective of seabird movements and interactions. This approach makes it difficult to assess the importance of fishery discards are to the population.

To overcome these limitations, tracking devices, such as satellite tags, have been employed to monitor seabird movements in relation to fishing vessels (Kays et al., 2015). These devices provide detailed data on seabird movements and their proximity to fishing fleets, offering a broader spatial and temporal understanding of their interactions. However, tracking data only reveals associations between seabirds and vessels, without confirming whether the birds are actively feeding on discards (Bernard et al., 2021). To address this, dietary studies are necessary to quantify the consumption seabirds do when attending discards, how much of the population relies on discards and to what extent these discards contribute to their overall diet.

Several methods have been employed to study seabird diets, ranging from invasive techniques like stomach content analysis to non-invasive methods such as faecal sample analysis. (Barrett et al., 2007; Duffy & Jackson, 1986). Invasive approaches can provide detailed dietary information but raise ethical concerns, particularly for species under conservation pressures. Non-invasive methods, such as observing feeding behaviour or the morphological analysis of regurgitates and faeces, offer alternatives but may not capture the full dietary spectrum. Direct observation tends to overestimate conspicuous species whereas morphological analyses often suffers from inaccuracies due to the difficulty of identifying digested organisms (Barrett et al., 2007; Bowser et al., 2013). Alternative techniques, such as stable isotope analyses or lipid and fatty acid analyses have presented methodological challenges when applied to field-based studies (Bowser et al., 2013; Braley et al., 2010). DNA-based methods have gained popularity in diet studies because they allow the identification of digested prey that can be identified using short DNA sequences unique for each prey (Bowser et al., 2013; Braley et al., 2010; Symondson, 2002). These DNA sequences can be identified from remains present in faeces, and the collection of faecal samples is relatively easy and non-invasive compared to other sampling methods (Bowser et al., 2013; Casper et al., 2007). Identifying prey DNA from faeces has shown significant promises in improving the study of marine animals' diet, particularly in cases where prey cannot be morphologically identified due to similar appearances, lack of hard remains, or extensive digestion, such as the case of most pelagic seabirds (Barrett et al., 2007; Bowser et al., 2013; Jarman et al., 2002).

2. OBJECTIVES

The primary aim of this study was to assess the diet of Cape Verde shearwaters, with a particular focus on the relevance of fishery discards as a food source. To achieve this, we applied DNA metabarcoding to faecal samples collected after shearwaters returned from a foraging trip that was tracked with GPS, some of which were also monitored using vessel radar detectors. These devices allowed us to infer the association of shearwaters with fishing vessels throughout their foraging activity. Our main hypothesis is that shearwaters associate to fishing vessels to feed on discards and thus, birds that associated to vessels will show a greater proportion of discard-derived prey in their faeces. Additionally, we accounted for some biological factors, such as sex and breeding period, which may influence the consumption of both, discards and natural prey. Lastly, as one of the first studies using DNA metabarcoding to analyse seabird diet, a secondary aim was to assess the efficiency and reliability of this method for studying seabird feeding habits and seabird association with fisheries.

3. MATERIALS AND METHODS

3.1. Species of study

Shearwaters get their name from the way they fly, they glide near the water's surface, creating the appearance that they are "shearing" the waves. Along with other seabirds like petrels, they belong to the order Procellariforms (Rajpar et al., 2018).

Figure 3.1 Cape Verde Shearwater (*Calonectris edwardsii*) in land (Left) and flying (Right). Authors: Projecto Vito volunteer and Robert Williams.

The species focused on this study is the Cape Verde shearwater, an endemic species of the Cape Verde archipelago (Figure 3.1). Due to its limited geographic range, this species has become increasingly important (Hazevoet, 1997).

On the IUCN Red List of Threatened Species, the Cape Verde shearwater was classified as near threatened in 2018. This classification reflects growing concerns about the long-term survival of the species (Paiva et al., 2015).

This bird is characterized by its brownish-grey dorsal plumage, with lighter tail coverts, while its ventral side is predominantly white. The beak is slim and compressed, with a yellowish-grey base and a darker tip. In terms of physical dimensions, it typically measures between 42 and 47 centimetres in length, with a wingspan ranging from 101 to 112 centimetres. The average weight varies between 420 and 540 grams, this large wingspan compared to their body size allows an efficient gliding over the ocean (Del Hoyo, 2020).

This species of seabird migrates over great distances and breeds only in Cape Verde islands. Breeding season lasts from late May to late September. Each pair lays a single egg in early June with no replacement egg. Both parents participate in the incubation, that lasts approximately

two months, and the chick-rearing (Navarro et al., 2007, 2009; Paiva et al., 2015; Xavier et al., 2024).

Following the breeding season, the shearwaters migrate to the northeastern coast of South America to spend the winter. The restricted distribution of this species has brought increased attention to its conservation due to the threats it faces on land and at sea (Hazevoet, 1997).

Previous studies indicates that diet of Cape Verde shearwater primarily consists of fish. These birds often associate with pelagic predators like tuna, which drive small fishes towards the surface, making them more accessible for the shearwaters (Weimerskirch, 2007).

During the breeding season, this shearwater adopts a dual-foraging strategy. They undertake long foraging trips to the more fruitful waters off the coast of Africa during the incubation stage (Paiva & Garthe, 2014). In contrast, their foraging trips are shorter and confined to less productive waters surrounding the Cape Verde islands during the chick-rearing phase. This change in foraging behaviour guarantees that they are able to feed their chicks more frequently (Paiva et al., 2015).

3.2. Zone of study

The Cape Verde archipelago (Figure 3.2), about 600 kilometres offshore the coast of Senegal in the Atlantic Ocean, serves as the research study area. Ten volcanic islands constitute this archipelago; each has a unique physical environment that is a home for a diverse range of habitats (Duarte & Romeiras, 2009).

Figure 3.2 Cape Verde archipelago, highlighting the specific location of the Curral Velho colony. Source: Google maps.

These islands are characterized by their arid tropical to semi-desert temperatures and great biodiversity. The region's unique geological and climatic characteristics add to its ecological

value, making it a crucial spot to research island biodiversity and conservation. In particular, the Curral Velho colony, located at coordinates 15º58''10,16 N and 22º47''22,84 W (Figure 3.2).

The Canary Current Large Marine Ecosystem (CCLME) has an impact on this area of the Atlantic (Vazquez et al., 2022). It is characterized by intense and continuous upwelling, because of these circumstances, a lot of small pelagic fish congregate, subsequently attracting a wide variety of predators. This area has received considerable interest for fishing efforts and has been recognized as a "World's fisheries-conservation hotspot" (Paiva et al., 2015).

3.3. Use of tracking devices

Three types of tracking devices were employed in this study (Figure 3.3): GPS Sputnik (Sextante technology, 20 g), GPS Axytrek (Technosmart, 17 g) and GPS CatLog (Perthold, 16 g). Each one vary in the type of data that collect. CatLog records only position data, while AxyTrek collects tracking data and also accelerometery. Going one step further, Sputnik records position, accelerometery and a radar signal, these devices were carefully positioned between the wings and parallel to the spine of the dorsal area to ensure a minimal disruption to the natural behaviour of the bird (Figure 3.3). The weight of the devices did not exceed 5% of the body weight, ensuring the ethical guidelines for the tracking studies (Navarro-Herrero et al., 2024; Reyes González et al., 2017).

Figure 3.3 Tracking devices deployed on Cory's shearwaters, *Calonectris borealis,* from left to right: GPS CatLog, GPS Axytrek and GPS XSputnik. Author: Marta Nadal.

The data obtained from these tracking devices was facilitated by Leia Navarro from the Seabird Ecology Lab. GPS data obtained with CatLog and Axytrek is cross-referenced with data from AIS or VMS. Nevertheless, there are few limitations, although access to vessels trajectories is

frequently restricted (Hinz et al., 2013; Pott & Wiedenfeld, 2017), and smaller vessels, which may not be equipped with VMS or AIS, can still be a significant impact on seabird's populations (Votier et al., 2023). These interactions were divided into two categories (Collet et al., 2015; Corbeau et al., 2021):

- Attendances: The seabird actively approaches within 1.5 km to a vessel with a minimum 10-minute duration.
- Encounters: The seabird is within 30 km of a vessel, at this distance bird is likely to notice it, with a minimum duration of 30 minutes.

Furthermore, Sputnik devices scan the surrounding 5 km for radio emissions radiated by marine radars (Weimerskirch et al., 2018). These radars use radio waves to identify any threats at sea, like other vessels, ice, rocks or land (Carneiro et al., 2022; Corbeau et al., 2019). Although it is typical for vessels to disable AIS in order to hide illicit activities or when traveling through regions where piracy is common, it is improbable that radar systems would be switched off due to the serious safety risk involved(Ford et al., 2018; Park et al., 2023). Despite these challenges, combining GPS, AIS, and radar detection provided a quantification of seabird-fishing vessel interactions.

3.4. Sample collection

In the years 2018, 2019 and 2021, fieldwork was done at the Cape Verde shearwater colony in Curral Velho, Cape Verde, during the breeding season, which extend from late June to late august. Fieldwork could not be carried out in 2020 due to the restrictions imposed by the SARS-COVID19 pandemic, and for that reason, no data is available for that year.

In order to take advantage of the period when adult shearwaters return to their nests to incubate eggs or feed their chicks, sampling was done at night. Individuals included in this study were captured at night from their nests, either by hand or, in the event that direct access was not feasible, with the help of a loop. Adult birds were fitted with tracking devices attached to the feathers on their back using adhesive tape. These devices were retrieved on average after 12.37 ± 5.67 days (considering all samples). For incubation samples, the devices were retrieved on average after 10.83 ± 3.26 days, while for chick rearing samples, were retrieved on average after 11.69 ± 7.01 days. To ensure that the birds' flight was not affected, the weight of these devices did not exceed 5% of the bird's body weight.

After the device was recovered, faecal samples were collected, which likely represent the prey consumed during the last days of the foraging trip. Digestion time in seabirds, can vary depending on the type of prey. For example, studies show that fish and cephalopods may take more than 12 hours to pass through the digestive system, with some hard prey remains persisting even longer (Alonso et al., 2018). Thus, it is reasonable to expect that the faeces sampled correspond to the prey consumed during the recorded foraging trip. Only individuals that had carried a GPS device were selected for faecal sampling. In order to obtain these faecal samples, the cloacal region was lightly massaged, and then the abdomen was lightly compressed in the direction of the cloaca until the bird started to expel faeces. After that, the samples were gathered and preserved in an ethanol-filled 2 ml vial (Figure 3.4).

Figure 3.4 Visualization of the obtention of faecal sample of a *Calonectris borealis* during 2020 Veneguera campaign. Author: Pablo Tresserras

3.5. Metabarcoding analysis

The procedures for DNA extraction, PCR amplification, library preparation and sequencing were carefully followed as described in (Antich et al., 2023) and (Wangensteen et al., 2018), this procedure was carried out by members of the Seabird Ecology Lab (Figure 3.5).

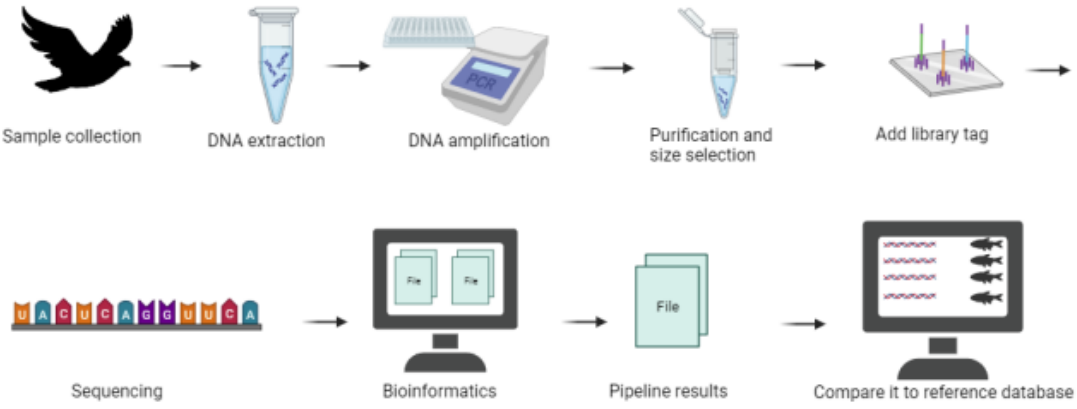

Figure 3.5 Schematization of the general steps of the protocol followed in this study.

Author: Marta Nadal, based on Wangensteen et al. 2018, using Biorender.

DNA metabarcoding is a powerful molecular tool used to analyse complex samples containing DNA from multiple species, allowing for the simultaneous identification of various organisms (Corell & Rodriguez-Ezpeleta, 2014) making it invaluable for studying environmental biodiversity (Snider et al., 2022; Valentini et al., 2009). Despite its efficiency, metabarcoding faces several challenges, including errors such as chimera formation, primer-dimer formation, tag-jumping, contaminations among others (Dunshea et al., 2008; Garafutdinov et al., 2020; Macher et al., 2023; Omelina et al., 2019; Schloss et al., 2011; Schnell et al., 2015). It is crucial to identify and eliminate various errors that can occur during the process to ensure the accuracy of the results.

3.5.1. DNA Extraction

Samples obtained during 2018, 2019 and 2021 campaigns were stored at the University of Barcelona until their analysis in 2023. Given the elevated level of uric acid present in seabird faeces, which may impede the Polymerase Chain Reaction (PCR) procedure, it is essential to choose a DNA extraction kit that efficiently eliminates such inhibitory agents to guarantee optimal PCR efficiency.

For this study, QIAGEN PowerSoil DNA Kit was used as it is designed especially to extract DNA from soil samples, which often contains a variety of PCR inhibitors. The extraction procedure was carried out un a sterile laminar flow cabinet with UV light sterilization between samples in order to avoid contamination.

Samples were mechanically disrupted and homogenized by adding the samples to a bead-beating tube. After inducing cell lysis, DNA is collected on a silica membrane in a spin column. Following that, the membrane's DNA is cleaned and rinsed so that the PCR amplification process starts.

3.5.2. PCR Amplification

For the PCR amplification, Leray-XT primer set (Wangensteen et al., 2018) was used, this set is known for its high level of degeneracy, enabling the amplification of approximately 313 base pairs of the cytochtome c oxidase subunit I (COI) gene. The sequences used were:

Forward, miCOIint-XT: 5'-GGWACWRGWTGRACWITITAYCCYCC-3'

Reverse, jgHCO2198: 5'-TAIACYTCIGGRTGICCRAARAAYCA-3'

The degenerated bases, which are W, R, Y and I, makes these primers particularly effective in amplifying a broad range of eukaryotic organisms, extending beyond metazoans.

- **W**: It can pair with Adenine and Thymine.
- **R**: It can pair with Adenine and Guanine.
- **Y**: It can pair with Cytosine and Thymine.
- **I**: Deoxy-inosine (I) can pair with all four natural bases.

A multiplexing strategy was used, where each sample was tagged with an 8-base pair barcode at the 5' end, with a minimum difference of 3 base pair for each sample, allowing for the simultaneous processing of several samples while preserving individual sampling identification, which helped speed the process and minimize resources. The same tag was used for both forward and reverse primers facilitating the elimination of inter-sample chimeras. The program OligoTag was used to create the tags (Boyer et al., 2016).

A total of 20 µL was used for each sample during the amplification process: The mixture contained: AmpliTaq® Gold MasterMix (Applied Biosystems, Foster City, CA, USA) 10 µL, forward and reverse 8-base tagged primers mix (2 µL of 5 µM), bovine serum albumin (20 mg/mL, 0.16 µL), molecular-grade water (5.84 µL) and DNA template (2 µL).

PCR procedure, one for each sample, included 10 minutes of initial denaturation at 95ºC, 35 cycles of further denaturation at 94ºC for 1 minute, hybridization at 45ºC for 1 minute, and elongation at 72ºC for another minute, ending with 5 minutes at 72ºC. Three PCR-blanks were run by amplifying the PCR mixture without the DNA template, as well as three negative controls that were run with ultrapure water (Milli-Q System, Merck Group, Darmstat, Germany).

Following PCR amplification, all products were pooled together. The distinct barcodes made pooling easier and allowed for group analysis while saving time and materials.

Subsequently, PCR products were purified and size-selected using the MinElute PCR purification columns. These columns concentrate the DNA while eliminating unwanted artifacts, such as primer dimers, nucleotides and fragments smaller than 70 base pairs.

3.5.3. Library preparation and sequencing

BIOO NEXTFLEX PCR-Free DNA-Seq Kit (Perkin-Elmer) was used to add adapters to the DNA fragments in order to prepare them for sequencing, this procedure is known as library preparation. These adapters let the DNA fragments adhere to the sequencing platform more easily. The actual sequencing procedure took about a month to finish and was carried out at the

CNAG sequencing facility employing an Illumina NovaSeq 6000 with 2x250 bp paired-end sequencing.

3.5.4. Bioinformatics analysis

MJOLNIR pipeline (Wangensteen, 2022), with default parameters customized for the COI Leray-XT marker, was used to process the sequencing data (Figure 3.3).

First, each sample was assigned to its original identifier by demultiplexing. Quality filtering was also used in this stage to exclude reads that had chimeric sequences or errors in amplification or sequencing. OBItools (Boyer et al., 2016) was used for filtering based on the read-length and paired-end alignment, using a phred quality score cutoff of 30. To eliminate chimeric sequences VSEARCH (Rognes et al., 2016) was used.

Second, sequences were categorized into MOTUs[1] using SWARM clustering method (Mahé et al., 2015). Taxonomic assignment of sequences within each MOTU was caried out using Ecotag algorithm (Boyer et al., 2016), owi_add_taxonomy (Wangensteen & Turon, 2016) and a custom reference database (Wangensteen, 2022). Ecotag assigned sequences to their most recent common ancestor within the reference set when exact matches could not be obtained.

Finally, to ensure data integrity LULU algorithm (Frøslev et al., 2017) was used to eliminate any pseudogenes, which are non-functional due to mutations, can complicate data analysis, so careful filtering and exclusion were necessary (Dunshea et al., 2008). Furthermore, given that samples were sequenced together with those from other seabird species, manual filtering was carried out to reduce the potential effects of "tag-jumping" during sequencing, which could lead to cross-contamination between species. Low-abundance sequences, potential contaminants and parasitic species that were considered unnecessary for the objectives of the study were also excluded from the dataset.

3.6. Data analysis

Each sample and the quantity of reads for every prey item in it were determined throughout the taxonomic assignment process. Furthermore, taxonomic data including the identity coefficient and sequence of each prey species was collected.

[1] Molecular Operational Taxonomic Units, with MOTUs the natural variations of a species' genome are represented. The largest distance between sequences that can be clustered together is d=13.

The accuracy of the taxonomic assignment during the pipeline was checked using the identity coefficient, numbers above 0.97 were considered accurate. Using the BLAST (NCBI) and Boldsystems database, manual verification was done for identity coefficients below 0.97. When a species match could not be found, matches at other taxonomic levels, such family, were sought.

Relative Read Abundance (RRA) was then calculated using taxonomic assignment results, where each prey item in a sample was represented by its relative significance on a scale from 0 to 1. Based on their phenology and habitat, three groups of prey species were classified (Annex I):

- Natural: Prey that the bird might be able to hunt independently.
- Discard: Prey that is unlikely to be found by the bird on its own; usually obtained by fishing waste or by-catch.
- Both: Prey that the bird could biologically hunt, but are frequently connected to fishing discards.

Once all species had been categorized, additional details obtained during field campaigns was integrated into the analysis. It includes information on sex, breeding stage, sample collection dates and interactions with fishing vessels obtained through tracking devices. The final dataset allowed to represent the relative importance of different prey types and the difference in discards consumption across various factors, such as proximity to fishing vessels, sex and breeding stage.

This analysis was conducted using R programming language, employing various packages, including tidyverse, pals, gridExtra, vegan, MASS, ggfortify, factoextra, readxl, broom, RColorBrewer, viridis, ggplot2, dplyr, and patchwork.

Data preprocessing involved filtering out irrelevant taxa (such as birds and certain phyla unrelated to prey) and transforming DNA read counts into relative read abundance (RRA) tables to normalize the data. The samples were then integrated with metadata, including information on bird sex, breeding stage, and interactions with fisheries.

Data visualization was carried out through stacked bar charts and pie charts to depict dietary composition, as well as boxplots to compare differences in the usage of various prey types based on factors such as sex, vessel interaction, and the duration of attendance at foraging areas.

A Shapiro-Wilk test was conducted to assess data normality. The results indicated that the distributions of relative read abundance variables were non-normal. Therefore, non-parametric statistical tests, such as the Kruskal-Wallis test and post hoc Wilcoxon tests with Holm correction, were used to evaluate significant differences in prey abundances based on factors like bird sex and interaction events

4. RESULTS

A total of 166 birds were sampled for this study. However, only 103 of these provided high-quality dietary sequencing data that could be analysed. The birds were categorized into two main groups based on their interaction events: 41 birds were classified under "attends," and 14 birds under "encounters." The average number of "attends" per sample was 21.26, while the average number of "encounters" per sample was 67.86. Out of the 166 birds sampled, 41 were female, 47 were male, and the rest had an undetermined sex. Regarding breeding stages, 24 birds were in the incubation stage, 12 were in the chick-rearing stage, and the remaining birds had an unknown breeding stage.

Attends by Sex and Breeding Stage

- When analysed by sex, females had an average of 21.2 "attends" per sample, males had an average of 22.6.
- When analysed by breeding stage, birds in the chick-rearing stage had an average of 12.1 "attends" per sample, those in the incubation stage had an average of 22.3.

Encounters by Sex and Breeding Stage

- When analysed by sex, females had an average of 39.6 “encounters” per sample, while males had a significantly higher average of 96.1.
- When analysed by breeding stage, birds in the chick-rearing stage experienced an average of 84.5 "encounters," those in the incubation stage had an average of 9.

4.1. Diet composition

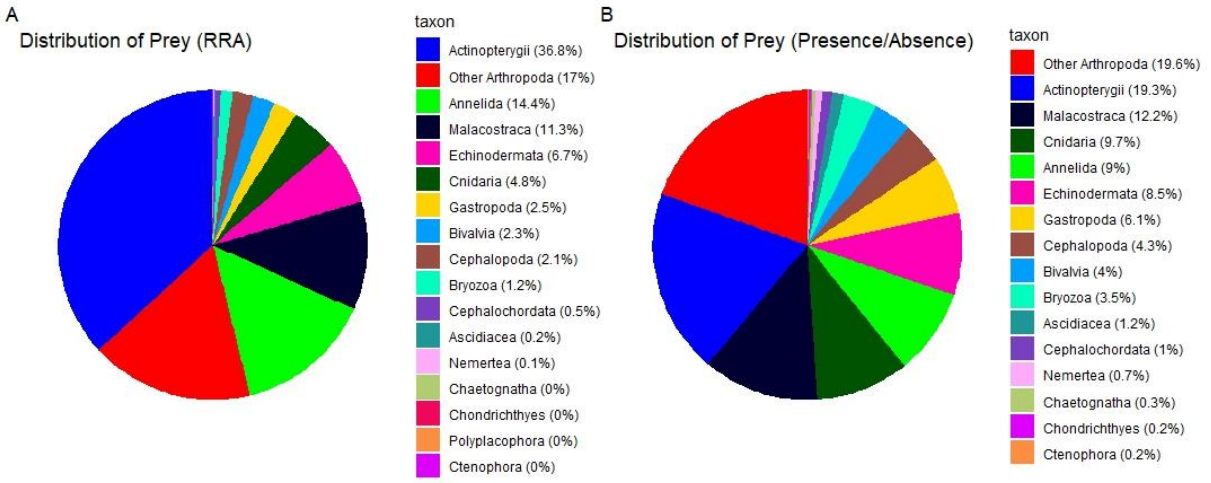

Figure 4.1 Proportion of prey taxa by class in the diet of Cape Verde shearwaters (*Calonectris edwardsii*) based on (A) Relative Read Abundance (RRA) and (B) Presence/Absence. Faecal samples (n=103) were collected at the end of feeding trips during the years 2018, 2019, and 2021, and analysed using DNA metabarcoding. (A) The RRA chart reflects the overall proportion of each taxonomic class in terms of quantity consumed, showing that the most abundant prey group is Actinopterygii (36.8%), followed by Other Arthropoda (17%), Annelida (14.4%), and Malacostraca (11.3%). (B) The Presence/Absence chart illustrates the proportion of detections for each taxonomic class, with Other Arthropoda (19.6%) and Actinopterygii (19.3%) being the most frequently detected taxa, followed by Malacostraca (12.2%) and Cnidaria (9.7%).

Data from 2018, 2019, and 2021 were combined to show the most prevalent prey groups in the diet of Cape Verde shearwaters using both Relative Read Abundance (RRA) and Presence/Absence data. Actinopterygii (bony fish) emerged as the most dominant prey category by RRA, accounting for 36.8% of the total diet. "Other Arthropoda," which made up 17%, included Maxillopods, Ostracods, and other unidentified arthropods. Annelida (14.4%), Malacostraca (11.3%), and Echinodermata (6.7%) also contributed significant portions to the diet (Figure 4.1A). Some taxa, such as Chaetognaths, Chondrichthyes, and Ctenophora, were either absent or poorly represented, contributing 0% to the RRA (Figure 4.1A).

In contrast, the Presence/Absence analysis, which indicates the percentage of the number of times a prey taxon was detected across all faeces, regardless of its number of RRA, showed a slightly different pattern. Other Arthropoda (19.6%) and Actinopterygii (19.3%) were the most frequently detected taxa, indicating their consistent presence in the diet. Other notable taxa included Malacostraca (12.2%), Cnidaria (9.7%), and Annelida (9%). Some taxa, like Chaetognatha and Chondrichthyes, were occasionally present but contributed little to the overall diet by quantity (Figure 4.1B).

Together, these analyses show that while Actinopterygii and Other Arthropoda are consistently present in the diet, the RRA data highlights that certain prey groups, like Actinopterygii,

dominate the diet in terms of relative quantity, while other taxa, such as Cnidaria and Echinodermata, may be detected frequently but in smaller quantities.

4.2. Foraging trips

A map summarizing the foraging trips made by shearwaters throughout the period they carried the tracking devices. It was possible to determine the zones where these birds were near vessels.

Considering tracking data across the study period, this map shows two significant zones of interaction (Figure 4.2):

- Cape Verde Archipelago
- West African coast

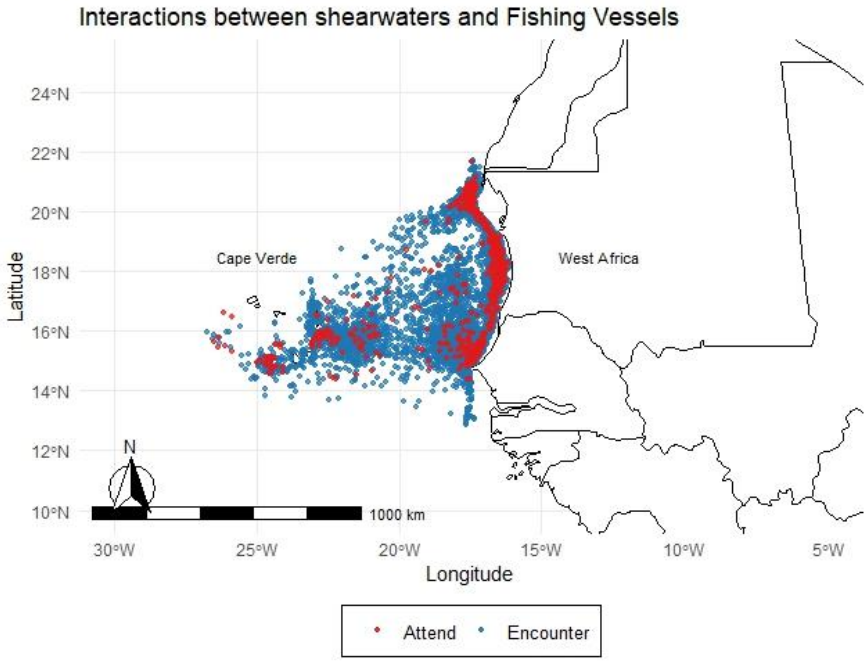

Figure 4.2 Spatial distribution of Cape Verde shearwater (*Calonectris edwardsii*) interactions with fishing vessels during foraging trips, based on data collected over 2018, 2019, and 2021. Interaction events were recorded using tracking devices fitted to the birds and cross-referenced with AIS data to classify two types of interactions: encounters (blue), where shearwaters were within 30 km of a vessel for at least 30 minutes, and attendances (red), where the birds actively approached within 1.5 km of a vessel for at least 10 minutes. The map highlights the geographic locations where these interactions occurred.

Encounters, blue dots, are more broadly distributed, however attendances, red dots, are strongly concentrated in these zones. This map provides crucial insights into spatial patterns of shearwater foraging behaviour.

4.3. Prey type analysis

Prey types are divided into three groups that were previously discussed, plus the unknown group for species which no information was available. The objective of this analysis is to understand the relative contribution of each type of prey to the overall diet of shearwaters.

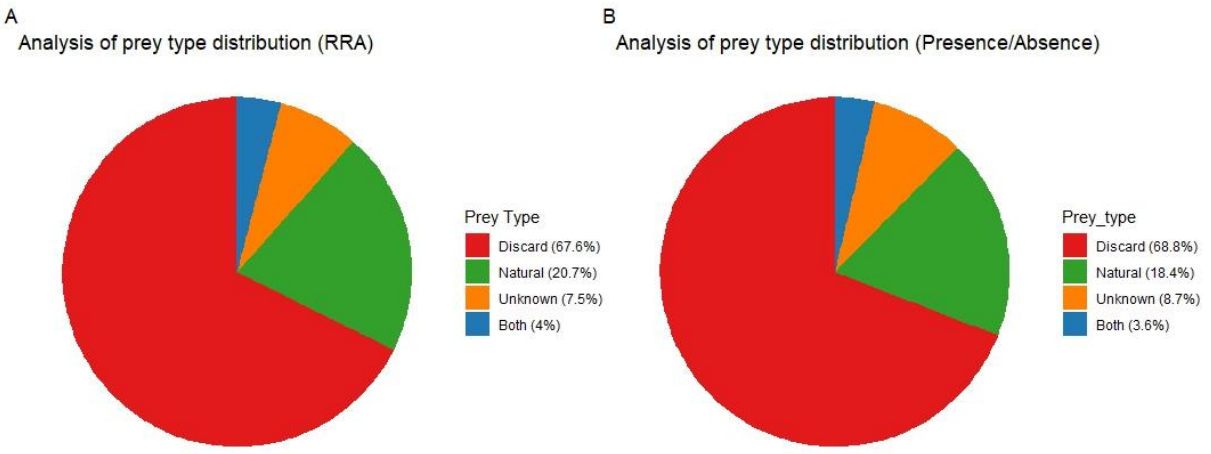

Figure 2.3 Proportion of prey types obtained from faecal samples of Cape Verde shearwaters (*Calonectris edwardsii*), based on (A) Relative Read Abundance (RRA) and (B) Presence/Absence analysis across the years 2018, 2019, and 2021. The charts categorize prey into four main types: 'Natural' (green – prey naturally caught by the birds), 'Discard' (red – prey derived from fishing vessel discards/offal), 'Unknown' (orange – prey with insufficient information to classify), and 'Both' (blue – prey that could originate from either natural sources or fishing activities). (A) reflects the proportion of each prey type in terms of quantity consumed, with discard prey dominating at 67.6%, followed by natural prey at 20.7%. (B) shows the presence/absence of prey types, with discard prey detected in 68.8% of samples, and natural prey in 18.4%. Faecal samples (n=103) were analysed using DNA metabarcoding to identify prey types.

To examine the composition of prey consumed by Cape Verde shearwaters, pie charts were created to show the distribution of prey types based on Relative Read Abundance (RRA) and Presence/Absence.

The RRA analysis indicates that discard prey represents the largest proportion of the diet, making up 67.6% of the total prey consumption. Natural prey accounts for 20.7%, while prey categorized as both discard and natural makes up 4%, and unknown prey constitutes 7.5%. This highlights that discard prey dominates the diet in terms of the amount consumed (Figure 4.3 A). In the Presence/Absence analysis, discard prey is also prevalent, detected in 68.8% of the samples. Natural prey was detected in 18.4% of samples, while unknown prey and both prey types were found in 8.7% and 3.6% of samples, respectively (Figure 4.3 B). This analysis demonstrates that discard prey is not only consumed in large quantities but also frequently detected across samples, emphasizing its importance in the diet of Cape Verde shearwaters.

4.3.1. Comparison of prey type composition between different interaction events

The interactions between shearwaters and vessels are divided into two categories to examine the kind of prey that seabirds consumed based on the type of event. In the following graph (Figure 4.4), A and B display the relative read abundance (RRA) of each prey type, while C and D show the presence/absence analysis to show how frequently each prey type was detected during these events. Samples in which interaction events could not be determined were not included for this analysis.

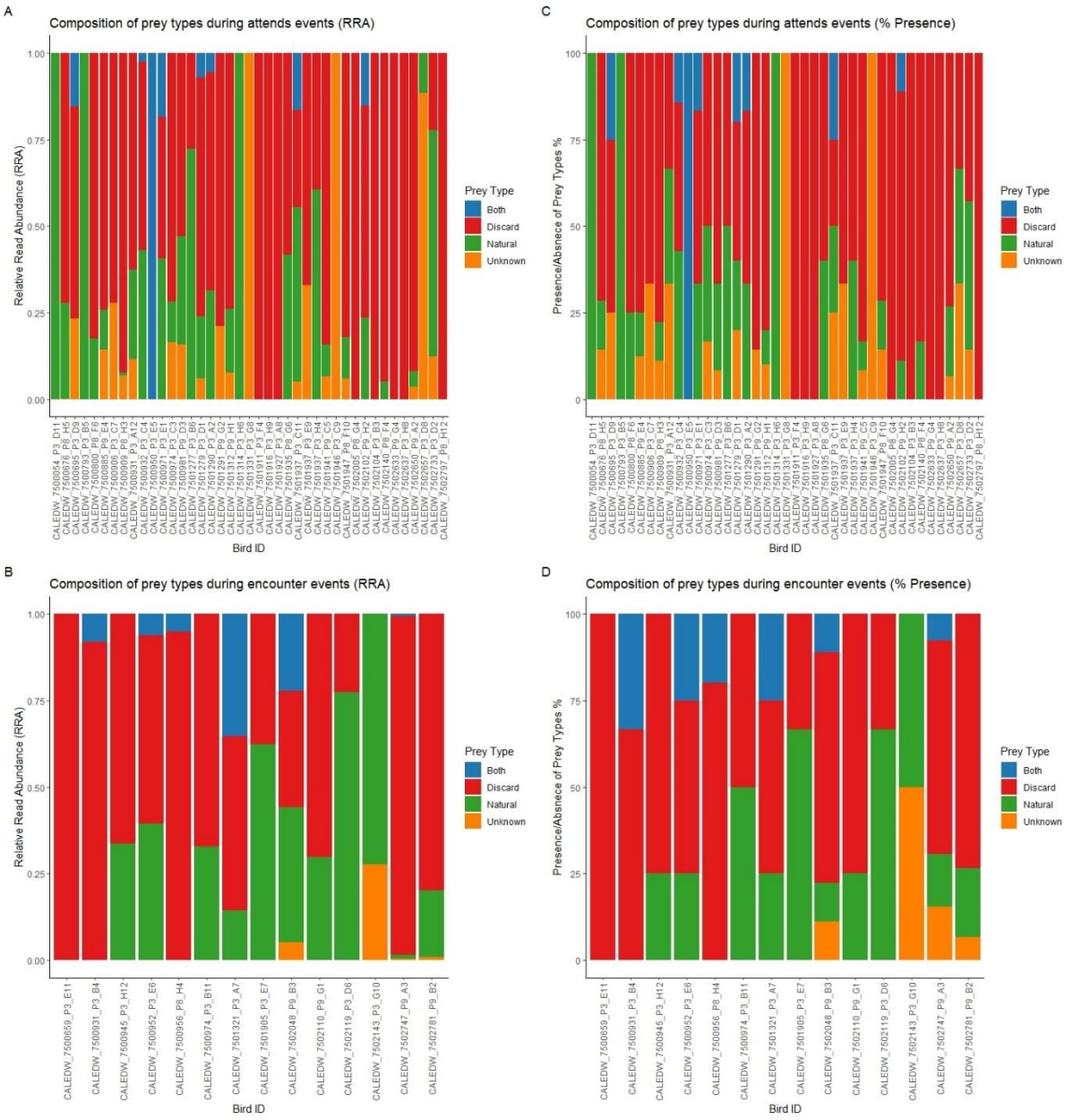

Figure 3.4 Relative Read Abundance (RRA) and Presence/Absence proportions of prey types (Natural – green, Discard – red, Both – blue, and Unknown – orange) per individual sampled (indicated by its ring number) during attend and encounter events. (A) shows the RRA of each prey type for individuals during attend events (n=41). (B) shows the RRA for individuals during encounter events (n=14). (C) shows the presence/absence proportions of prey types during attend events, and (D) shows the presence/absence proportions during encounter events. Data was combined from the years 2018, 2019, and 2021.

A boxplot was made to show the differences in the consumption of discards prey compared to natural prey for each form of interactions in order to see if there is pattern in the consumption of each type of prey (Figure 4.5). Additionally, a Kruskal-Wallis test was performed to determine if the differences in prey consumption are statistically significant by analysing the p-value.

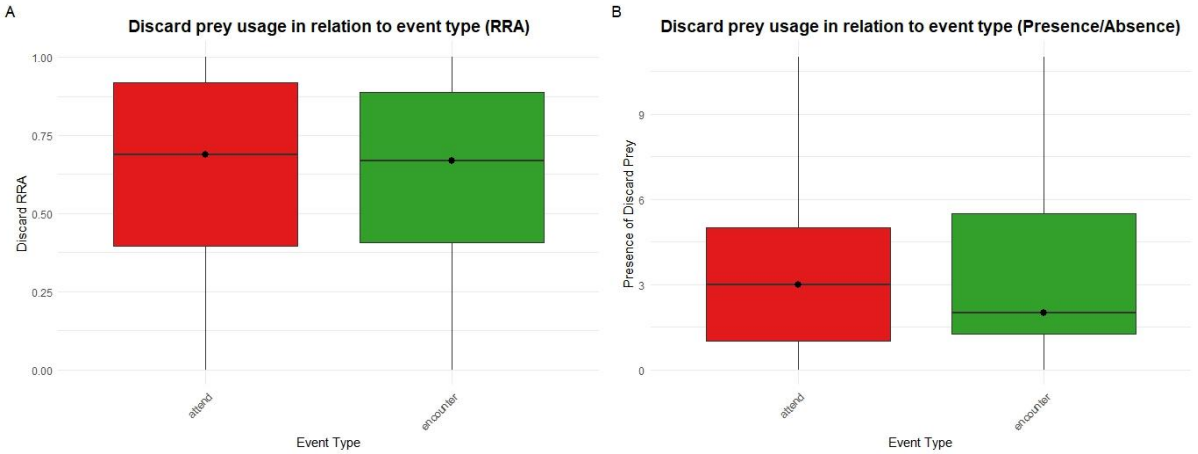

Figure 4.5 Discard prey usage in relation to event type based on fecal samples containing at least one attendance event (n=38) versus those with only encounters (n=14). (A) represents discard usage measured by Relative Read Abundance (RRA) across event types. (B) shows the presence/absence of discard prey by event type. Kruskal-Wallis tests revealed no statistically significant differences in discard usage for RRA (χ^2 = 0.04546, p-value = 0.8312) or presence/absence of discard prey (χ^2 = 0.0015242, p-value = 0.9689).

To investigate whether discard prey usage differs between event types (trips with attendance vs. encounters), two Kruskal-Wallis tests were performed. The test results for discard prey usage measured by Relative Read Abundance (RRA) showed a chi-squared value of 0.04546 and a p-value of 0.8312, indicating that there is no statistically significant difference in discard usage between the two event types. Similarly, the test for the presence/absence of discard prey returned a chi-squared value of 0.0015242 and a p-value of 0.9689, confirming no significant difference in the presence of discard prey between attendance and encounter trips.

4.3.2. Prey type composition by attendance – sampling interval

There were no significant differences between the groups in the initial analysis of prey consumption. This raised the chance that prey eaten during the attendance events, may not have been reflected in the samples.

4.3.2.1. Four days interval

In order to address this, attendance events were split into two groups: those that happened less than 4 days after the event and those that happened more than 4 days after the event (Figure 4.6).

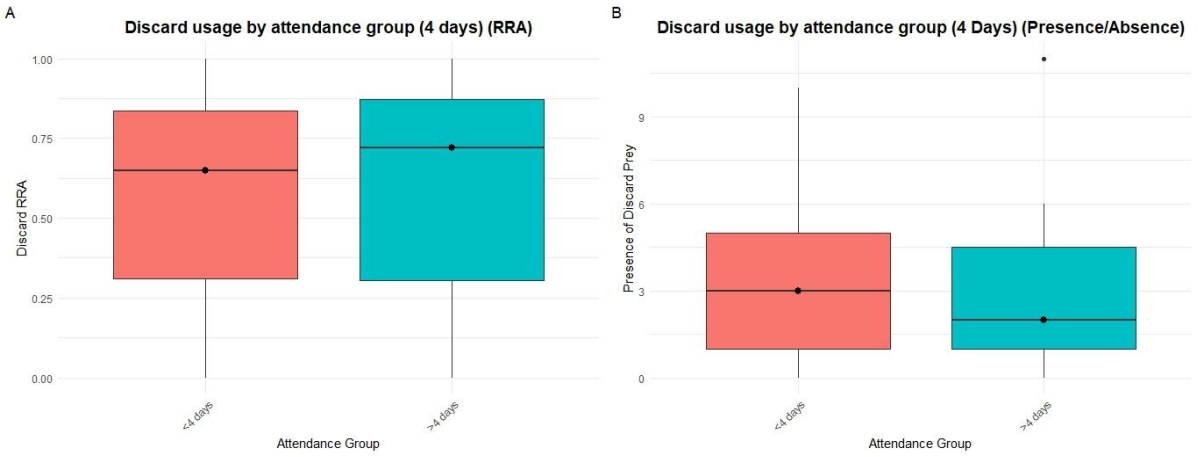

Figure 4.6 Boxplots showing discard prey usage by attendance group, categorized by the time since the last attendance event (<4 days n=34, >4 days n=7). (A) represents discard usage measured by Relative Read Abundance (RRA. (B) shows the presence/absence of discard prey. Kruskal-Wallis tests revealed no statistically significant differences in discard usage for RRA ($\chi^2 = 0.0076$, p-value = 0.93) or presence/absence of discard prey ($\chi^2 = 0.0111$, p-value = 0.92).

To investigate whether the timing of attendance events affects discard prey usage, two Kruskal-Wallis tests were conducted. The results for discard prey usage measured by Relative Read Abundance (RRA) returned a chi-squared value of 0.0076 and a p-value of 0.93, indicating no statistically significant difference in discard usage between the two groups. Similarly, the test for the presence/absence of discard prey showed a chi-squared value of 0.0111 and a p-value of 0.92, confirming no significant difference in the presence of discard prey based on the timing of the last attendance event.

4.3.2.2. Two days interval

The analysis was replicated by dividing the attendance events into two groups again: those that took place more than two days before sample collection and those that happened within two days (Figure 4.7). This division aimed to determine whether consumption of natural prey and discard may be affected by an even shorter time gap between the interaction events and sample collection.

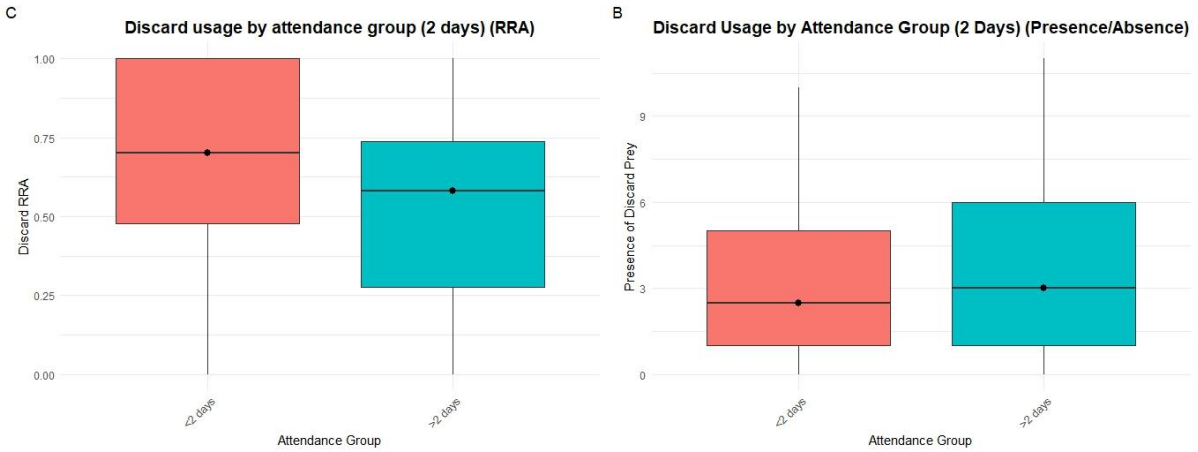

Figure 4.7 Boxplots showing discard prey usage by attendance group, categorized by whether the sample was collected within 2 days of the last attendance event (<2 days n=24, >2 days n=17). **(A)** represents discard usage measured by Relative Read Abundance (RRA). **(B)** shows the presence/absence of discard prey. Kruskal-Wallis tests revealed no statistically significant differences in discard usage for RRA ($\chi^2 = 2.7692$, $p = 0.096$) or presence/absence of discard prey ($\chi^2 = 0.0029$, $p = 0.96$).

Kruskal-Wallis tests revealed no statistically significant differences. The test for discard usage measured by Relative Read Abundance (RRA) showed a chi-squared value of 2.7692 and a p-value of 0.096, while the test for the presence/absence of discard prey returned a chi-squared value of 0.0029 and a p-value of 0.96, indicating no significant difference between the two groups.

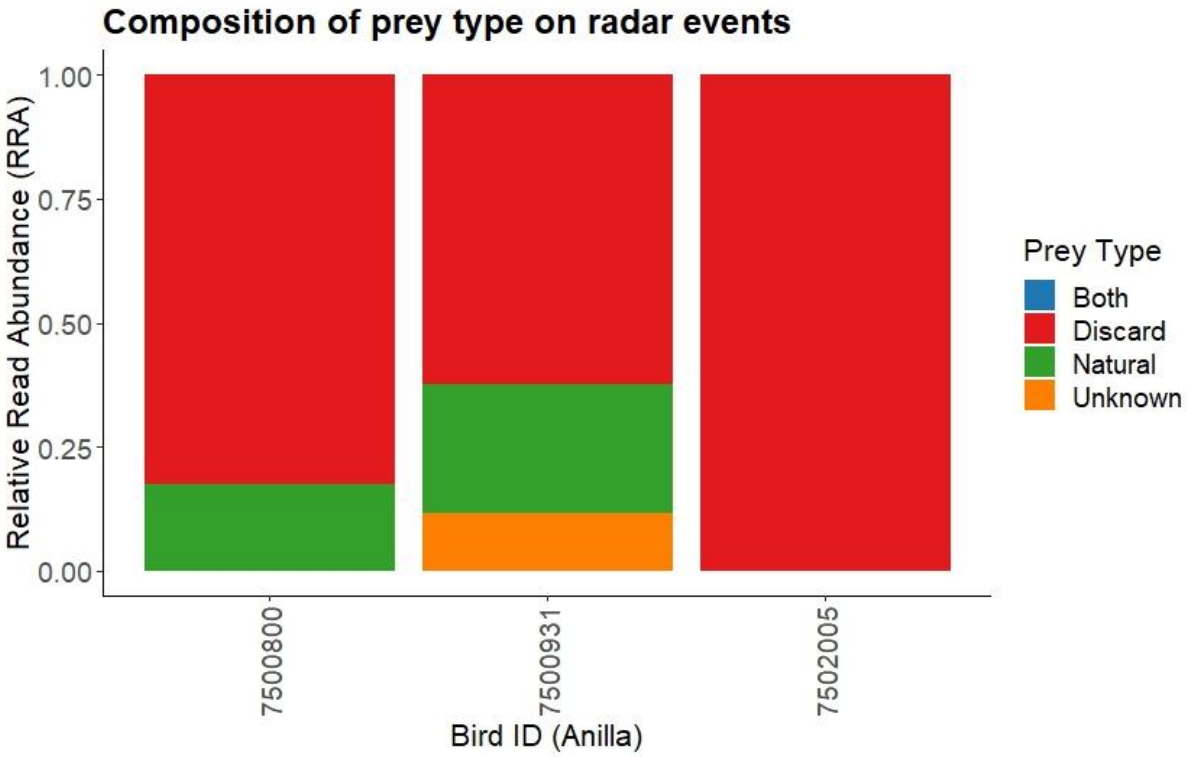

Figure 4.8 Prey type (Natural – green, Discard – red, Both – blue, or Unknown – orange) RRA proportions of Cape Verde shearwater (*Calonectris edwardsii*) specimens during radar-detected interactions with fishing vessels (n=3). Samples were collected in 2018, 2019, and 2021. Subjects are identified by bird ID (code composed of species name, ring number, plate number used during DNA extraction, and position number on the plate).

Data coming from Sputnik devices, those who can detect radar emissions, was also examined, however since the number of samples analysed with an associated radar was very small (n=3) their significance is questionable, a bar plot representing prey type in terms of RRA was generated, it can be observed that the majority of the diet composition of these individuals were discard preys (Figure 4.8).

4.3.3. Biological analysis

4.3.3.1. Comparison of prey type consumption by sex

To look at potential variations in prey eating habits based on sex, an analysis of discard and natural prey consumption was conducted across sexes (Figure 4.9).

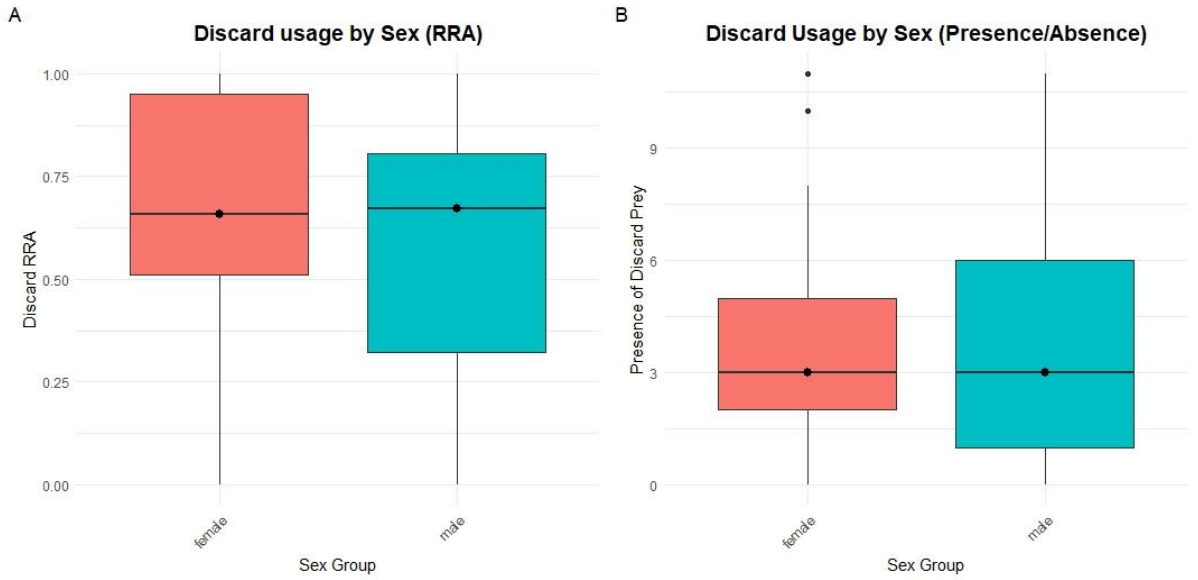

Figure 4.9 Discard usage by sex (female n=41 vs male n=47) . (A) represents discard usage measured by Relative Read Abundance (RRA) . (B) shows the presence/absence of discard prey in the diet by sex. There are some outliers for females, but the median values are similar between males and females. Kruskal-Wallis tests revealed no statistically significant differences between sexes for discard usage in terms of RRA ($\chi^2 = 1.2073$, $p = 0.27$) or presence/absence of discard prey ($\chi^2 = 1.076$, $p = 0.30$).

To assess whether there are significant differences in discard prey usage between male and female shearwaters, Kruskal-Wallis tests were conducted. The results for discard usage measured by Relative Read Abundance (RRA) yielded a chi-squared value of 1.2073 and a p-value of 0.27 indicating that there is no statistically significant difference in discard usage between sexes.

Similarly, the test results for the presence/absence of discard prey showed a chi-squared value of 1.076 and a p-value of 0.30, further suggesting that the presence of discard prey in the diet does not significantly differ between male and female shearwaters.

4.3.3.2. Comparison of prey type consumption by breed stage

It is known that depending on the breeding stage, seabirds alter their foraging behaviour. To explore whether these differences influence on prey type consumption, shearwaters were categorized into two different groups depending on the breeding stage when the sample was obtained, incubation and chick-rearing (Figure 4.10).

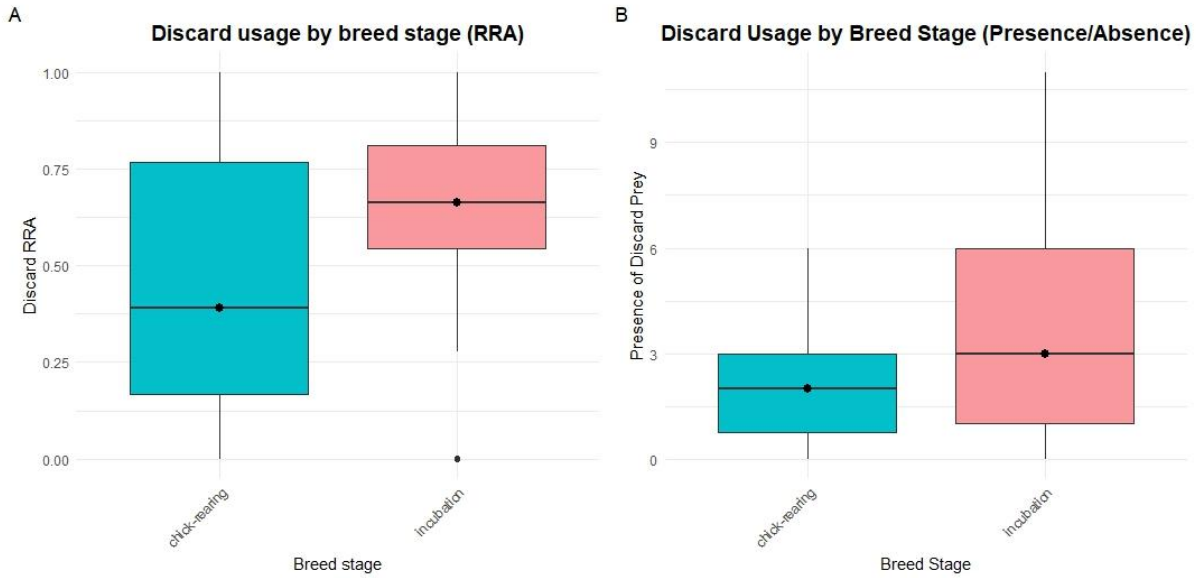

Figure 4.10 Boxplots showing discard usage by breeding stage (chick-rearing n=12 vs incubation n=27, total n=39). (A) represents discard usage measured by Relative Read Abundance (RRA) across two breeding stages, with incubation showing a higher median RRA compared to chick-rearing, suggesting greater discard usage during incubation. (B) shows the presence/absence of discard prey in each breeding stage. The incubation stage has a higher median presence of discard prey compared to chick-rearing. A Kruskal-Wallis test revealed a significant difference in discard usage between breeding stages in terms of RRA ($\chi^2 = 7.5915$, p-value = 0.02), but no significant difference in terms of the presence of discard prey ($\chi^2 = 3.0531$, p-value = 0.08).

To determine if there are significant differences in the consumption of discard and natural prey during different breeding stages, two Kruskal-Wallis tests were conducted. The test results for discard prey usage (measured by Relative Read Abundance, RRA) showed a chi-squared value of 7.5915 and a p-value of 0.02, indicating a statistically significant difference in discard usage between breeding stages. This suggests that shearwaters during the incubation stage may have consumed more discards compared to the chick-rearing stage.

In contrast, the test results for the presence/absence of discard prey did not reveal a statistically significant difference between breeding stages, with a chi-squared value of 3.0531 and a p-value of 0.08.

5. DISCUSSION

This research aimed to explore the dietary habits of the Cape Verde shearwater, with a focus on the reliance on fisheries discards along the coast of Cape Verde archipelago and the northwest coast of Africa. This research represents the first attempt to combine the information from DNA metabarcoding of faeces with that from the previous foraging trip tracked with GPS and analysed for the association with fishing activity. By examining the diet of these birds over three breeding seasons, this approach allowed for a deeper understanding of the diet composition and the influence of some biological factors, although we could not detect a relation between vessel interactions and the proportion of discards in the diet.

Previous studies indicate that Cape Verde shearwaters mainly feed on Actinopterygii and cephalopods (Paiva et al., 2015), a similar pattern shown by other closely related shearwater species whose diets are primarily based on Actinopterygii, crustaceans and cephalopods (Reyes-González & González-Solís, 2016). In this study, actinopterygians emerged as the most important prey group, representing 36.8% in terms of relative read abundance (RRA) and 19.3% in the frequency of presence and absence analysis. Arthropods, including malacostraca and other arthropods, also played a significant role. Despite these findings, further research is needed to better understand the diet composition of Cape Verde shearwaters.

Focusing on the prey type analysis, we observe a clear predominance of fishery discards in both metrics analysed, underscoring the importance of discards as a food source for this species. In the RRA analysis, prey presumably obtained from discards made up 67.6% of the total diet, while in the presence/absence analysis, they accounted for 68.8%. This suggests that, although Cape Verde shearwaters have access to natural prey, fishery discards represent a significant portion of their diet, likely due to their higher availability and ease of access in areas with fishing activity. This reliance on discards highlights the potential vulnerability of the species to changes in fisheries management practices. While measures to reduce overfishing and promote more sustainable practices are beneficial, they may inadvertently reduce an important food source for seabirds (Bicknell et al., 2013; Votter et al., 2004). Additionally, a small percentage of samples were classified as "unknown" or "both" (a combination of natural prey and discards), further emphasizing the need for more research to better understand the origin of the prey consumed and the impact of discards on the feeding behaviour of shearwaters. The reduction of available discards could intensify existing conservation challenges for this species. If the

availability of discards decreases seabirds may face an increased competition for natural prey sources or be forced to travel larger distances to find its preys.

Combining data from tracking devices with the prey type analysis, shearwaters were classified into two categories: those that had a close interaction with a fishing vessel (attendance) and those with a more distant proximity (encounter). Our objective was to determine whether shearwaters with attendance events showed a higher proportion of discard prey in their diet. However, the initial analysis did not reveal a significant relationship.

The influence of the time frame between the sample collection and the interaction with fishing vessels is a critical factor to consider in diet analysis. While there are limited studies specifically addressing the time lag between vessel interactions and prey consumption in seabirds, it is known that different prey types can take varying amounts of time to pass through the digestive system. Alonso et al. (2018) showed that fish and cephalopods can take more than 12 hours to transit through the digestive system of seabirds, with some hard prey remains persisting for even longer periods. This suggests that the timing of prey ingestion relative to sample collection is important, further research on the digestion timeframes of these species is needed to better understand prey consumption in Cape Verde shearwater.

Given the possibility that the time between the attendance event and sample collection could have influenced the results, two additional analyses were performed. The first restricted the analysis to birds where the attendance event occurred within 4 days prior to sample collection, but again no significant relationship was found. In the second analysis, the time window was further reduced to 2 days, which approached significance but remained non-significant.

Interestingly, in this study, we identified instances where discard prey species were found in shearwaters that, based on tracking data, did not have close interactions with fishing vessels (attendance). This raises questions about the origin of these discard prey. One possible explanation is the misclassification of prey types due to gaps in our understanding of the behaviour and biology of some prey species. Certain species, which we classified as discards, may have unstudied life cycles, or undergo vertical migrations that make them naturally available to the shearwaters, thereby positioning them within their natural prey spectrum rather than as fishery discards (Votier et al., 2010). This suggests that some prey we assumed were associated with fishery activity could, in fact, be part of the birds' natural diet.

Another hypothesis involves illegal, unregulated, or unreported (IUU) fishing, which may not have been detected in our tracking data but still provides discards that the shearwaters utilize.

Studies have shown that IUU fishing is prevalent in areas such as West African waters, where vessel transponders may be deliberately turned off, making it difficult to track fishing activity through conventional means (Navarro-Herrero et al., 2024). These hidden operations present a significant challenge in accurately assessing seabird interactions with fisheries and the true extent of discard utilization.

In this study, we did not find any significant sex-related differences in the consumption of fishery discards. Both the RRA analysis and the presence/absence analysis indicated no statistically significant differences. These findings are consistent with previous studies on other shearwater species, which also report no significant differences in discard consumption between males and females (Navarro et al., 2007, 2009). While seabirds generally exhibit sexual differences in metabolic rates, with females typically foraging more intensively and exhibiting higher activity at night (De Felipe et al., 2019; Reyes-González et al., 2021) these patterns did not translate into differences in the use of fishery discards in our sample. Therefore, despite potential biological differences between the sexes, our data suggest that both male and female shearwaters exploit discards similarly.

Our analysis revealed that breeding stage may influence prey type consumption in Cape Verde shearwaters. Compared to the chick-rearing phase, there was a greater reliance on fishery discards during the incubation phase, with a significant result in the RRA analysis, though the presence/absence analysis did not reach statistical significance. Previous studies have demonstrated that shearwaters adopt a dual-foraging strategy during the breeding season, alternating between long trips to distant foraging grounds to maximize food intake, and shorter trips closer to the colony to meet the immediate energy demands (Paiva et al., 2015). In other shearwater species, it has been observed that during the chick-rearing stage, adults tend to return to pre-laying weight levels (Navarro et al., 2007), this could explain the higher reliance on discards during the incubation phase, when the energetic demands of adults are greater due to prolonged fasting periods during incubation shifts. During the chick-rearing phase, adults may prioritize foraging for higher-quality prey to provide better nutrition for their chicks (Navarro et al., 2007; Paiva et al., 2015).

In summary, most of the analyses did not yield statistically significant differences in the use of discards. This lack of significance can be attributed to several methodological limitations inherent to the DNA metabarcoding technique and other factors that may have influenced the results. One of the most sensitive steps in the DNA metabarcoding process is the extraction of

DNA from the samples. In this study, we used the QIAGEN PowerSoil Pro extraction kit, following the protocol strictly. This kit was probably not the best option as only 103 out of the initial 166 samples provided good dietary sequencing data. This means that 37.95% of the samples had to be excluded due to poor DNA extraction results. Incomplete reference databases may have led to misidentifications of species, as many remain unstudied or unknown (Alonso et al., 2014; Clare, 2014). Only a limited number of species and genera are represented in these databases, which often results in prey being identified only at higher taxonomic levels, such as family, rather than at the species level (Alonso et al., 2014). Additionally, there is also bias introduced by the primers used in the analysis. Universal primers can favour the amplification of certain taxonomic groups, leading to an overrepresentation of some prey while others remain underrepresented (Alonso et al., 2014; Clare, 2014).

Although DNA metabarcoding is an effective tool for analysing environmental DNA, it presents limitations. For instance, it does not allow for the differentiation between whether a prey item is an adult, larva, or juvenile. These life stages can occupy different ecological niches, which could lead to errors when classifying prey types. To mitigate this issue in future studies, a combination of DNA metabarcoding with other methods, such as morphological analysis of stomach contents, could be employed to improve the accuracy of prey identification (Alonso et al., 2014; van der Loos & Nijland, 2021). The ability to quantify prey using molecular methods remains limited, which may lead to an overrepresentation of certain prey species while underestimating others (Clare, 2014).

In this study we used two different methods to analyse the results: Relative Read Abundance (RRA) to quantify the relative presence of prey in the faecal samples, and presence/absence analysis. Both methods have limitations. For instance, RRA can overestimate the abundance of some prey due to differences in DNA extraction efficiency, leading to potential biases in the quantification of certain species (Clare, 2014). On the other hand, while presence/absence analysis avoids this bias, it tends to overestimate the importance of rare prey items in the samples, as they are given equal weight to more frequently detected species (Ando et al., 2023).

6. CONCLUSIONS

In conclusion, the majority of the tests performed did not reveal statistically significant differences in the use of discards, which can be attributed to several methodological and experimental limitations. A key area for improvement is the DNA metabarcoding methodology. While this technique is highly effective in identifying prey composition, it is limited in its ability to distinguish between life stages (larva, juvenile, or adult), which may result in misclassification of prey as discards. This issue is particularly relevant in species with complex life cycles or vertical migrations. To address this limitation, future studies should consider complementing DNA metabarcoding with traditional morphological analysis or other molecular approaches, which would allow for more accurate prey identification, including life stage differentiation.

Another major challenge encountered was the reduction in sample size as more datasets were integrated, particularly when combining tracking data (AIS) and reproductive status. This reduction diminished the statistical power of the study, limiting the ability to detect significant patterns. To overcome this issue, future research should focus on increasing sample sizes by ensuring that as much data as possible is collected during fieldwork, such as the breeding stage and sex of individuals, to reduce data loss and improve the robustness of the analyses.

Despite these limitations, this study reveals that almost 68% of the diet of Cape Verde shearwaters, based on RRA (Relative Read Abundance), consists of fishery discards, demonstrating a significant reliance on these resources. This high percentage highlights the potential vulnerability of the population to changes in fisheries management practices.

This study offers a novel combination of DNA metabarcoding with GPS tracking data to examine the diet of Cape Verde shearwaters across different breeding seasons and interactions with fishing vessels. This approach provides valuable insights into the species' reliance on fishery discards and highlights the potential vulnerability of the population to changes in fisheries management practices. Future studies addressing these methodological challenges will contribute to a more comprehensive understanding of the foraging ecology of seabirds in a changing marine environment.

7. BIBLIOGRAPHY

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Annex I

Table 1 Annex: The table lists prey items identified in Cape Verde shearwaters faeces. Taxa are classified by phylum, class, and the lowest taxonomic assignment determined through the analysis. For each taxon, the total number of reads, which is the sum of all samples analysed, is provided. The column "Best identity" show the accuracy of the taxonomic match between sequences, with 1 being the highest and 0 the lowest. Prey items are categorized as originating from fishing vessel discards, naturally captured prey, both sources, or unknown if insufficient information was available to make a classification. To classify prey ecological characteristics, such habitat and behaviour, were considered.

Taxonomic assignment	Class	Phylum	Best identity	Total reads	Prey type
Euclymene robusta	Polychaeta	Annelida	0,8703	2374	Discard
Gyptis propinqua	Polychaeta	Annelida	0,9872	7829	Discard
Platynereis dumerilii	Polychaeta	Annelida	1	33970	Discard
Platynereis dumerilii	Polychaeta	Annelida	1	3896	Discard
Platynereis dumerilii	Polychaeta	Annelida	0,9462	210	Discard
Phyllodoce sp. CMC01	Polychaeta	Annelida	0,9968	1958	Discard
Bylgides groenlandicus	Polychaeta	Annelida	0,9968	23	Discard
Harmothoe sp. CMC01	Polychaeta	Annelida	0,9968	146	Discard
Myrianida rubropunctata	Polychaeta	Annelida	0,9968	1407	Discard
Odontosyllis fulgurans	Polychaeta	Annelida	0,8168	3637	Discard
Proceraea aurantiaca	Polychaeta	Annelida	0,9904	667	Discard
Proceraea paraurantiaca	Polychaeta	Annelida	0,9872	20492	Discard
Syllidae	Polychaeta	Annelida	0,8308	844	Discard
Syllidae	Polychaeta	Annelida	0,8012	934	Discard
Syllidae	Polychaeta	Annelida	0,8474	244	Discard
Syllidae	Polychaeta	Annelida	0,8073	55	Discard
Phyllodocida	Polychaeta	Annelida	0,8297	8362	Discard
Phyllodocida	Polychaeta	Annelida	0,8544	4469	Discard
Phyllodocida	Polychaeta	Annelida	0,9936	2155	Discard
Phyllodocida	Polychaeta	Annelida	0,8119	1305	Discard
Phyllodocida	Polychaeta	Annelida	0,8188	636	Discard
Phyllodocida	Polychaeta	Annelida	0,9936	43	Discard
Galathowenia oculata	Polychaeta	Annelida	0,9873	29	Discard
Sabellaria alveolata	Polychaeta	Annelida	1	4247	Discard
Chone infundibuliformis	Polychaeta	Annelida	0,9872	2843	Discard
Hydroides elegans	Polychaeta	Annelida	0,9904	8391	Discard
Hydroides elegans	Polychaeta	Annelida	0,7723	47	Discard
Hydroides	Polychaeta	Annelida	0,6893	3298	Discard
Sabellida	Polychaeta	Annelida	0,8100	2596	Discard
Laonice cirrata	Polychaeta	Annelida	0,9808	12364	Discard

Polydora cornuta	Polychaeta	Annelida	0,7771	437	Discard
Spionida	Polychaeta	Annelida	0,7901	543	Discard
Terebellidae	Polychaeta	Annelida	0,8423	3113	Discard
Terebellida	Polychaeta	Annelida	0,8082	1658	Discard
Terebellida	Polychaeta	Annelida	0,7906	5706	Discard
	Polychaeta	Annelida	0,8164	82311	Discard
	Polychaeta	Annelida	0,8464	1105	Discard
	Polychaeta	Annelida	0,8333	4224	Discard
	Polychaeta	Annelida	0,8170	17554	Discard
	Polychaeta	Annelida	0,8213	4128	Discard
	Polychaeta	Annelida	0,8280	103	Discard
	Polychaeta	Annelida	0,7656	946	Discard
Aspidosiphon	Sipuncula	Annelida	0,8292	121	Discard
		Annelida	0,8188	7602	Discard
Ampithoe rubricata	Malacostraca	Arthropoda	0,9936	1829	Discard
Sunamphitoe pelagica	Malacostraca	Arthropoda	0,9414	252	Discard
Aora typica	Malacostraca	Arthropoda	1	3155	Discard
Microdeutopus chelifer	Malacostraca	Arthropoda	1	428	Discard
Microdeutopus sp. SFAM14-002	Malacostraca	Arthropoda	0,9968	49598	Discard
Apherusa bispinosa	Malacostraca	Arthropoda	0,9773	2519	Discard
Caprella acanthifera	Malacostraca	Arthropoda	0,9936	543	Discard
Caprella acanthifera	Malacostraca	Arthropoda	0,8433	334	Discard
Phtisica marina	Malacostraca	Arthropoda	0,9935	5645	Discard
Phtisica	Malacostraca	Arthropoda	0,9223	4839	Discard
Pseudoprotella phasma	Malacostraca	Arthropoda	1	27763	Discard
Caprellidae	Malacostraca	Arthropoda	0,8414	2077	Discard
Laticorophium baconi	Malacostraca	Arthropoda	1	1031	Discard
Monocorophium acherusicum	Malacostraca	Arthropoda	1	122	Discard
Dexamine spiniventris	Malacostraca	Arthropoda	1	7753	Discard
Echinogammarus longisetosus	Malacostraca	Arthropoda	0,8949	3285	Discard
Gammaridae sp. KML 32	Malacostraca	Arthropoda	0,9872	152	Discard
Themisto libellula	Malacostraca	Arthropoda	0,9968	1348	Natural
Ericthonius punctatus	Malacostraca	Arthropoda	0,8801	247	Discard
Jassa falcata	Malacostraca	Arthropoda	0,9968	74	Discard
Jassa slatteryi	Malacostraca	Arthropoda	1	5093	Discard
Ischyroceridae	Malacostraca	Arthropoda	0,8307	1486	Discard
Ischyroceridae	Malacostraca	Arthropoda	0,8679	673	Discard
Microprotopidae	Malacostraca	Arthropoda	0,8006	688	Discard
Niphargus pachypus	Malacostraca	Arthropoda	0,8106	519	Discard
Photidae	Malacostraca	Arthropoda	0,8673	607	Discard
Stenothoe monoculoides	Malacostraca	Arthropoda	0,8107	78	Discard
Alpheidae	Malacostraca	Arthropoda	0,8423	607	Discard

Acanthonyx lunulatus	Malacostraca	Arthropoda	0,9746	10148	Discard
Galathea intermedia	Malacostraca	Arthropoda	0,9968	1949	Discard
Galathea spinosorostris	Malacostraca	Arthropoda	0,9900	149	Discard
Hippolytidae	Malacostraca	Arthropoda	0,8491	15171	Discard
Eurynome spinosa	Malacostraca	Arthropoda	1	273	Discard
Palaemonidae	Malacostraca	Arthropoda	0,8391	181	Discard
Plesionika heterocarpus	Malacostraca	Arthropoda	0,9968	499	Discard
Plesionika narval	Malacostraca	Arthropoda	0,9968	1251	Discard
Pasiphaea tarda	Malacostraca	Arthropoda	1	20	Discard
Parapenaeus longirostris	Malacostraca	Arthropoda	0,9968	15	Discard
Pilumnus hirtellus	Malacostraca	Arthropoda	1	703	Discard
Pilumnus villosissimus	Malacostraca	Arthropoda	0,9968	1733	Discard
Euphausia gibboides	Malacostraca	Arthropoda	0,9904	2681	Natural
Euphausia krohni	Malacostraca	Arthropoda	1	2898	Natural
Meganyctiphanes norvegica	Malacostraca	Arthropoda	0,9904	1893	Natural
Cilicaea sp. 72	Malacostraca	Arthropoda	1	1436	Discard
Isopoda	Malacostraca	Arthropoda	0,9744	397	Discard
Isopoda	Malacostraca	Arthropoda	0,8885	294	Discard
Isopoda	Malacostraca	Arthropoda	0,8376	12	Discard
Mysida	Malacostraca	Arthropoda	1	4543	Natural
Acartia tonsa	Maxillopoda	Arthropoda	0,9936	2482	Natural
Acartia tonsa	Maxillopoda	Arthropoda	1	197	Natural
Paracartia grani	Maxillopoda	Arthropoda	1	1216	Natural
Gaetanus tenuispinus	Maxillopoda	Arthropoda	0,9968	754	Natural
Calanus hyperboreus	Maxillopoda	Arthropoda	1	2930	Natural
Nannocalanus minor	Maxillopoda	Arthropoda	0,9968	792	Natural
Microcalanus pusillus	Maxillopoda	Arthropoda	1	277	Natural
Paraeuchaeta norvegica	Maxillopoda	Arthropoda	1	464	Natural
Metridia longa	Maxillopoda	Arthropoda	0,9904	113	Natural
Temora stylifera	Maxillopoda	Arthropoda	0,9776	2080	Natural
Calanoida	Maxillopoda	Arthropoda	0,7944	9688	Natural
Calanoida	Maxillopoda	Arthropoda	0,8513	5081	Natural
Calanoida	Maxillopoda	Arthropoda	0,9872	44	Natural
Calanoida	Maxillopoda	Arthropoda	0,8522	398	Natural
Calanoida	Maxillopoda	Arthropoda	0,8196	92	Natural
Oithona similis	Maxillopoda	Arthropoda	1	707	Natural
Oithona similis	Maxillopoda	Arthropoda	1	1031	Natural
Oithona similis	Maxillopoda	Arthropoda	0,8694	799	Natural
Schizopera	Maxillopoda	Arthropoda	0,8031	20883	Natural
Schizopera	Maxillopoda	Arthropoda	0,8173	1777	Natural
Harpacticoida	Maxillopoda	Arthropoda	0,8117	9132	Natural
Harpacticoida	Maxillopoda	Arthropoda	0,8287	619	Natural
Harpacticoida	Maxillopoda	Arthropoda	0,8281	3646	Natural
Harpacticoida	Maxillopoda	Arthropoda	0,8365	848	Natural

Harpacticoida	Maxillopoda	Arthropoda	0,7771	425	Natural
Harpacticoida	Maxillopoda	Arthropoda	0,8302	218	Natural
Triconia borealis	Maxillopoda	Arthropoda	0,9936	140	Natural
Poecilostomatoida	Maxillopoda	Arthropoda	0,8411	4365	Natural
Balanus trigonus	Maxillopoda	Arthropoda	1	33253	Discard
Verruca stroemia	Maxillopoda	Arthropoda	0,9432	150	Discard
Sessilia	Maxillopoda	Arthropoda	0,9361	1040	Discard
Sessilia	Maxillopoda	Arthropoda	0,9968	326	Discard
Hexanauplia	Maxillopoda	Arthropoda	0,7914	9039	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,8307	2684	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,7950	3378	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,7723	3435	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,8307	578	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,7931	3270	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,7890	169	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,7975	899	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,8019	181	Unknown
Hexanauplia	Maxillopoda	Arthropoda	0,7754	32	Unknown
Neocopepoda	Maxillopoda	Arthropoda	0,8313	1394	Natural
Neocopepoda	Maxillopoda	Arthropoda	0,8162	159	Natural
Neocopepoda	Maxillopoda	Arthropoda	0,7883	124	Natural
Podoplea	Maxillopoda	Arthropoda	0,8449	5973	Natural
		Arthropoda	0,8469	607	Unknown
		Arthropoda	0,8625	6205	Unknown
		Arthropoda	0,7702	1867	Unknown
		Arthropoda	0,7737	5794	Unknown
		Arthropoda	0,8762	2619	Unknown
		Arthropoda	0,8433	1416	Unknown
		Arthropoda	0,8774	13	Unknown
		Arthropoda	0,8080	161	Unknown
		Arthropoda	0,8401	50	Unknown
		Arthropoda	0,8500	127	Unknown
		Arthropoda	0,8469	590	Unknown
		Arthropoda	0,8176	173	Unknown
		Arthropoda	0,9016	52	Unknown
Crustacea		Arthropoda	0,8297	8662	Unknown
Crustacea		Arthropoda	0,8193	1281	Unknown
Pancrustacea		Arthropoda	0,7799	388	Unknown
Pancrustacea		Arthropoda	0,8000	29	Unknown
Pancrustacea		Arthropoda	0,7857	180	Unknown
Pancrustacea		Arthropoda	0,7913	455	Unknown
Pancrustacea		Arthropoda	0,7919	38	Unknown
Chorizopora brongniartii	Gymnolaemata	Bryozoa	0,8594	374	Discard
Hippomenella vellicata	Gymnolaemata	Bryozoa	0,8173	12009	Discard

Celleporella angusta	Gymnolaemata	Bryozoa	0,9936	146	Discard
Watersipora subtorquata	Gymnolaemata	Bryozoa	0,9872	406	Discard
Cheilostomatida	Gymnolaemata	Bryozoa	0,8100	145	Discard
Vesiculariidae	Gymnolaemata	Bryozoa	0,9904	1093	Discard
Cyclostomatida	Stenolaemata	Bryozoa	0,8270	7317	Discard
Cyclostomatida	Stenolaemata	Bryozoa	0,7778	762	Discard
Sagitta elegans	Sagittoidea	Chaetognatha	0,9194	77	Natural
Spadella cephaloptera	Sagittoidea	Chaetognatha	0,9840	164	Discard
Anguilla anguilla	Actinopterygii	Chordata	0,9936	19388	Natural
Atherina boyeri	Actinopterygii	Chordata	0,9968	438	Natural
Atherina hepsetus	Actinopterygii	Chordata	0,9967	1629	Natural
Synodus saurus	Actinopterygii	Chordata	1	403	Discard
Exocoetus obtusirostris	Actinopterygii	Chordata	1	89184	Natural
Exocoetus volitans	Actinopterygii	Chordata	1	75	Natural
Exocoetus	Actinopterygii	Chordata	0,9159	1191	Natural
Scomberesox	Actinopterygii	Chordata	0,9150	5655	Natural
Parablennius tentacularis	Actinopterygii	Chordata	0,9968	151	Discard
Clinitrachus argentatus	Actinopterygii	Chordata	0,9709	5806	Discard
Coryphaena equiselis	Actinopterygii	Chordata	1	14234	Discard
Gadiculus argenteus	Actinopterygii	Chordata	1	6232	Discard
Gaidropsarus ensis	Actinopterygii	Chordata	0,9650	361	Discard
Coelorinchus caelorhincus	Actinopterygii	Chordata	1	11640	Discard
Gobius incognitus	Actinopterygii	Chordata	1	9035	Discard
Zebrus zebrus	Actinopterygii	Chordata	0,9741	6	Discard
Apogon imberbis	Actinopterygii	Chordata	0,9968	841	Discard
Pteragogus trispilus	Actinopterygii	Chordata	0,9794	551	Discard
Thalassoma pavo	Actinopterygii	Chordata	0,9904	180	Discard
Desmodema polystictum	Actinopterygii	Chordata	1	20498	Discard
Melanocetus johnsonii	Actinopterygii	Chordata	0,9840	928	Discard
Chelon labrosus	Actinopterygii	Chordata	1	5959	Both
Diaphus dumerilii	Actinopterygii	Chordata	1	3197	Both
Diaphus dumerilii	Actinopterygii	Chordata	0,9903	3784	Both
Electrona risso	Actinopterygii	Chordata	1	3835	Discard
Hygophum benoiti	Actinopterygii	Chordata	1	33353	Discard
Hygophum macrochir	Actinopterygii	Chordata	1	292	Discard
Notoscopelus bolini	Actinopterygii	Chordata	0,9904	3454	Discard
Brotula multibarbata	Actinopterygii	Chordata	0,9935	24169	Discard
Epigonus constanciae	Actinopterygii	Chordata	0,9903	14	Discard
Serranus sanctaehelenae	Actinopterygii	Chordata	1	13	Discard
Citharus linguatula	Actinopterygii	Chordata	1	2285	Discard
Chiasmodon niger	Actinopterygii	Chordata	1	2845	Discard
Cubiceps pauciradiatus	Actinopterygii	Chordata	0,9902	5623	Discard
Nomeus gronovii	Actinopterygii	Chordata	0,9868	5639	Discard
Lepidopus caudatus	Actinopterygii	Chordata	0,9712	6976	Discard

Spicara smaris	Actinopterygii	Chordata	0,9968	18354	Both
Diplodus sargus	Actinopterygii	Chordata	0,9936	886	Both
Oblada melanura	Actinopterygii	Chordata	1	36841	Both
Oblada melanura	Actinopterygii	Chordata	0,9904	1711	Both
Gonostoma denudatum	Actinopterygii	Chordata	0,9904	3562	Discard
Cyclothone pseudopallida	Actinopterygii	Chordata	1	39	Discard
Ichthyococcus ovatus	Actinopterygii	Chordata	0,9968	5917	Discard
Vinciguerria nimbaria	Actinopterygii	Chordata	1	12611	Discard
Argyropelecus affinis	Actinopterygii	Chordata	0,9904	6645	Discard
Argyropelecus affinis	Actinopterygii	Chordata	0,9022	817	Discard
Argyropelecus olfersii	Actinopterygii	Chordata	0,9967	11971	Discard
Argyropelecus	Actinopterygii	Chordata	0,9183	1119	Discard
Polyipnus clarus	Actinopterygii	Chordata	0,9904	9897	Discard
Polyipnus	Actinopterygii	Chordata	0,9331	313	Discard
Sternoptyx diaphana	Actinopterygii	Chordata	1	11661	Discard
Sternoptyx	Actinopterygii	Chordata	0,9007	1114	Discard
Astronesthes richardsoni	Actinopterygii	Chordata	0,9903	14	Discard
Bathophilus pawneei	Actinopterygii	Chordata	0,9936	7462	Discard
Macroramphosus scolopax	Actinopterygii	Chordata	1	1699	Discard
Mullus barbatus	Actinopterygii	Chordata	0,9936	742	Discard
Lagocephalus sceleratus	Actinopterygii	Chordata	1	9930	Discard
Lagocephalus suezensis	Actinopterygii	Chordata	0,9713	1347	Discard
Diplosoma listerianum	Ascidiacea	Chordata	1	1951	Discard
Morchellium argus	Ascidiacea	Chordata	1	704	Discard
Aplousobranchia	Ascidiacea	Chordata	0,7905	30	Discard
Botryllus schlosseri	Ascidiacea	Chordata	0,9936	588	Natural
Styela plicata	Ascidiacea	Chordata	0,9968	138	Natural
Stolidobranchia	Ascidiacea	Chordata	0,8255	828	Unknown
Branchiostoma lanceolatum	Cephalochordata	Chordata	0,9935	10793	Discard
Prionace glauca	Chondrichthyes	Chordata	1	2139	Discard
Sagartiogeton laceratus	Anthozoa	Cnidaria	0,9936	474	Discard
Cornularia cornucopiae	Anthozoa	Cnidaria	0,9968	376	Discard
Paralemnalia sp. A CSM-2013	Anthozoa	Cnidaria	1	135	Discard
Parasphaerasclera	Anthozoa	Cnidaria	0,9808	1674	Discard
Alcyonacea	Anthozoa	Cnidaria	0,9617	10519	Discard
Alcyonacea	Anthozoa	Cnidaria	0,8056	176	Discard
Corynactis californica	Anthozoa	Cnidaria	0,9650	2906	Discard
Anthozoa	Anthozoa	Cnidaria	0,9522	2552	Discard
Carybdea xaymacana	Cubozoa	Cnidaria	0,8094	35	Natural
Clava multicornis	Hydrozoa	Cnidaria	0,8766	2168	Discard
Campanularia hincksii	Hydrozoa	Cnidaria	0,9936	10871	Discard
Clytia hemisphaerica	Hydrozoa	Cnidaria	0,9968	64	Discard
Plumularia setacea	Hydrozoa	Cnidaria	0,8694	1845	Discard

Leptothecata	Hydrozoa	Cnidaria	0,8921	1113	Discard
Leptothecata	Hydrozoa	Cnidaria	0,8892	1430	Discard
Leptothecata	Hydrozoa	Cnidaria	0,8794	965	Discard
Leptothecata	Hydrozoa	Cnidaria	0,8917	1386	Discard
Leptothecata	Hydrozoa	Cnidaria	0,9712	823	Discard
Leptothecata	Hydrozoa	Cnidaria	0,9299	187	Discard
Leptothecata	Hydrozoa	Cnidaria	0,8829	54	Discard
Hydroidolina	Hydrozoa	Cnidaria	0,8738	5782	Discard
Hydroidolina	Hydrozoa	Cnidaria	0,8406	3400	Discard
Hydroidolina	Hydrozoa	Cnidaria	0,8766	1253	Discard
Hydroidolina	Hydrozoa	Cnidaria	0,8822	154	Discard
Hydroidolina	Hydrozoa	Cnidaria	0,8576	51	Discard
	Hydrozoa	Cnidaria	0,8375	5830	Discard
	Hydrozoa	Cnidaria	0,7562	408	Discard
Mnemiopsis leidyi	Tentaculata	Ctenophora	0,8135	363	Natural
Anseropoda placenta	Asteroidea	Echinodermata	0,9904	1247	Discard
Arbacia lixula	Echinoidea	Echinodermata	0,9968	1063	Discard
Gracilechinus acutus	Echinoidea	Echinodermata	0,9936	27821	Discard
Gracilechinus multidentatus	Echinoidea	Echinodermata	0,9744	283	Discard
Paracentrotus lividus	Echinoidea	Echinodermata	1	50447	Discard
Psammechinus miliaris	Echinoidea	Echinodermata	1	751	Discard
Dendrochirotida	Holothuroidea	Echinodermata	0,8333	352	Discard
Molpadia musculus	Holothuroidea	Echinodermata	0,9143	714	Discard
Ophiactis savignyi	Ophiuroidea	Echinodermata	0,9105	4379	Discard
Ophiocomina nigra	Ophiuroidea	Echinodermata	0,9936	54696	Discard
Ophiopholis aculeata	Ophiuroidea	Echinodermata	0,9159	19	Discard
Ciliatocardium ciliatum	Bivalvia	Mollusca	0,9968	312	Discard
Macoma calcarea	Bivalvia	Mollusca	0,9936	195	Discard
Mytilus edulis/galloprovincialis	Bivalvia	Mollusca	0,9904	13184	Discard
Mytilus edulis/galloprovincialis	Bivalvia	Mollusca	0,9968	305	Discard
Mytilidae	Bivalvia	Mollusca	0,8646	2212	Discard
Anguipecten	Bivalvia	Mollusca	0,8371	2795	Discard
Veneridae	Bivalvia	Mollusca	0,8339	261	Discard
Loligo vulgaris	Cephalopoda	Mollusca	0,9968	210	Discard
Gonatus steenstrupi	Cephalopoda	Mollusca	1	17206	Discard
Histioteuthis meleagroteuthis	Cephalopoda	Mollusca	1	1003	Discard
Stigmatoteuthis hoylei	Cephalopoda	Mollusca	0,9808	258	Discard
Onykia carriboea	Cephalopoda	Mollusca	1	16830	Discard
Eulimidae	Gastropoda	Mollusca	0,8119	4749	Discard
Littorina littorea	Gastropoda	Mollusca	1	1670	Natural
Pusillina sarsii	Gastropoda	Mollusca	0,9967	2983	Discard
Rissoa parva	Gastropoda	Mollusca	0,9777	4573	Discard

Tonna	Gastropoda	Mollusca	0,9457	70	Discard
Tritia incrassata	Gastropoda	Mollusca	0,9553	66	Discard
Tritia incrassata	Gastropoda	Mollusca	0,9840	401	Discard
Tritia incrassata	Gastropoda	Mollusca	1	197	Discard
Tritia reticulata	Gastropoda	Mollusca	1	2771	Discard
Aegires punctilucens	Gastropoda	Mollusca	0,9936	669	Discard
Doto koenneckeri	Gastropoda	Mollusca	0,9521	5370	Discard
Doto koenneckeri	Gastropoda	Mollusca	0,9457	45	Discard
Knoutsodonta sp. B GF-2017	Gastropoda	Mollusca	0,9868	2687	Discard
Polycerella emertoni	Gastropoda	Mollusca	0,9935	103	Discard
Patella rustica	Gastropoda	Mollusca	0,9968	20052	Natural
Patella vulgata	Gastropoda	Mollusca	1	20	Natural
Creseis virgula conica	Gastropoda	Mollusca	0,9968	2688	Natural
Heliconoides inflatus	Gastropoda	Mollusca	0,9872	48	Natural
Elysia gordanae	Gastropoda	Mollusca	0,9771	292	Discard
Calliostoma virescens	Gastropoda	Mollusca	0,9521	4455	Discard
Euthyneura	Gastropoda	Mollusca	0,8302	22	Discard
Acanthochitona discrepans	Polyplacophora	Mollusca	1	143	Discard
Parvicirrus dubius	Anopla	Nemertea	0,8801	470	Discard
		Nemertea	0,9076	597	Discard