Flying under the radar: DNA barcoding ray wings in Greece detects protected species and umbrella labelling terms

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Abstract

Mislabeling of seafood products and marketing of protected species remains a worldwide issue despite the labeling regulations set at a local, European and International level. DNA barcoding has proven to be the most popular and accurate method of detection of fraudulent seafood products. This study investigated the batoid meat market of Greece, the mislabeling rates and the protected species occurrence. A total of 114 ray products were collected from fishmongers, open markets, supermarkets, and restaurants across eight Greek cities. The cytochrome oxidase subunit I (COI) gene was used to analyze samples, and the sequences were compared against genetic databases for species identification. At least 13 species across nine genera were identified. The results did not indicate significant differences in species utilization among cities, retailers, and labels. However, in the pairwise comparisons, Athens differed from all other locations and a similar trend was followed by the label "salachi". Moderate mislabeling levels were recorded (13.5%), while 3.5% of the identified samples belonged to species with prohibitions on landings, confirming an ongoing market for protected species. Overall, 19.8% of the samples originated from species that are locally listed in threatened categories of the IUCN Red List of species.

Keywords

Batoidea, Elasmobranch, Seafood labeling, Mitochondrial DNA, Species identification, Greek market

1. Introduction

Catches of elasmobranchs (sharks, rays, and skates) have increased over the last 40 years, threatening many species with the risk of extinction (Davidson et al., 2016). Yet, they comprise less than 1% of the world fisheries catches (FAO, 2014, 2016) and are characterised by poor catch reporting, whilst the subsequent lack of data poses a significant obstacle to their effective conservation and management (Cashion et al., 2019). This is well illustrated in the European Union (EU), where landings of different ray species were grouped under a single category, effectively masking serious population declines of vulnerable species under more abundant groups (Dulvy et al., 2000). The issue was addressed in 2013 with the introduction of new regulation (CEC, 2103) that required species specific landings to be recorded. However, the global landings of elasmobranchs have been estimated to be far outweighed by the incidental and discarded catch (Clarke et al., 2006), and significantly underestimated by catches from illegal, unregulated and unreported (IUU) fisheries (Helyar et al., 2014). These problems around the sparsity of species-specific catch data, poor reporting, and underestimation of catches remain a critical issue (Davidson et al., 2016; Cashion et al., 2019). Data deficiency and ambiguity renders stock assessment difficult and limits effective conservation (Cashion et al., 2019).

Historically, the Mediterranean Sea presented a high diversity and abundance of elasmobranchs with approximately 88 species being recorded (Cavanagh & Gibson 2007; Coll et al., 2010; Serena et al., 2020). More recently, the Mediterranean elasmobranchs have seen their regional status worsen, with 53% to 71% of the species being at risk of extinction (Dulvy et al., 2014). At least 66 species of elasmobranchs have been reported in the seas around Greece (Papaconstantinou, 2014), including 30 rays (Papaconstantinou, 2014; Chatzispyrou et al., 2020). Rays and skates (of the superorder Batoidea) are cartilaginous fish, with a distinctive flattened dorso-ventrally body and pectoral fins fused to the head (Compagno, 1973; 1999a). Their biological characteristics, such as late sexual maturity, prolonged gestation period, low fertility, and long-life span, make them especially vulnerable to anthropogenic pressures (Seitz & Poulakis, 2006; Field et al., 2009; Ferretti et al., 2010; Dulvy et al., 2014). Many batoid populations are declining, and overfishing appears to be the principal threat (Dulvy et al., 2014). An increasing number of species are protected by international, European and/or Greek laws and legislation (Supplementary Table 1). However, in the Greek market, their meat is generally classified in two categories, namely “Vatos” and “Galeos” and sold as such (Pazartzi et al., 2019). Batoid meat is mainly consumed in the mainland of Northern Greece and the islands of the North Aegean Sea, whilst is less common along the South Aegean and the Ionian seas. It is considered a delicacy and the main ingredient in a variety of traditional plates.

Morphological identification is impossible for the majority of batoid meat in Greece, due to processing, a practice similar to shark meat (Pazartzi et al., 2019). All external

traits and characteristics are removed and only the wings (the large, muscular pectoral fins) are kept and sold. The removal of skin, head, and tail poses a challenge to species identification based on morphological features, allowing the trade of protected species even within countries with firm trade regulations in the food industry. Extensive processing practices can increase species substitution, which is commonly seen in the elasmobranch meat market (Pazartzi et al., 2019; Minoudi et al., 2020). Even where morphological characters remain intact, threatened species from the Red List of the International Union for Conservation of Nature (IUCN) are often found on vendor displays in Greece and internationally (Holmes et al., 2009; Pazartzi et al., 2019; Minoudi et al., 2020). The transparency of local seafood marketing is also reduced with the wide-spread use of non-specific terms in labeling and lack of labeling regulation (Cashion et al., 2019). In the Greek fish market, most rays and skates are traded under broad labels such as “Vatos”, “Rina”, “Ray”, “Aetos”, “Rinovatos”, “Trigona”, “Salachi”, and “*Raja* spp.”. Despite the strict legislation in the case of shark meat and “Galeos”, where only *Mustelus* spp. products are sold under this label (Official Government Gazette 475/Issue B’/27-3-2015, No. 1750/32219 under EU 1379/2013), broad “umbrella” labeling for batoids with a variety of species being grouped under a single category is permitted (Official Government Gazette 475/Issue B’/27-3-2015). For example, all *Raja* spp. and *Rostroraja* spp. products can be sold under the label “vatos”, and *Leucoraja spp*. as “Strogilovatos”. Additionally, three species are sold under a species specific label; *Dipturus batis* is marketed as “Gkrizogaleos”, *Dipturus oxyrinchus* as “Nona”, and *Dasyatis pastinaca* as “Vatotrigona” (Official Government Gazette 475/Issue B’/27-3-2015). These issues are compounded by low consumer awareness that the most common label of “vatos” is used for ray and skate products, similar to labels around shark products with the terms “galeos” in Greece (Pazartzi et al., 2019) and “caçao” meat in Brazil (Bornatowski et al., 2015).

Mislabeling occurs when one species is substituted and traded under the name of another (Rasmussen & Morrissey, 2008). It is a persistent problem, and it has been documented worldwide in a variety of commercial species (Galal-Khallaf et al., 2014). It is also considered to be a global problem in the seafood industry (Von de Heyden et al., 2010; Hanner et al., 2011). Mislabeling has been associated with fraud, i.e. the intentional substitution of lower value products to consumers, and potentially has health implications associated with allergens and heavy metal loads associated with different species (Nagalakshmi et al., 2016; Xiong et al., 2019). It can also lead to data falsification and misrepresentation of species exploitation with negative effects on species and population conservation (Cawthorn et al., 2015; Kroetz et al., 2020), allowing protected or prohibited species to enter the supply chain. Accurate identification of marketed species is critical as it assists the consumer to make informed decisions and responsibly participate in a regulated trade, avoiding the purchase of threatened or protected species (Moretti et al., 2003). Unintentional and/or fraudulent mislabeling are still under investigation in the Greek seafood industry (Minoudi et al., 2020). Despite the Regulation (EU) No. 1379/2013, which requires to inform consumers with the product's commercial and scientific name, the geographical area, production method, and fishing gear, mislabelling remains common in Greece (Garcia-Vazquez et al., 2011; Pazartzi et al., 2019; Minoudi et al., 2020).

The aim of this study is to investigate the batoid meat trade in Greece and aid in the detection of illegally traded species that are protected by international and national legislation. DNA barcoding, i.e. sequencing approximately 650 base pairs (bp) from the 5′ end of the mitochondrial Cytochrome Oxidase Subunit I (COI) gene (Meyer & Paulay, 2005), was used to identify species being sold under the umbrella terms used for batoid species ("Vatos", "Rina", "Ray", "Aetos", "Rinovatos", "Trigona", "Salachi", and "*Raja* spp."). Additionally, the patterns of species utilization between different retailer types and cities were investigated. Samples were collected from a range of retailers such as fishmongers, open markets, and supermarkets in nine different Greek cities. The implications of the findings in the context of batoid conservation in Greece will also be explored.

2.Materials and methods

2.1. Sample collection and storage

A total of 114 tissue samples were collected from products sold under a range of labels, such as "vatos", "rina", "raya", "trigona", "*R. clavata*", "vatos achnistos", "salachi" (Supplementary Fig. 1). They were obtained from fishmongers, open markets (i.e. street retailers), and supermarkets, while one cooked sample was collected from a restaurant, located in eight Greek cities (Alexandroupolis: n = 13, Athens: n = 9, Kavala: n = 25, Katerini: n = 1, Komotene: n = 14, Mytilene: n = 14, Rethymno: n = 3, Thessaloniki: n = 35) between October 2017 and May 2021 (Fig. 1, Supplementary Table 1). Only one tissue sample per retailer was collected to avoid sampling the same individual fish and maintain the independence of each sample. Approximately 1-5 g of tissue were obtained and preserved in 95% ethanol. Samples were stored at −20 °C until further analysis and details such as sampling location, date, label, and price were recorded.

2.2. DNA extraction, amplification and sequencing

Genomic DNA was extracted from approximately 25 mg of tissue, using the Chelex 100 DNA extraction protocol (Walsh et al., 1991; Estoup et al., 1996). The tissue was placed in 500 µl of a 10% Chelex solution with 7 µl of Proteinase. The mixture was heated at 55 °C for 75 min and vortexed every 15 min. Subsequently, the vial was heated at 95 °C for10 min.

The COI gene was utilised, as it has been repeatedly used in elasmobranch DNA barcoding studies, producing reliable amplification and sequencing (Serra-Pereira et. al., 2010; Ferrette et al., 2019; Sudibyo et al., 2020). A 670 bp segment of the COI gene was amplified by polymerase chain reaction (PCR) using the following primers: Forward FishF2: 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGAT -3' and Reverse–FishR2: 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAG-3' (Ivanova, Zemlak, Hanner, & Hebert, 2007) with M13 tails (M13F: TGTAAAACGACGGCCAGT and M13R: CAGGAAACAGCTATGAC) (Messing, 1983) to maximise the useful length of subsequent sequence reads. For the amplification of the COI gene, PCR cycling conditions included an initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 52 °C for 40 s, 72 °C for 50 s, and a final extension at 72 °C for 10 min. PCR was conducted in 25 µl volumes and the reaction mixtures contained 2µl of DNA, 5 µl of Buffer (KAPA

Biosystems, South Africa), 1.5 µl of $MgCl_2$ (KAPA Biosystems, South Africa), 0,5 µl of dNTPs (Promega, Madison, WI USA), 1 µl of each primer, 0.15µl of taq DNA polymerase (KAPA Biosystems, South Africa), and 14.6 µl of molecular grade water. All PCR runs included negative controls. Amplicons were purified using polyethylene glycol (PEG) precipitation protocol (Sambrook & Russell, 2001).

Additionally, for the cooked sample a 168 bp segment of the COI gene was amplified by polymerase chain reaction (PCR) utilizing two primers: COI_MINI-F2-M13: 5′-'TGTAAAACGACGGCCAGT'ATRAAACCMCCHGCAATYTCHCA-3', and Forward FishR2 (Wannell et al., 2020) PCR for each COI minibarcode was conducted in a volume of 40 µl containing: 2 µl of DNA, 8 µl of Buffer (KAPA Biosystems, South Africa), 2.4 µl of $MgCl_2$ (KAPA Biosystems, South Africa), 0.8 µl of dNTPs (Promega, Madison, WI USA), 1.2 µl of each primer, 0.1 µl of taq (KAPA Biosystems, South Africa), and 24.3 µl of molecular grade water. PCR cycling conditions included an initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 52 °C for 40 s, 72 °C for 50 s, and a final extension at 72 °C for 10 min. PCR products were sequenced commercially (Macrogen, The Netherlands).

2.3. Sequence analysis and species identification

Sequences were manually checked and edited using ProSeq 3.0 (Filatov, 2002) and BioEdit 7.2.6 (Hall, 1999). All sequences were translated and checked for the presence of stop codons and mitochondrial pseudogene (NUMTs) and compared against GenBank with nucleotide BLAST (blastn) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). They were also compared against the BOLD database (Species Level Barcode Records, http://www.boldsystems.org/). All sequences are available in Supplementary Table 2. The identity threshold was set at 98% for both databases, and only sequences with high homology (≥ 98%) were deemed acceptable (and species were identified with the top match) and included in the analysis (Supplementary Table 1) (Barbuto et al., 2010; Armani et al., 2015; Pazartzi et al., 2019). Sequences that did not fulfil the criteria were disregarded.

2.4. Phylogeny

Construction of a phylogenetic tree was carried out in MEGA 11.0 (Tamura et al., 2021). Sequences were aligned using ClustalW software as implemented in MEGA. Phylogenetic analysis was performed using the Neighbor-joining (NJ) method (Saitou & Nei, 1987) with the Kimura 2-parameter model (K2P) (Kimura, 1980) and 1,000 bootstrap replicates.

2.5. Statistical analyses

Statistical analyses were conducted in R 3.5.1 (https://cran.r-project.org/) and PAST-6 (Hammer, Harper, & Ryan, 2001), following Griffiths et al. (2013). We tested for any effect of the city/location and the label on patterns of batoids on sale. Each sample/data represented one point, and a non-parametric analysis of similarity (ANOSIM) using the Bray-Curtis distance measure was performed. The contribution

of variables to similarity (SIMPER analysis) was also calculated. Due to small sample sizes, Rethymno and Katerini were excluded from the analysis, and samples collected from coastal fishermen were combined with those from open markets. Samples collected under the label "vatos", "vatos/*Raja* spp.", "vatos achnistos" and "*R. clavata*" were combined under the category "vatos". Similarly, "vatos/trigona" and "trigona" were grouped as "trigona", and the labels "rina" and "vatos/rina" were classified as "rina". The relationship between the sampling location and the marketing of prohibited and mislabeled species was examined using chi-square tests. Principal Component Analysis (PCA) was only used to visualize the data, as it is not the recommended analysis for categorical datasets. For this analysis, the Bray-Curtis distance measure was calculated for samples collected at the same city and label.

3. Results

3.1. DNA extraction, amplification and sequencing evaluation

In total, 111 batoid products were successfully identified out of 114 samples. Sequence lengths varied between 101 (sample B43, Supplementary Tables 1-2) and 665 bp, with an average length of 490 bp (Supplementary Table 2). Most barcode searches on both BLAST and BOLD resulted in similar, clear top matches with an average of 99.5% confidence on species assignment (Supplementary Table 1). For the processed sample obtained from the restaurant, a fragment of 177 bp produced clear top matches with confident species assignment of 99.32% (sample B113, *Raja clavata*, Supplementary Table 1).

3.2. Species identification

At least 13 species across nine genera (*Aetomylaeus*, *Dasyatis*, *Dipturus, Gymnura, Leucoraja, Mobula, Myliobatis, Raja, Rostroraja),* and 5 families (Dasyatidae, Gymnuridae, Mobulidae, Myliobatidae, Rajidae) were identified (Supplementary Table 1). The most frequent species traded was the thornback ray (*R. clavata,* n = 77, 69.4%), followed by the rough ray (*Raja radula*, n = 10, 8.1%) and individuals of the genus *Dasyatis* (grouped due to taxonomic ambiguity in databases, *Dasyatis* spp., n = 6) (Fig. 2). The brown ray (*Raja miraletus,* n = 5) and the bull ray (*Aetomylaeus bovinus,* n = 4) were not very common. In cases of identification inconsistencies due to top multiple matches, species with Indian, Atlantic, and Pacific geographic distributions were deemed as unlikely. From 111 identified samples, 15 cases (13.5%) of mislabeling were detected between the name reported on the label and the species identified (Supplementary Table 1).

Among the samples, species protected by international and national legislation (CITES Appendix II, Barcelona Convention: Annex II, CMS Appendix I & II, Ministerial Order 67/1981 (Official Government Gazette 23A)) were identified and accounted for 3.6% of the samples (four cases). Additionally, any species being listed as Vulnerable, Endangered, and Critically Endangered in the Red List of threatened

species of the International Union for Conservation of Nature (IUCN) for the Mediterranean Sea (Dulvy, Allen, Ralph, & Walls, 2016), were identified and accounted for 19.8% of the samples (22 cases, Fig. 3). More specifically, the Critically Endangered sandy ray (*Leucoraja circularis,* n = 1) and the spiny butterfly ray (*Gymnura altavela,* n = 1), along with the Endangered spinetail devil ray (*Mobula mobular*, n = 1) and the bottlenose skate (*Rostroraja alba,* n = 1) were reported (Supplementary Table 1).

3.3. Phylogeny

The phylogenetic tree (Supplementary Fig. 2) was constructed with the objective to serve as corroborating evidence, and it confirmed the validity of the analysis generated by the BLAST and BOLD databases.

3.4 Species comparison among cities and retailer types

Mytilene showed the highest diversity of species identified (number of species = 6), followed by Athens (number of species = 5), whereas Thessaloniki, Kavala and Komotene had the same number (four) (Table 1, Supplementary Table 1). The highest level of mislabeling was detected in Mytilene (n = 7), followed by Athens and Rethymno with 3 cases, and Komotene (n = 2). No cases of mislabeling were identified in Alexandroupolis, Kavala, and Thessaloniki (Table1, Supplementary Table 1).

Species utilization in comparison with the labels was not significant (ANOSIM, $R = 0.109$, $p = 0.060$, Supplementary Table 3), although the p-value is close to the 95% confidence interval. The label “salachi" was differentiated from all other labels in the pairwise comparisons ($p < 0.01$, Supplementary Table 3); this was mainly driven by *R. clavata* and *Dasyatis* spp. (SIMPER analysis, Supplementary Table 3). A very similar trend was followed in the comparison of species utilization among the different cities (ANOSIM, $R = 0.055$, $p = 0.057$, Supplementary Table 3), again with a p-value very close to the 95% confidence interval. Athens was significantly different to all other locations ($p < 0.05$), driven mainly by *R. clavata* and *Dasyatis* spp. (SIMPER analysis, Supplementary Table 3). The comparison of species utilization between different retailers was not significant (ANOSIM, $R = 0.042$, $p = 0.062$). However, the sample sizes are very small for some retailers (Supplementary Table 3). The PCA analysis illustrated the differences between cities and between labels, corroborating the ANOSIM results (Supplementary Figs. 3a-3b). The protected species *M. mobular* and *G. altavela* were both found in Athens, under the label “salachi” and were absent from the rest of the sampling locations. Finally, the chi-square tests indicated that the presence of mislabeled and protected species is location dependent ($p < 0.0001$ and $p = 0.033$) (Supplementary Table 4).

4. Discussion

4.1. DNA barcoding and species identification

The use of the mitochondrial COI gene was largely successful, as has been demonstrated by previous studies on a range of elasmobranch meat products (Haque, Das & Biswas, 2019; Hellberg, Isaacs & Hernandez, 2019; Choo et al., 2021), generating 111 DNA barcodes out of 114 samples. These revealed the sale of at least 13 species of batoids sold under a variety of commercial terms in Greece. This number corresponds to 34.2% of the 38 species currently found in the Mediterranean (Serena et al., 2020), and 43.3% of the 30 species recorded in Greece (Papaconstantinou, 2014; Chatzispyrou et al., 2020). The thornback ray (*R. clavata*) was by far the most identified species, representing 69.4% of the samples analysed. The second most abundant species identified was *R. radula* (8.1%).

Species level identification was possible in 96.4% of the samples. This was not possible for most samples from the Dasyatidae family (Supplementary Table 1), mainly due to the taxonomic ambiguity and misnomers in the existing databases. For better taxonomic resolution and accuracy in databases such as BOLD and GenBank, there is a need for continuous updating of the lodged sequences and curation of international repositories (Wannell et al., 2020). Among the top matches for the Dasyatidae samples (homology ≥ 98%) were *D. pastinaca, D. tortonesei, D. marmorata,* and *Taeniura grabata*. In the past, the taxonomic status of *D. pastinaca* and *D. tortonesei* has been questioned (Tortonèse, 1987; Compagno, 1999b; Serena, 2005), although more recent studies suggest that they are two distinct species (Saadaoui et al., 2016; Vella & Vella, 2021). Among elasmobranchs, difficulties in species identification are occasionally reported with the COI gene, which is partially attributed to the low levels of genetic variation among species (Lopez, Ryburn, Fedrigo, & Naylor, 2006; Veríssimo, Zaera-Perez, & Leslie, 2017; Almerón-Souza et al., 2018). Previous studies suggested that dual markers might be required for the successful species level identification of elasmobranch samples, especially where the COI region fails to amplify (Feitosa et al., 2018; Pazartzi et al., 2019; Marchetti et al., 2020).

Due to the scarcity of available research, whether seafood mislabeling is considered common in Greece is not yet known and is currently under investigation (Garcia-Vazquez et al., 2011; Pazartzi et al., 2019; Minoudi et al., 2020). Pazartzi et al. (2019) demonstrated high levels of mislabeling at around 56% in shark filets. Conversely, Minoudi et al. (2020) reported lower levels of mislabeling among elasmobranchs. This investigation on rays and skates showed a similar trend, with 15 mislabeling cases detected (13.5%, Supplementary Table 1).

In this study, four species (*Leucoraja circularis, R. alba, G. altavela*, and *M. mobular*) that are currently protected by international and national legislation were identified, and therefore were illegally caught and landed in Greek markets. All four species are listed in Barcelona Convention Annex II, and one was additionally listed in CITES appendix II and CMS: Appendix I & II . Protected species accounted only for the

3.6% of the samples, a considerably low percentage in comparison with the global trends, where unregulated elasmobranch meat landings and commercialization are considered more common (Appleyard, White, Vieira, & Sabub, 2018; Fields et al., 2018; Palumbi et al., 2018). As expected, all protected batoids species encountered in the Mediterranean, are listed as either Endangered or Critically Endangered in the Red List of threatened species of the IUCN for the Mediterranean (Dulvy, Allen, Ralph, & Walls, 2016). In view of their overall low abundance in the Mediterranean and limited records in the Greek seas (12 and 1 individuals of *G. altavela* and *R. alba*, respectively, in 2554 hauls in the North Aegean for 2018-2019; National Data Collection Framework for the Greek Fisheries), the relatively low number of protected species among our samples may simply reflect their rarity, rather than the successful implementation of the legal protection and prohibitions on landings.

4.2. Differences among location, retailers and label types

The present research is an attempt to investigate the batoid products sold in the Greek market. Previous studies investigating the mislabeling levels in the seafood market of Greece were mainly focused on shark products (Pazartzi et al., 2019; Minoudi et al., 2020). Our analysis was not able to identify significant differences between locations, retailers and label types. However, the pairwise comparisons identified significant differences between “salachi" and all other labels, as well as between Athens and all other locations. Interestingly, the labeling of batoid meat appears to be mostly affected by the location rather than the labeling legislation that currently exists in Greece. The term “vatos” appears to be more common in the North of the country, and usually refers to skates, mainly species of the Rajidae family. In the South (i.e. Athens), where batoid meat consumption is less common, with some areas not consuming it at all, the most popular designation is the term “salachi”. Similarly, the term “trigona” was only encountered in the island of Lesvos (Mytilene) in the North Aegean Sea (Supplementary Fig. 4). Moreover, higher mislabeling rates were recorded in Athens and Mytilene compared to all other locations, as a single term is mainly used in markets to describe all batoid meat products (salachi and trigona respectively). Whereas in northern Greece, mislabeling cases were not frequent, and the term “vatos” was the most utilised label, which is the legal designation for all *Raja* spp. and *Rostroraja* spp. species.

4.3. Conservation issues

Extensive processing practices of the batoid meat in the seafood industry, combined with broad labelling regulations and poor catch reporting, allow species substitution and commercialization of endangered species (Cashion et al., 2019; Pazartzi et al., 2019). While species level identification on batoid landings in Greece remains rare, different ray species are combined under a single category, effectively masking population declines, despite EU regulations (Dulvy et al., 2000; Griffiths, et al., 2013; Pazartzi et al., 2019). Such practices have significant negative effects on the conservation and management efforts of fragile populations (Sadovy de Mitcheson et al., 2018). Thus, DNA barcoding could aid the conservation and management efforts for batoids in the Mediterranean through improving species-specific catch-data.

At present, 47% of the IUCN-listed batoids species of the Mediterranean are classified in threatened categories. In the current study, nine out of the 13 species identified are listed in threatened categories (CR, EN, VU) by IUCN, locally in the Mediterranean (Dulvy et al., 2016), representing 19.8% of samples. This study demonstrates that vulnerable, threatened, and even in some cases protected rays and skates are finding their way into the Greek seafood market. Additionally, current legislation leaves vulnerable species with declining populations as *A. bovinus* (CR), *R. radula* (EN), *D. pastinaca* (VU), *D. tortonesei* (VU), and *Myliobatis aquila* (VU), unprotected. These species are excluded from both national and international legal frameworks, while their populations are declining due to current practices.

5. Conclusions

This study assessed the species composition, level of mislabeling and numbers of protected species in batoid meat products in Greece, using DNA barcoding. Moderate rates of mislabeling and protected species were detected compared to previous studies for elasmobranchs worldwide and nationwide. The higher mislabeling rates were detected in the South of the country and the islands, where a single term is predominantly used for all batoid meat products. This type of mislabeling could be unintentional and has occurred due to marketing practices in the area and the low consumer awareness. However, the discovery of four rare, protected species of the Mediterranean demonstrates that protected rays and skates find their way into the consumers' plate. Their low occurrence in the market is probably related to their overall rarity and low abundance in the Mediterranean Sea.

Despite the low mislabeling rate and the low numbers of protected species in the market, improvements can be made to increase the transparency of the seafood industry, as well as consumer awareness and conservation efforts. Labelling legislation should be improved, and become more species specific, leading to the expansion of the list of species protected. Indeed, this study is one of the very few conducted in the country investigating the transparency of the seafood market. In future, it is important to implement similar DNA barcoding studies including more regions and market types, i.e. restaurants, where the lack of labelling regulations renders species substitution easier.

Conflicts of interest

The authors declare that they have no conflict of interest.

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