

Metabarcoding of Mediterranean monk seal (*Monachus monachus*) scats from the isolated population of Madeira reveals a diverse piscivorous diet

Subramaniam Vijayakumar¹, Ashlie J. McIvor^{2,3}, Gonçalo Espregueira Themudo¹, Rosa Pires^{4*}, Philip Francis Thomsen⁵, Ana Dinis^{2,6}, João Canning-Clode^{2,6,7}, João Carneiro^{1**}, Paula F. Campos¹

1. CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal
2. MARE – Marine and Environmental Sciences Centre / ARNET – Aquatic Research Network, Regional Agency for the Development of Research, Technology and Innovation (ARDITI), Funchal, Madeira Island, Portugal
3. Department of Biology, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal
4. Instituto das Florestas e Conservação da Natureza, IP-RAM, Funchal, Portugal
5. Department of Biology, Aarhus University, Aarhus, Denmark
6. Faculty of Life Sciences, University of Madeira, Funchal, Portugal
7. Smithsonian Environmental Research Centre, Edgewater, MD, USA

*Current affiliation: Serviço de Ambiente e Ação Climática de Santa Maria, Açores, Portugal

** Current affiliation: Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal.

Corresponding author: Paula F. Campos, email: pcampos@ciimar.up.pt/
campos.f.paula@gmail.com

Abstract

The Mediterranean monk seal (*Monachus monachus*) is one of the world's most endangered marine mammals, and information on its feeding ecology is needed to support conservation planning. Here, high-throughput DNA metabarcoding of scat samples from Madeira was used to characterise the diet of this geographically isolated population. Fourteen samples were analysed using universal fish primers targeting the mitochondrial 12S rRNA gene, enabling detection of both bony and cartilaginous fishes. Across samples, 38 prey taxa were identified, including records not previously reported in monk seal diet studies, such as *Fistularia petimba*, as well as cartilaginous fish such as *Bathytoshia centroura*, *Dasyatis pastinaca*, and *Torpedo marmorata*. Frequency of occurrence indicated a diet dominated by demersal and reef-associated taxa, with frequent detection of Sparidae, alongside evidence of opportunistic prey use. Overall, these results provide a high-resolution dietary profile for monk seals in Madeira and demonstrate the utility of scat metabarcoding for monitoring trophic ecology in small, threatened populations. This approach offers a scalable framework for assessing dietary shifts over time and informing adaptive management in human-impacted coastal ecosystems.

Keywords: Trophic Ecology, DNA Metabarcoding, Piscivory, Conservation Genomics, Pinniped

1. Introduction

The Mediterranean monk seal (*Monachus monachus*) is the world's most threatened pinniped and has experienced severe historical declines driven by persecution, habitat loss, disturbance, and fisheries interaction (e.g., Karamanlidis *et al.*, 2016a). Until the beginning of the 20th century its distribution covered a vast range including the Mediterranean, Marmara and Black Seas as well as the Atlantic coast of Africa, from Morocco to Gambia and the Macaronesian Islands. At present breeding is restricted to a small number of geographically isolated subpopulations in the eastern Mediterranean and the central-eastern Atlantic. Recent assessments indicate encouraging signs of recovery in some areas, but the global population remains small and spatially fragmented, sustaining continued conservation concern.

Historically, the Mediterranean monk seal has undergone a drastic loss of genetic diversity resulting in four genetically distinct populations: Cabo Blanco, Madeira, the eastern Mediterranean, and a largely extinct western Mediterranean group (Rey-Iglesia *et al.*, 2020; Salmons *et al.*, 2022). Currently, only two rare mitochondrial haplotypes remain, which represent only ca. 25% of the species' historical mitochondrial variation (Karamanlidis *et al.*, 2016b; Rey-Iglesia *et al.*, 2020). Genetic evidence also indicates past gene flow between North Atlantic and Mediterranean seals, suggesting that these populations once formed a continuous group in the western Mediterranean (Rey-Iglesia *et al.*, 2020). Compared to other pinniped species, the Mediterranean monk seal ranks among the lowest in genetic diversity, a condition likely caused by a severe population bottleneck that reduced its effective population size to fewer than 50 individuals. This bottleneck has significantly increased the risks of inbreeding and related genetic problems (Parsons, 2024).

The Madeiran population of Mediterranean monk seals exhibits an exceptionally low level of genetic diversity, reflecting the population's long history of isolation and decline. Specifically, all sampled individuals share the exact same mitochondrial genome, with no variation detected over the past 200 years (Rey-Iglesia *et al.*, 2020). This lack of mitochondrial diversity is matched by very low nuclear genetic diversity as well, Madeira's seals show heterozygosity levels around $H_e \approx 0.04$, which is among the lowest ever recorded in seals (Salmons *et al.*, 2022). These findings suggest a strong genetic bottleneck, likely driven by a combination of small population size, prolonged isolation, and inbreeding.

Compared to other monk seal populations in the eastern Mediterranean, such as those in the Ionian and Aegean Seas, which still retain some genetic structure and variation, the Madeiran population stands out as particularly vulnerable (McIvor *et al.*, 2023; Parsons, 2024).

Although there has been a modest increase in numbers in recent years, thanks largely to local conservation efforts, the genetic risks remain high (Pires *et al.*, 2023). Initiatives like the LIFE Madeira Monk Seal project have been central to this recovery, implementing measures such as habitat protection, restricting human access to sensitive areas, and using non-invasive tools like genetic monitoring to track individual seals and assess population trends (Pires, Aparicio, & Fernandez de Larrinoa, 2020).

Understanding the diet of the Mediterranean monk seal is crucial for conservation efforts aimed at protecting this critically endangered species. By studying their diet, we can gain insights into the species habitat use, prey preferences, overall ecological role within marine ecosystems and also possible human-seal interactions. Most information available so far has been based on the study of stomach contents from stranded animals and anecdotal sightings (e.g., Salman, Bilecenoglu, & Güçlüsoy, 2001; Pierce *et al.*, 2011; Pires, Aparicio, & Fernandez de Larrinoa, 2019; Moreira Santos *et al.*, 2026). More recently, complementary approaches using Baited Remote Underwater Video systems (BRUVs) have begun to provide promising, non-invasive insights into monk seal foraging behaviour and prey associations, helping to overcome some of the limitations of traditional methods (Ashlie J. McIvor, 2024).

According to previous studies, the Mediterranean monk seal exhibits a diverse and regionally variable diet, largely shaped by the availability of local prey. For instance, studies on seals along the Greek coast between 1997 and 2008 documented a wide prey spectrum, identifying 530 prey items across 71 specimens. Cephalopods, particularly the common octopus (*Octopus vulgaris*) and horned octopus (*Eledone cirrhosa*), constitute nearly half of their diet. Fish species, especially coastal and reef-associated sea breams, also form a significant portion, while crustaceans and other molluscs contribute smaller amounts (Salman *et al.*, 2001; Pierce *et al.*, 2011). However, stomach content analysis has important limitations. It relies on opportunistic sampling and only reflects recently ingested, often partially digested prey, which can bias species identification. Additionally, results may be influenced by the cause of death and provide only a narrow snapshot in space and time, limiting broader ecological inference. Complementing these findings, stable isotope analysis has revealed that approximately 60% of the monk seal's diet originates from coastal and seabed habitats, emphasizing their strong dependence on near-shore environments (Karamanlidis *et al.*, 2014). As opportunistic feeders, monk seals occasionally prey on larger animals such as green sea turtles (*Chelonia mydas*), demonstrating adaptive feeding strategies (Tonay *et al.*, 2016). This dietary flexibility is crucial for their survival, especially given current habitat fragmentation and increasing human pressures, such as fishing, especially in the Mediterranean (Pierce *et al.*, 2011; Karamanlidis

et al., 2014). While stable isotope analysis provides valuable integrative insights into trophic levels and habitat use over longer timescales, confirming reliance on benthic and neritic prey (Karamanlidis *et al.*, 2014) it lacks the taxonomic resolution necessary to identify specific prey species. This limitation highlights the need for complementary methods to achieve detailed dietary profiling.

High-throughput DNA metabarcoding has become a widely used approach for reconstructing predator diets from scat material by amplifying short genetic markers from degraded prey DNA and assigning sequences to reference databases (Deagle *et al.*, 2019). This technique has been successfully applied to other pinnipeds, such as the Australian sea lion (*Neophoca cinerea*), where it revealed diverse prey items, including soft-bodied taxa and unexpected species, while also uncovering spatial variation in dietary habits (Berry *et al.*, 2017). Our approach leverages universal genetic markers targeting fish and high-throughput sequencing technologies to detect degraded or trace amounts of DNA, offering a significant advantage over morphology-based techniques (Deagle *et al.*, 2019).

In this study, DNA metabarcoding was applied to Mediterranean monk seal scats collected from Madeira and Desertas Islands to characterise the fish component of diet in this isolated subpopulation. The resulting dataset provides an updated, high-resolution baseline for trophic ecology in the Madeira monk seal subpopulation and demonstrates the utility of metabarcoding for non-invasive dietary assessment in a threatened pinniped.

2. Methods

2.1 Sample collection

Between December 2014 and July 2021, 16 Mediterranean monk seal (*Monachus monachus*) scat samples were collected during systematic monitoring surveys on Madeira and the Desertas Islands as part of the Madeira monk seal conservation program. Samples were collected by IFCN-IP Madeira staff and contributing authors, from coastal caves and haul-out beaches used for resting and breeding. Scats were collected only when seals were absent to minimise potential disturbance. Samples were preserved in ethanol until laboratory processing. Collection sites included Cabo de S. Lourenço and Gruta da Atalaia (Madeira), and Tabaqueiro, Vermelhas, Gruta dos Lobos, and Lanço do Rico (Desertas) (Figure 1; Table 1). From these only 14 were available for genetic analyses.

2.2 DNA extraction and contamination control

DNA extractions were conducted at CIIMAR/UP in a molecular laboratory in which fish genomics work had not previously been performed. Scat material was dried at 37°C, to eliminate traces of ethanol, and subsampled for molecular analyses. To reduce host DNA and external contamination, only the inner portion of each scat was processed. DNA was extracted using the QIAamp Fast DNA stool kit (Qiagen, Germany) with the following modifications: in step 2 instead of 1min vortexing we have done two cycles of 1min at 4000rpm in a bead beater homogenizer (Precellys, Bertin Instruments, France) to ensure better sample homogenization, with samples placed on ice between cycles; samples were eluted in EB buffer and incubated in a thermomixer at 37°C for 10min to increase DNA yields. For most specimens at least two different replicates were made to increase sample representation and extraction blanks were included in every batch.

2.3 PCR amplification, indexing, and sequencing

Polymerase chain reactions (PCR) amplifications were performed in a dedicated UV-irradiated PCR cabinet using the MiFish primer sets (Miya *et al.*, 2015; Taberlet *et al.*, 2018). To simultaneously amplify teleost and elasmobranch prey DNA, the MiFish-U and MiFish-E primer pairs were combined within each PCR reaction: MiFish-U-F (5'-GTCGGTAAACTCGTGCCAGC-3') and MiFish-U-R (5'-CATAGTGGGGTATCTAATCCCAGTTTG-3'), and MiFish-E-F (5'-GTTGGTAAATCTCGTGCCAGC-3') and MiFish-E-R (5'-CATAGTGGGGTATCTAATCCTAGTTTG-3'). These primers target a short fragment of the

mitochondrial 12S rRNA gene, amplifying a region of circa 170bp for bony fish and circa 180 bp for elasmobranchs, respectively. Each primer was tagged with a unique 6-nucleotide sample-specific sequence generated in OligoTag (Coissac, 2012) and preceded by two or three random nucleotides at the 5' end to increase sequence complexity during sequencing (De Barba *et al.*, 2014; Sigsgaard *et al.*, 2020). Several negative controls were included in each PCR set-up. PCR amplifications were carried out in 25 µl reactions containing 12,5 µl Supreme NZYTaQ II 2× Green Master Mix (NZYTech, Lisbon, Portugal), 0,5 µl of each primer (10µM; four primers total), 9,5 µL molecular-grade water, and 1 µl DNA extract. Thermal cycling conditions were 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min followed by 72 °C for 7 min. Amplicons were visualized on 1.5% agarose gels under UV light after electrophoresis.

Each sample was amplified in quadruplicate to reduce stochastic amplification effects and PCR bias. For each sample, 5µL from each replicate PCR was pooled (20µL total) and purified using the PureLink PCR purification kit (Invitrogen, Carlsbad, USA). Purified amplicons were pooled equimolarly into three tag-unique pools (Supplementary table 1) and submitted to Novogene for double strand DNA library preparation and 150bp PE sequencing on a partial lane of an Illumina NovaSeq 6000 platform.

2.4 Bioinformatics processing and OTU generation

Raw pair-end reads were demultiplexed by sample-specific 6-nt tags (allowing a 15% error rate) and processed using Cutadapt (Martin, 2011). Reads lacking valid tag sequences were discarded, and tag sequences were removed prior to downstream processing. Primer sequences were trimmed from the 5' ends of reads, allowing a 10% error rate to accommodate sequencing error and primer variation. Paired-end reads were merged using VSEARCH(Rognes *et al.*, 2016), requiring a lenient minimum overlap of 10 bp to maximize data retention for error correction, and allowing up to five mismatches; staggered alignments were permitted. For each biological sample, merged reads from PCR replicates were pooled prior to OTU inference. To eliminate any short, spurious artifacts generated by the lenient merging overlap, these pooled reads were strictly filtered to retain near full-length amplicons (≥ 150 bp) and to remove reads with high expected error (maxee = 2.0). Filtered reads were dereplicated (retaining singletons), screened for chimeras using UCHIME de novo (Edgar *et al.*, 2011), and clustered into OTUs at 97% sequence similarity. OTU centroid sequences were retained for downstream taxonomic assignment. Custom scripts used to automate demultiplexing and OTU generation are available

on GitHub (repository: <https://github.com/SUBRAMANIAM96/MonkSeal-Diet-Metabarcoding>, version v1.0.0, accessed March 2026). Full command-line calls and pipeline documentation are provided in the Supplementary Material.

2.5 Taxonomic assignment and curation

Taxonomic assignment of OTU sequences was performed by BLAST searches against the comprehensive NCBI nucleotide database using high-performance computing resources at CIIMAR. Matches were retained when sequence identity was $\geq 98\%$ and e-value $\leq 1e-5$, and only alignments meeting a minimum query coverage threshold of 100% were considered for taxonomic annotation.

BLAST outputs generated using BLAST were parsed using a custom R script (filter_best_hits.R) to remove duplicate accession IDs, retaining only the entry with the highest percentage identity for each accession ID along with its corresponding OTU number, and taxonomy was retrieved from accession identifiers using a custom Python script (fetch_taxonomy.py). Assigned taxa were then classified as either (i) recorded from the Madeira Archipelago or (ii) not previously recorded from the Madeira Archipelago based on the annotated checklist of Madeira's marine fauna (Biscoito, Ribeiro, & Freitas, 2018), the Madeira Marine Biodiversity Database, maintained by MARE-Madeira research institute, and FishBase (Froese & Pauly, 2025). Family-level classifications were cross-checked using Eschmeyer's Catalog of Fishes (Fricke, Eschmeyer, & van der Laan, 2026) supplemented with regional checklists (Wirtz, Fricke, & Biscoito, 2008). Summary tables of taxonomic assignments across all retained samples are provided in the Supplementary Material. Custom scripts used for BLAST parsing and taxonomy retrieval are publicly available on GitHub (repository: <https://github.com/SUBRAMANIAM96/MonkSeal-Diet-Metabarcoding>, version v1.0.0, accessed March 2026).

2.6 Dietary metrics

Frequency of occurrence (FO) was calculated for each prey taxon as the proportion of retained scat samples ($n = 14$) in which the taxon was detected. FO provides a presence-based summary of dietary composition that is commonly used in pinniped dietary studies and metabarcoding applications (Imorou *et al.*, 2019). FO was calculated separately for taxa recorded from the Madeira Archipelago and taxa not previously recorded locally (based on regional occurrence sources). Species with higher FO values are interpreted as more consistently consumed prey, while those with lower values represent occasional or incidental dietary components.

$$FO = \left(\frac{\text{Number of samples containing the taxon}}{\text{Total number of samples (14)}} \right) * 100$$

3. Results

A total of 8,468,273 raw sequencing reads were obtained across the 14 scat samples, which generated **7,628** Operational taxonomic units (OTUs) before taxonomic filtering. OTU richness per retained sample ranged from **179** to 960 (Table 2), with DES_07 showing the highest richness and DES_02 the lowest.

Taxonomic assignment of fish-associated sequence features initially yielded **7628** OTUs. Of these, **193** OTUs were successfully assigned to fish at $\geq 98\%$ identity, while 7435 OTUs remained unassigned or were assigned with low confidence. The high-confidence assignments result into 51 unique fish taxa as potential prey items. Of these, 38 taxa correspond to species previously documented from the Madeira Archipelago (Table 3). Frequently detected taxa include European conger (*Conger conger*), rough tail stingray (*Bathytoshia centroura*), Mediterranean moray (*Muraena helena*), axillary seabream (*Pagellus acarne*), and red porgy (*Pagrus pagrus*). Elasmobranch DNA was detected in multiple samples. Specifically, rough tail stingray (*Bathytoshia centroura*) was detected in 13 out of 14 samples, tortoise stingray (*Dasyatis tortonesei*) in 6 out of 14 samples, marbled electric ray (*Torpedo marmorata*) in 6 out of 14 samples, and common stingray (*Dasyatis pastinaca*) in 2 out of 14 samples (Table 3).

The 38 regionally documented taxa spanned 24 families (Figure 2). Sparidae was the most species-rich family, represented by seven species such as axillary seabream (*Pagellus acarne*), red porgy (*Pagrus pagrus*), pink dentex (*Dentex gibbosus*), white seabream (*Diplodus sargus*), and gilt-head seabream (*Sparus aurata*). Labridae was also represented (e.g., Mediterranean rainbow wrasse, *Coris julis*; and ornate wrasse, *Thalassoma pavo*), along with Congridae (bandtooth conger, *Ariosoma balearicum*; and European conger, *Conger conger*) and Muraenidae (Mediterranean moray, *Muraena helena*; black moray, *Muraena augusti*; and fangtooth moray, *Enchelycore anatina*).

In addition to taxa recorded regionally, 13 taxa matched species for which published records are not currently available for the Madeira or Desertas Islands (e.g., yellowtail amberjack, *Seriola lalandi*, detected in 11 out of 14 samples). While some of these species are known from other parts of the eastern Atlantic or nearby regions, there are currently no published records confirming their presence in Madeira or Desertas waters (Table 3). Their presence in the dataset is attributed to bioinformatic misassignments, where the lack of reference DNA for local endemic species forces the algorithm to match with closely related foreign taxa. They were retained in the dataset pending independent verification.

DNA from other taxa that did not represent possible dietary items, including the studied species (monk seal) and the Madeiran wall lizard (*Teira dugesii*), was also detected in the scat samples. These findings underscore the taxonomic diversity targeted by the MiFish primers, which amplify not only fish DNA but also DNA from other vertebrates present in the environment. Notably, the Madeiran wall lizard (*Teira dugesii*) was detected in 3 out of 14 samples, and monk seal DNA was detected in all samples. To ensure dietary composition focused solely on fish prey, these non-target OTUs were excluded from downstream analyses.

Among the taxa recorded from Madeira, the European conger (*Conger conger*) and rough tail stingray (*Bathytoshia centroura*) were the most frequently detected, appearing in 13 out of 14 samples (FO = 92.9%), followed closely by the Mediterranean moray (*Muraena helena*, 85.7%). Other highly frequent taxa included the axillary seabream (*Pagellus acarne*) and red porgy (*Pagrus pagrus*) at 78.6%. Mid-frequency taxa (detected in 35% to 65% of samples) included the grey triggerfish (*Balistes caprisus*, 64.3%), European hake (*Merluccius merluccius*, 64.3%), European seabass (*Dicentrarchus labrax*, 42.9%), white seabream (*Diplodus sargus*, 42.9%), European pilchard (*Sardina pilchardus*, 42.9%), and gilt-head seabream (*Sparus aurata*, 35.7%). Lower-frequency taxa ranged from 28.6% down to 7.1%, such as the blue jack mackerel (*Trachurus picturatus*) and chub mackerel (*Scomber colias*), each detected in a single sample (FO = 7.1%).

For taxa lacking published regional records, FO values ranged from 78.6% to 7.1%. Complete FO values are provided in Table 3.

4. Discussion

This study provides the first fish-focused dietary characterisation of the isolated Madeira population of the Mediterranean monk seal using scat DNA metabarcoding. By applying MiFish 12S primers to scat samples collected across Madeira and the Desertas Islands and summarising detections using frequency of occurrence, a diverse prey assemblage was recovered, including 38 taxa recorded from the Madeira Archipelago and additional taxa not previously reported locally. Elasmobranch DNA was detected repeatedly across samples, indicating that cartilaginous fishes form a consistent component of the detectable prey spectrum. Our findings demonstrate the value of non-invasive metabarcoding for generating high-resolution baseline dietary data in a small, disturbance-sensitive pinniped population, while also establishing a foundation for interpreting prey use in relation to local habitats and human activities.

Although this sample size may appear small, it reflects the considerable difficulty of obtaining such material. Moreover, the local population consists of only around 27 (Pires *et al.*, 2023) individuals, and the caves used by the seals are often flooded at high tide, which washes away scats if not collected during low tide (Pires, Neves, & Karamanlidis, 2007). Access to the caves at low tide was not always possible, as the animals were present in the caves. To minimize disturbance to this already extremely vulnerable population, all samples were collected only in the absence of animals.

Most dietary studies on the Mediterranean monk seal (*Monachus monachus*) have focused on populations in the Aegean Sea and eastern Mediterranean, primarily relying on traditional stomach content and stable isotope analyses (Salman *et al.*, 2001; Karamanlidis *et al.*, 2011, 2014; Pierce *et al.*, 2011; Tonay *et al.*, 2016). Our study is the first to apply a DNA metabarcoding approach using MiFish primers to analyse fish species in the diet of the monk seal population from Madeira. This non-invasive technique allowed us to achieve unprecedented taxonomic resolution, detecting prey species often missed or indistinguishable through conventional methods.

The diversity of fish species detected in the Mediterranean monk seal diet around Madeira confirms broad prey detection. The seals consume a wide variety of prey spanning multiple ecological niches, including benthic, demersal, and pelagic species. Many benthic and reef-associated fishes, such as *Ariosoma balearicum*, *Conger conger*, *Muraena helena*, and *Muraena augusti*, inhabit rocky substrates and crevices, with detections dominated by coastal and benthic-associated taxa, consistent with coastal foraging reported for the species (Pires,

2020). Additionally, commercially important demersal species like *Dicentrarchus labrax* (European seabass), *Merluccius merluccius* (European hake), *Mullus surmuletus* (red mullet), and several *Pagellus* species are abundant on continental shelves and soft-bottom habitats, and their presence in the diet indicates the seals' exploitation of these environments. Pelagic and schooling fishes such as *Sardina pilchardus* (European pilchard), *Scomber colias* (Atlantic chub mackerel), and *Trachurus trachurus* (Atlantic horse mackerel) further demonstrate the seals' capacity to forage across different water columns and habitats. Given that Mediterranean monk seals primarily forage in coastal and shelf waters, this mix of prey types underscores their ecological flexibility and ability to exploit a broad range of marine habitats. Such dietary adaptability is crucial for their survival in a dynamic and changing marine environment, especially amid pressures from habitat degradation, fishing, and climate variability (Karamanlidis *et al.*, 2016a). Overall, the seal's diet incorporates both benthic and pelagic prey, emphasizing their role as generalist predators within the Madeira marine ecosystem and highlighting the importance of conserving diverse marine habitats to support their foraging needs.

We observed considerable overlap with prey species previously reported in Mediterranean monk seal diets, including *Ariosoma balearicum*, *Conger conger*, *Dicentrarchus labrax*, *Merluccius merluccius*, *Diplodus sargus*, *Pagellus acarne*, *Bathytoshia centroura*, *Pagellus erythrinus*, *Scorpaena scrofa*, *Muraena helena*, *Pagellus bogaraveo*, *Pagrus pagrus*, *Sparus aurata*, and *Coris julis* (Salman *et al.*, 2001; Cebrian, Fatsea, & Mytilineou, 2011; Karamanlidis *et al.*, 2011). This overlap not only confirms the consistency of monk seal diet across regions but also the accuracy and reliability of our metabarcoding approach in detecting key dietary components. Our study further improved species-level identification within genera previously reported more broadly. For example, while earlier research identified *Dentex* sp ((e.g., Dede, A.; Tonay, M. A.; Öztürk, 2015) we specifically detected *Dentex gibbosus*. Similarly, in the case of *Mullus* spp (Pierce *et al.*, 2011), our metabarcoding approach allowed us to pinpoint *Mullus surmuletus* as a significant prey species, demonstrating the ability of metabarcoding to distinguish between closely related species.

Moreover, by using the MiFish-E primer set targeting elasmobranch fish DNA, we detected cartilaginous fish species such as *Bathytoshia centroura*, *Torpedo marmorata*, *Dasyatis tortonesei*, and *Dasyatis pastinaca*. Elasmobranchs have previously been reported as prey, but they were not identified to a specific taxonomic level, likely because their cartilage-based skeletons are highly susceptible to degradation by stomach acids, which makes them difficult to detect using traditional morphological analysis. This discovery expands the known trophic

niche of the species and highlights the power of molecular techniques to reveal overlooked prey.

Significantly, our study advances the understanding of the Mediterranean monk seal diet by revealing a far more diverse array of prey species than previously documented. Unlike earlier research that reported a limited prey spectrum, something that might be explained by the less sophisticated methodology used, we identified a broad suite of species not previously recorded in monk seal diets, including *Chelon labrosus*, *Fistularia petimba*, *Sardina pilchardus*, *Kyphosus sectatrix*, *Trisopterus luscus*, *Pontinus kuhlii*, *Balistes capriscus*, *Thalassoma pavo*, *Epinephelus marginatus*, *Lepidopus caudatus*, *Tripterygion delaisi*, *Muraena augusti*, *Scomber colias*, *Enchelycore anatina*, *Gephyroberyx darwinii*, and *Helicolenus dactylopterus*. By overcoming the limitations of digestion bias and morphological identification inherent in traditional diet studies, metabarcoding reveals a higher taxonomic resolution and clearer picture of monk seal fish diet than morphology-based methods (Valentini, Pompanon, & Taberlet, 2009; Deagle *et al.*, 2019). This enhanced understanding is crucial for advancing ecological knowledge and informing future research on the species' feeding strategies and habitat use in Madeira.

While not the focus of our current study, Mediterranean monk seals have also been documented to prey on cephalopods, as well as sea turtles. Observations of aggressive and predatory interactions with loggerhead turtles (*Caretta caretta*) have been reported by Margaritoulis, Karavellas, & Irvine (1996) and Margaritoulis & Touliatou (2011), and predation on the green turtle (*Chelonia mydas*) has also been reported (Tonay *et al.*, 2016).

Non-native species: Findings and limitations

A total of 13 taxa lacking published regional records were detected in the Mediterranean monk seal scat samples. This highlights some of the challenges that are faced when identifying prey species from these complex samples. The DNA metabarcoding approach is limited by the incompleteness and inaccuracies of local reference databases. These gaps lead to bioinformatic misassignments, where the algorithm forces a match with a closely related foreign species because the local endemic species is missing from the database (Armani *et al.*, 2021). For example, Pacific and geographically distant species such as *Pontinus macrocephalus* and *Helicolenus avius* were identified in the samples; however, this is highly likely due to their 12S

rRNA genetic similarity with the documented native species *Pontinus kuhlii* and *Helicolenus dactylopterus*, respectively.

Teira dugesii (Madeiran wall lizard), was also frequently detected in the scat samples (3/14). This is likely due to its high abundance and wide distribution across Madeira and Desertas Islands, where it is the most common terrestrial vertebrate (Silva-Rocha *et al.*, 2016). Its range significantly overlaps with the haul-out sites of Mediterranean monk seals, increasing the likelihood of its presence in scat samples, not as a primary prey item, but rather as a secondary or environmental trace.

Fisheries and aquaculture interactions

Of the 38 fish species identified as documented prey of the Mediterranean monk seal, several are heavily targeted by both artisanal and recreational fisheries around the Madeira Archipelago (Figure 3). Pelagic species such as the European pilchard (*Sardina pilchardus*) and Atlantic chub mackerel (*Scomber colias*) are widely exploited by the local seine fleet, while demersal species like the red porgy (*Pagrus pagrus*) and blackspot seabream (*Pagellus bogaraveo*) are also commonly fished. Notably, the pink dentex (*Dentex gibbosus*) and red porgy are among the most frequently caught species in artisanal and shore-based recreational fisheries, highlighting their significant commercial and subsistence value in the region (Martinez-Escauriaza *et al.*, 2021). The white seabream (*Diplodus sargus*), a common target in spearfishing, also appears frequently in the seal's diet. Other overlapping species include the striped red mullet (*Mullus surmuletus*) and the axillary seabream (*Pagellus acarne*), often caught using artisanal methods such as handlines and nets, as shown by catch-per-unit-effort studies (Martinez-Escauriaza *et al.*, 2021). Additionally, species like the Mediterranean rainbow wrasse (*Coris julis*), the ornate wrasse (*Thalassoma pavo*), red scorpionfish (*Scorpaena scrofa*), and the grey triggerfish (*Balistes capriscus*) are regularly captured in nearshore artisanal and recreational fisheries, frequently through spearfishing and small traps. Gilthead seabream (*Sparus aurata*), a species that emerged as a moderate component in the Mediterranean monk seal diet (5/14 samples; FO = 35.7%), is also the main species farmed in local aquaculture facilities around Madeira. Due to periodic releases and escapes from offshore cages, a wild population has become established in the archipelago (Alves & Alves, 2002; Parretti *et al.*, 2023). While minimal interaction has been reported between seals and aquaculture in Madeira, anecdotal reports of cage damage and predation by seals do exist (Pires, Neves, & Karamanlidis, 2008; Amaral & Andrade, 2025). Together, these findings may

also provide an additional indication, complementing previous citizen-science that the introduced gilthead seabream is occurring in the wild (Parretti *et al.*, 2023) at sufficient abundance to be routinely incorporated into the natural diet of Mediterranean monk seals.

Also, all scat samples in which *Sparus aurata* was present also contained a range of other prey species. This suggests that seals are predominantly foraging on wild, naturally occurring *S. aurata* alongside other species, rather than exclusively targeting farmed fish. If seals were frequently raiding cages, we would expect a much lower diversity of prey in their diet, reflecting specialized foraging on a single, abundant food source. These complex ecological interactions underscore the importance of integrated coastal zone management strategies that reconcile aquaculture development with the habitat requirements and conservation priorities of the Mediterranean monk seal.

Integrated Approaches to Sustainable Natural Resource Management

Our study underscores the need for an integrated approach for managing natural resources in ecologically dynamic systems such as Madeira's coastal waters. Several detected taxa are targeted by fisheries and aquaculture occurs locally, suggesting potential for resource overlap. Rather than relying solely on conventional measures like protected areas or uniform restrictions, our findings show that management frameworks should be flexible and responsive to real-world interactions between people and nature.

Metabarcoding analysis, as implemented in our research, provides insights into the dietary habits of the Mediterranean monk seal, allowing resource managers to refine their strategies with species-level precision.

Innovative Conservation Methodologies for Monk Seal Protection

The application of advanced molecular techniques, particularly DNA metabarcoding, marks a significant advancement in conservation practice (Thomsen, Jensen, & Sigsgaard, 2024). Traditional morphological analyses of diet are limited in their ability to detect soft-bodied or rare prey, but our dual-primer approach revealed a much broader spectrum of prey species, including previously unreported cartilaginous fishes. This depth of dietary resolution moves conservation planning from generalized interventions toward highly specific, evidence-based actions that reflect the seals' foraging behaviour and habitat use.

These molecular insights facilitate the identification of conservation priorities, such as the need to protect habitats supporting the seals' preferred prey and the implementation of restoration efforts where necessary. By engaging stakeholders, including fishers, aquaculture industries, and coastal communities, in the development and application of these targeted management strategies, we ensure that conservation measures are both practical and respected, thereby enhancing their effectiveness and long-term sustainability.

Conclusions

In this study, we have for the first time, used DNA metabarcoding analyses to examine the diet of the world's most endangered pinniped, the Mediterranean monk seal.

Frequency of occurrence (FO) analysis revealed well-defined foraging patterns among the seals, with a predominance of preferred, energy-rich species in their diet. Specifically, species such as the European conger (*Conger conger*) and rough tail stingray (*Bathytoshia centroura*) were present in nearly all samples (FO = 92.9%). This suggests a foraging strategy centred on reliable prey sources. Nevertheless, the detection of secondary and occasional prey species indicates flexible and opportunistic feeding behaviour, enabling the seals to adjust to changes in prey availability within their environment.

The diet was largely composed of bottom-dwelling and reef-associated fish, with a strong preference for species in the Sparidae family, likely reflecting both prey availability and the seals' capacity to exploit diverse coastal habitats. This ecological versatility emphasizes their role as adaptable predators and underscores the importance of conserving a variety of habitats to support their foraging needs and long-term survival in a changing environment.

Our analysis also detected species not previously reported in these waters, such as *Fistularia petimba*. The presence of *Sparus aurata* may suggest some interaction with nearby aquaculture, though the predominance of wild prey indicates that aquaculture is not a primary food source. Notably, we observed dietary overlap with species targeted by local fisheries, suggesting potential competition.

These findings provide valuable ecological insights into Mediterranean monk seals around Madeira and offer a robust foundation for conservation and management strategies aimed at enhancing the species' recovery and resilience in the face of environmental and anthropogenic pressures.

Acknowledgements

This research was funded by national funds through FCT, Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UIDB/04423/2025 (<https://doi.org/10.54499/UIDB/04423/2025>), UID/PRR/04423/2025 (<https://doi.org/10.54499/UID/PRR/04423/2025>), LA/P/0101/2020 (<https://doi.org/10.54499/LA/P/0101/2020>); UID/50016/2025 and LA/P/0076/2020 (<https://doi.org/10.54499/LA/P/0076/2020>); UIDB/04050/2020 (UMinho CBMA - <https://doi.org/10.54499/UIDB/04050/2020>). AJM received the support of a fellowship (LCF/BQ/DI20/11780037) from “la Caixa” Foundation (ID 100010434) and the Early Career Researcher Fellowship Grant from EuroMarine. JCC was funded by national funds through FCT under the Scientific Employment Stimulus Institutional Call (CEECINST/00098/2018). PFC was partially supported by national funds through FCT, I.P., under the Scientific Employment Stimulus Initiative, references CEECIND/01799/2017, 2023.05877.CEECIND and SARDINOMICS 2022.03142.PTDC. JC was supported by 2023.15056.TENURE.043, funded by national funds through FCT.

We would also like to thank IFCN biologists and nature wardens for their contributions towards sample collection.

CRedit author statement

Paula F. Campos: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – Reviewing and Editing. Subramaniam Vijayakumar: Data curation, Formal analysis, Software, Visualization, Writing – Original draft preparation. Ashlie J. McIvor: Sample collection, Conceptualization, Data curation, Formal analysis, Writing – Reviewing and Editing. Gonçalo Espregueira Themudo: Methodology, Validation, Formal analysis, Data curation, Supervision, Writing – Reviewing and Editing. Rosa Pires: Writing – Reviewing and Editing. Philip Francis Thomsen: Validation, Writing – Reviewing and Editing. Ana Dinis: Writing – Reviewing and Editing. João Canning-Clode: Writing – Reviewing and Editing. João Carneiro: Methodology, Validation, Supervision, Writing – Reviewing and Editing.

Bibliography

- Alves FMA & Alves CMAA. 2002.** Two new records of seabreams (Pisces:Sparidae) from the Madeira Archipelago.
- Amaral AC & Andrade CAP. 2025.** The Conservation of the Endangered *Monachus monachus*: Could Maritime Workers Contribute to Its Study? *Environments - MDPI* **12**: 207.
- Armani A, Guardone L, La Castellana R, Gianfaldoni D, Guidi A & Castigliego L. 2021.** DNA barcoding reveals commercial and health issues in ethnic seafood sold on the Italian market. *FOOD CONTROL* **55**: 206–214.
- Ashlie J. McIvor. 2024.** Identifying marine predator conservation priorities in an Oceanic Archipelago.
- De Barba M, Miquel C, Boyer F, Mercier C, Rioux D, Coissac E & Taberlet P. 2014.** DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Molecular Ecology Resources* **14**: 306–323.
- Berry TE, Osterrieder SK, Murray DC, Coghlan ML, Richardson AJ, Grealy AK, Stat M, Bejder L & Bunce M. 2017.** <sc>DNA</sc> metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecology and Evolution* **7**: 5435–5453.
- Biscoito M, Ribeiro C & Freitas M. 2018.** Annotated checklist of the fishes of the archipelago of Madeira (NE Atlantic): I-Chondrichthyes. *Zootaxa* **4429**: 459–494.
- Cebrian D, Fatsea H & Mytilineou C. 2011.** *Some data on biometry and stomach content of a Mediterranean monk seal found in Santorini Island (Greece)*. . Monaco.
- Coissac E. 2012.** OligoTag: A Program for Designing Sets of Tags for Next-Generation Sequencing of Multiplexed Samples. 13–31.
- Deagle BE, Thomas AC, McInnes JC, Clarke LJ, Vesterinen EJ, Clare EL, Kartzinel TR & Eveson JP. 2019.** Counting with <sc>DNA</sc> in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology* **28**: 391–406.
- Dede, A.; Tonay, M. A.; Öztürk B. 2015.** Mediterranean Monk Seal *Monachus monachus* (Hermann, 1779) in the Aegean Sea. In: Katağan, T.; Tokaç, A.; Beşiktepe, S.; Öztürk B, ed. *he Aegean Sea Marine biodiversity, Fisheries, Conservation and Governance*. Turkish Marine Research Foundation, 612–636.
- Edgar RC, Haas BJ, Clemente JC, Quince C & Knight R. 2011.** UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.
- Fricke R, Eschmeyer WN & van der Laan R. 2026.** Eschmeyer’s Catalog of Fishes . (<http://www.calacademy.org/scientists/catalog-of-fishes-family-group-names/>). *Electronic version accessed 04/06/2026*.
- Froese R & Pauly D. 2025.** FishBase.
- Imorou RS, Adite A, Sossoukpe E & Abou Y. 2019.** Length-weight models and condition factors of fishes from Okpara Stream, Oueme River, Northern-Benin. *International Journal of Forest, Animal and Fisheries Research* **3**: 65–80.
- Karamanlidis AA, Kallianiotis A, Psaradellis M & Adamantopoulou S. 2011.** Stomach contents of a subadult Mediterranean monk seal (*Monachus monachus*) from the Aegean Sea. *Aquatic Mammals* **37**: 280–283.
- Karamanlidis AA, Curtis PJ, Hirons AC, Psaradellis M, Dendrinis P & Hopkins JB. 2014.** Stable Isotopes Confirm a Coastal Diet for Critically Endangered Mediterranean Monk Seals. *Isotopes in Environmental and Health Studies* **50**: 332.
- Karamanlidis AA, Dendrinis P, de Larrinoa PF, Gücü AC, Johnson WM, Kiraç CO & Pires R. 2016a.** The Mediterranean monk seal *Monachus monachus*: status, biology, threats, and conservation priorities. *Mammal Review* **46**: 92–105.
- Karamanlidis AA, Gaughran S, Aguilar A, Dendrinis P, Huber D, Pires R, Schultz J, Skrbinšek T & Amato G. 2016b.** Shaping species conservation strategies using mtDNA

analysis: The case of the elusive Mediterranean monk seal (*Monachus monachus*). *Biological Conservation* **193**: 71–79.

Margaritoulis D, Karavellas D & Irvine C. 1996. Predation of adult loggerheads by Mediterranean monk seals. In: *Proceedings of the Fifteenth Annual Symposium on Sea Turtle Biology and Conservation, NOAA Technical Memorandum NMFS-SEFSC-387.*, 193–196.

Margaritoulis D & Touliaou S. 2011. Mediterranean monk seals present an ongoing threat for loggerhead sea turtles in Zakynthos. *Marine Turtle Newsletter*: 18.

Martinez-Escauriaza R, Pita P, de Gouveia MLF, Gouveia NMA, Teixeira E, de Freitas M & Hermida M. 2021. Analysis of Big Game Fishing Catches of Blue Marlin (*Makaira nigricans*) in the Madeira Archipelago (Eastern Atlantic) and Factors that Affect Its Presence. *Sustainability* **2021, Vol. 13, Page 8975** **13**: 8975.

McIvor AJ, Pires R, Lopes C, Raimundo J, Campos PF, Pais MP, Canning-Clode J & Dinis A. 2023. Assessing microplastic exposure of the Critically Endangered Mediterranean monk seal (*Monachus monachus*) on a remote oceanic island. *Science of The Total Environment* **856**: 159077.

Moreira Santos I, Netels R, McIvor A & Campos PF. 2026. Feeding Ecology and Conservation of the Mediterranean Monk Seal, (*Monachus monachus*).

Parretti P, Monteiro JG, Gizzi F, Martínez-Escauriaza R, Alves F, Chebaane S, Almeida S, Pessanha Pais M, Almada F, Fernandez M, Nogueira N, Andrade C & Canning-Clode J. 2023. Citizen Science and Expert Judgement: A Cost-Efficient Combination to Monitor and Assess the Invasiveness of Non-Indigenous Fish Escapees. *Journal of Marine Science and Engineering* **11**: 438.

Parsons ECM. 2024. Mediterranean monk seal (*Monachus monachus*) 5-Year Review: Summary and Evaluation.

Pierce G, Hernandez_Milian G, Begoña Santos M, Dendrinis P, Psaradellis M, Tounta E, Androukaki E & Edridge A. 2011. Diet of the Monk Seal (*Monachus monachus*) in Greek Waters. *Aquatic Mammals* **37**: 284–297.

Pires R. 2020. *LIFE Madeira Monk Seal project: Conservation of the Mediterranean monk seal in Madeira*. Funchal.

Pires R, Aparicio F, Baker J, Pereira S, Caires N, Cedenilla M, Harting A, Menezes D & Fernández de Larrinoa P. 2023. First demographic parameter estimates for the Mediterranean monk seal population at Madeira, Portugal. *Endangered Species Research* **51**: 269–283.

Pires R, Aparício F & Fernandez de Larrinoa P. 2019. *Plano de Ação para a Conservação da população de Lobo-marinho no Arquipélago da Madeira*. Instituto das Florestas e Conservação da Natureza, IP-RAM.

Pires R, Neves HC & Karamanlidis AA. 2007. Activity Patterns of the Mediterranean Monk Seal (*Monachus monachus*) in the Archipelago of Madeira. *Aquatic Mammals* **33**: 327–336.

Pires R, Neves HC & Karamanlidis AA. 2008. The Critically Endangered Mediterranean monk seal *Monachus monachus* in the archipelago of Madeira: priorities for conservation. *Oryx* **42**: 278–285.

Rey-Iglesia A, Gaubert P, Espregueira Themudo G, Pires R, De la Fuente C, Freitas L, Aguilar A, Borrell A, Krakhmalnaya T, Vasconcelos R & Campos PF. 2020. Mitogenomics of the endangered Mediterranean monk seal (*Monachus monachus*) reveals dramatic loss of diversity and supports historical gene flow between Atlantic and eastern Mediterranean populations. *Zoological Journal of the Linnean Society* **191**: 1147–1159.

Rognes T, Flouri T, Nichols B, Quince C & Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

- Salman A, Bilecenoglu M & Güçlüsoy H. 2001.** Stomach contents of two Mediterranean monk seals (*Monachus monachus*) from the Aegean Sea, Turkey. *Journal of the Marine Biological Association of the UK* **81**: 719–720.
- Salmona J, Dayon J, Lecompte E, Karamanlidis AA, Aguilar A, Fernandez de Larrinoa P, Pires R, Mo G, Panou A, Agnesi S, Borrell A, Danyer E, Öztürk B, Tonay AM, Anestis AK, González LM, Dendrinis P & Gaubert P. 2022.** The antique genetic plight of the Mediterranean monk seal (*Monachus monachus*). *Proceedings of the Royal Society B: Biological Sciences* **289**.
- Sigsgaard EE, Torquato F, Frøslev TG, Moore ABM, Sørensen JM, Range P, Ben-Hamadou R, Bach SS, Møller PR & Thomsen PF. 2020.** Using vertebrate environmental DNA from seawater in biomonitoring of marine habitats. *Conservation Biology* **34**: 697–710.
- Silva-Rocha I, Sá-Sousa P, Fariña B & Carretero MA. 2016.** Molecular analysis confirms Madeira as source for insular and continental introduced populations of *Teira dugesii* (Sauria: Lacertidae). *Salamandra* **52**: 269–272.
- Thomsen PF, Jensen MR & Sigsgaard EE. 2024.** A vision for global eDNA-based monitoring in a changing world. *Cell* **187**: 4444–4448.
- Tonay AM, Danyer E, Dede A, Öztürk B & Öztürk AA. 2016.** The stomach content of a Mediterranean Monk Seal (*Monachus monachus*): finding of Green Turtle (*Chelonia mydas*) remains. *Zoology in the Middle East* **62**: 212–216.
- Valentini A, Pompanon F & Taberlet P. 2009.** DNA barcoding for ecologists. *Trends in Ecology & Evolution* **24**: 110–117.
- Wirtz P, Fricke R & Biscoito MJ. 2008.** The coastal fishes of Madeira Island-new records and an annotated check-list. *Zootaxa* **1715**.

Table 1. Details of *Monachus monachus* scat samples included in this study. Information provided includes sample ID, collection island and site, and date of collection. Samples marked “N” were excluded from downstream metabarcoding analyses following quality-control filtering. Note: no sample is labelled DES_09.

Sample ID	Collection Island	Collection site	Collection date	Included in analysis
DES_01	Desertas	Tabaqueiro	19/11/2020	Y
DES_02	Desertas	Tabaqueiro	19/11/2020	Y
DES_03	Desertas	Tabaqueiro	30/04/2021	Y
DES_04	Desertas	Tabaqueiro	01/05/2021	N
DES_05	Desertas	Tabaqueiro	06/04/2021	Y
DES_06	Desertas	Tabaqueiro	06/04/2021	Y
DES_07	Desertas	Tabaqueiro	06/04/2021	Y
DES_08	Madeira	Gruta da Atalaia	29/12/2014	Y
DES_10	Desertas	Gruta dos Lobos	09/04/2015	Y
DES_11	Desertas	Gruta dos Lobos	18/02/2015	Y
DES_12	Desertas	Vermelhas	13/06/2015	Y
DES_13	Desertas	Vermelhas	13/06/2015	Y
DES_14	Desertas	Lanço do Rico	17/06/2015	N
DES_15	Madeira	Ponta de S. Lourenço, Vaticano	22/06/2016	Y
DES_16	Madeira	Ponta de S. Lourenço, Vaticano	01/08/2017	Y
DES_17	Desertas	Tabaqueiro	17/04/2021	Y

Table 2 - Number of operational taxonomic units (OTU) detected per sample after bioinformatic processing (14 samples retained for downstream analysis).

Sample name	Number of OTUs
DES_01	441
DES_02	179
DES_03	779
DES_04	NA
DES_05	478
DES_06	348
DES_07	960
DES_08	479
DES_10	731
DES_11	512
DES_12	478
DES_13	624
DES_14	NA
DES_15	793
DES_16	379
DES_17	447

Table 3. Frequency of occurrence of each species identified using molecular analysis from Mediterranean monk seal scat, categorized by documented presence in the Madeira Archipelago.

Documented in Madeira	FO%	Not Documented in Madeira	FO%
<i>Conger conger</i>	92.9	<i>Seriola lalandi</i>	78.6
<i>Bathytoshia centroura</i>	92.9	<i>Gnathophis bathytos</i>	50
<i>Muraena helena</i>	85.7	<i>Helicolenus avius</i>	50
<i>Pagellus acarne</i>	78.6	<i>Polymixia sazónovi</i>	42.9
<i>Pagrus pagrus</i>	78.6	<i>Pontinus macrocephalus</i>	42.9
<i>Balistes caprisus</i>	64.3	<i>Helicolenus hilgendorfi</i>	35.7
<i>Gephyroberyx darwini</i>	64.3	<i>Sphoeroides spengleri</i>	21.4
<i>Merluccius merluccius</i>	64.3	<i>Kyphosus pacificus</i>	14.3
<i>Trisopterus luscus</i>	64.3	<i>Phycis chesteri</i>	14.3
<i>Dentex gibbosus</i>	50	<i>Diplodus kotschy</i>	7.1
<i>Pontinus kuhlii</i>	50	<i>Himantura microphthalmia</i>	7.1
<i>Dasyatis tortonesei</i>	42.9	<i>Hoplostethus crassispinus</i>	7.1
<i>Diplodus sargus</i>	42.9	<i>Merluccius capensis</i>	7.1
<i>Dicentrarchus labrax</i>	42.9	--	--
<i>Enchelycore anatina</i>	42.9	--	--
<i>Kyphosus sectatrix</i>	42.9	--	--
<i>Sardina pilchardus</i>	42.9	--	--
<i>Torpedo marmorata</i>	42.9	--	--
<i>Ariosoma balearicum</i>	35.7	--	--
<i>Sparus aurata</i>	35.7	--	--
<i>Epinephelus marginatus</i>	35.7	--	--
<i>Lepidopus caudatus</i>	35.7	--	--
<i>Mullus surmuletus</i>	28.6	--	--
<i>Trachurus trachurus</i>	28.6	--	--
<i>Fistularia petimba</i>	21.4	--	--
<i>Chelon labrosus</i>	21.4	--	--
<i>Scorpaena scrofa</i>	21.4	--	--
<i>Tripterygion delaisi</i>	21.4	--	--
<i>Dasyatis pastinaca</i>	14.3	--	--
<i>Phycis phycis</i>	14.3	--	--
<i>Thalassoma pavo</i>	14.3	--	--
<i>Coris julis</i>	7.1	--	--
<i>Helicolenus dactylopterus</i>	7.1	--	--
<i>Muraena augusti</i>	7.1	--	--
<i>Pagellus bogaraveo</i>	7.1	--	--
<i>Pagellus erythrinus</i>	7.1	--	--
<i>Scomber colias</i>	7.1	--	--

<i>Trachurus picturatus</i>	7.1	--	--
-----------------------------	-----	----	----

Figure 1- Geographic location of the collected Mediterranean monk seal scat samples: four in Desertas Islands and two in Madeira Island .

Figure 2 – Distribution of prey species by family, illustrating the diversity of taxa consumed. Silhouettes derived from Phylopic.org.

Figure 3 - Circular graph showing the overlap between commercially important fish species, aquaculture and the Mediterranean monk seal's diet.

- Local Fisheries
- Aquaculture
- Monk Seal Diet





