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Stable isotopes confirm a coastal diet for critically endangered Mediterranean monk seals[†]

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Understanding the ecology and behaviour of endangered species is essential for developing effective management and conservation strategies. We used stable isotope analysis to investigate the foraging behaviour of critically endangered Mediterranean monk seals (*Monachus monachus*) in Greece. We measured carbon and nitrogen isotope ratios (expressed as δ^{13}C and δ^{15}N values, respectively) derived from the hair of deceased adult and juvenile seals and the muscle of their known prey to quantify their diets. We tested the hypothesis that monk seals primarily foraged for prey that occupy coastal habitats in Greece. We compared isotope values from seal hair to their coastal and pelagic prey (after correcting all prey for isotopic discrimination) and used these isotopic data and a stable isotope mixing model to estimate the proportion of coastal and pelagic resources consumed by seals. As predicted, we found that seals had similar δ^{13}C values as many coastal prey species and higher δ^{13}C values than pelagic species; these results, in conjunction with mean dietary estimates (coastal = 61 % vs. pelagic = 39 %), suggest that seals have a diverse diet comprising prey from multiple trophic levels that primarily occupy the coast. Marine resource managers should consider using the results from this study to inform the future management of coastal habitats in Greece to protect Mediterranean monk seals.

Keywords: carbon-13; diet; endangered species; food web; foraging; Greece; isotope ecology; *Monachus monachus*; nitrogen-15; seals

1. Introduction

Knowledge of animal ecology and behaviour is of key importance when designing management and conservation plans for free-ranging species [1–4]. The Mediterranean monk seal (*Monachus monachus;* hereafter, monk seal) is the rarest pinniped and one of the most critically endangered marine mammals on Earth [5]. It is estimated that fewer than 600 individuals survive in three isolated subpopulations in the archipelago of Madeira and the Cabo Blanco region in the northern Atlantic Ocean, and in the eastern Mediterranean Sea [6–9]. The largest and most important population of approximately 300 individuals survives in the Ionian and Aegean seas of Greece

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[8,10]. Small population units can be found also along the Aegean and Mediterranean coastal areas of Turkey [11,12]. Monk seals are primarily threatened by habitat destruction and fragmentation, and fisheries–seal conflicts (i.e. deliberate killing by fisherman, drowning through accidental entanglement in fishing gear) [6].

Limited information is available about the behaviour of monk seals. Data indicate, however, that monk seals rely on suitable coastal caves for resting, pupping, and raising newborns [13,14]. At sea, monk seals are known to travel great distances (e.g. ∼288 km in three months; maximum straight distance travelled ∼78 km [15]) and dive to depths of ∼200 m to forage [16]. Stomach content analysis of dead monk seals revealed that they have a heterogeneous diet consisting of bony fish, cephalopods, and crustaceans [17–21]. In Greece, monk seals are known to forage for more than 530 prey species (i.e. 50 % Cephalopods, 48 % fish, 1.5 % non-cephalopod molluscs, 0.4 % Crustaceans) [21]; the common octopus (*Octopus vulgaris*; ∼34 %) and bony fish from the family Sparidae (∼28 %) being identified in the stomachs of monk seals most frequently [21]. Results from this study [21] and others [20,22] are consistent with the known movement patterns of six monk seals in Greece [15,16]. Collectively, results from these past studies suggest that monk seals forage primarily on the continental shelf along the coast.

Although results from past diet studies provide a baseline of foods consumed by monk seals, they provide limited information about their foraging behaviour. Many foods have been likely overestimated as diet components because indigestible materials (e.g. cephalopod beaks, crustaceans, and fish otoliths) were commonly identified in stomachs, while other foods have been likely underestimated because their fleshy materials (e.g. meat from snails, mussels, and fish) were digested [23–28]. In simple terms, current inferences related to the diets of monk seals are drawn from likely biased samples of undigested foods consumed before seals died [23,29].

Stable isotope analysis (SIA) can be used to estimate the diets of animals over the long term by relating the isotopes in their tissues to those measured in their foods. Depending on the type of tissue examined, isotope values may reflect the diet over a period of a few days and weeks (e.g. blood serum, hair [30]) to years (e.g. vibrissae and bone, [31]). Consumer tissues typically become enriched in ^{15}N and ^{13}C relative to their prey due to the process of isotopic discrimination (i.e. small offsets of isotope values between dietary sources and animal tissues due to metabolic and digestive processes) [32–36]. Nitrogen isotope values (δ^{15}N) derived from consumer tissues often increase in a predictable fashion at each trophic step [23,29,37–41]. This expected increase can elucidate the trophic position of consumers and their prey in food webs [33,42–45]. Carbon isotope values (δ^{13}C), on the other hand, are often used to identify or confirm the diet or foraging location of consumers because δ^{13}C values can differ for prey that occupy different habitats. In marine ecosystems, the relative abundance of heavy carbon isotopes (^{13}C) has been used to distinguish between habitats where phytoplankton is the only source of organic carbon (i.e. pelagic and offshore habitats) and habitats where macrophytes are a relevant source of organic carbon (i.e. vegetated onshore coastal habitats enriched in ^{13}C) [46,47]. As a result, δ^{13}C values are generally higher in the tissues of marine consumers that forage for resources near the coast vs. off-shore habitats [48].

So far, only one study conducted off the northwest coast of Africa [49] has used SIA to investigate the diet of the Mediterranean monk seal. The study identified monk seals as a coastal consumer [49]; however, its results are not necessarily applicable to monk seals in Greece because the Atlantic Ocean and Mediterranean Sea have different isotopic baselines [49,50], and the demographic structure [8,9] and behaviour [16,51] of monk seals are also different between regions.

In this study, we used δ^{13}C and δ^{15}N values derived from monk seal hair and muscle from their prey to test two predictions deduced from the hypothesis that deceased monk seals in Greece primarily foraged in coastal habitats: (1) seals have similar δ^{13}C values as known coastal prey and (2) seals have elevated δ^{13}C values when compared to pelagic prey. We also used a stable isotope mixing model to estimate the diets of seals using these isotopic data.

2. Methods

2.1. *Sampling*

We used hair samples collected opportunistically from deceased monk seals ($n = 23$; 11 males, 11 females, 1 unknown sex) in the Ionian ($n = 3$) and Aegean seas ($n = 20$) in Greece in 1996–2008 (Figure 1). Isotopes in full length hair will generally reflect the average diet of monk seals over several weeks [52] because monk seals undergo a well-known moulting cycle that occurs once per year [53]. We only used hair from adult ($n = 19$) and juvenile seals ($n = 4$) that were in a good dietary condition (i.e. blubber thickness at thorax for adults >5 cm, and juveniles >3 cm) at the time of their death. We did not sample seals <2 years old because nursing pups feed on their mothers' milk, enriching ^{15}N in their tissues [54]. In order to distinguish juveniles from moulted, lactating pups (due to the absence of characteristic external morphological features), we estimated the age of juvenile seals by counting growth layer groups in the cementum adjacent to the root tip using unprocessed longitudinal or transverse sections. The mean age of juveniles was 3.3 years [55]. We distinguished juveniles from adults based on the characteristic differences of their pelage [53].

Figure 1. Map of Greece indicating the locations where 23 deceased Mediterranean monk seals were sampled in 1996–2008. Shaded areas indicate the four locations where known monk seal prey species were collected in 2008.

We purchased known coastal and pelagic monk seal foods ($n = 73$) from three local fish markets around the Aegean Sea and one research vessel from the Hellenic Centre for Marine Research; all foods were collected in 2008 within the core distribution of the Mediterranean monk seal in Greece (Figure 1). We measured the fork length, carapace width, and body mass of one to five individuals of each species ($n = 32$). We sampled a 2×2 cm piece of white muscle or tentacle (from cephalopods) from each specimen, rinsing each repeatedly with distilled water. We then froze all foods until preparation for SIA.

2.2. *Stable isotope analysis*

We removed seal hair from the skin and rinsed it in a 2:1 chloroform–methanol solution. We then rinsed each sample five times with distilled water and air-dried samples for at least one week. We freeze-dried subsamples of muscle from each specimen; then crushed each sample with a mortar and pestle, passing the materials through a 500 μm screen to ensure a high-level of homogenization. We did not extract lipids from food samples because fat content was <1 % [56]. We weighed ∼20 whole hairs (0.8–1.2 mg) from each seal and ∼0.5 mg of muscle from each seal food into tin cups separately.

The Water Resource Sciences Lab (UBC, Canada) analysed tissue samples using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer. Samples were combusted and the tissue carbon and nitrogen converted to CO_2 and N_2, which were separated chromatographically with a Euro EA. Reaction columns contained isotope grade combustion and reduction reagents, and gases were ultra high purity grade. Ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in the gases were measured with a Micromass IsoPrime isotope ratio mass spectrometer with standard reference gasses (CO_2 and N_2), and calibrated to National Institute of Standards and Technology calibration standards (8547 ammonium sulphate, and 8542 sucrose for $^{15}N/^{14}N$ and $^{13}C/^{12}C$, respectively). Stable isotope ratios are expressed in delta (δ) notation as parts per thousand or per mil (‰). SIA results are reported and defined as

$$\delta^j X = \frac{(^jX/^iX)_{\text{sample}}}{(^jX/^iX)_{\text{standard}}} - 1, \tag{1}$$

where ^jX is the heavier isotope (^{13}C, ^{15}N) and ^iX the lighter isotope (^{12}C, ^{14}N) in the analytical sample and international measurement standard [57]; reference standards are Vienna Peedee Belemnite for C and atmospheric N_2 for N. Replicate standard reference materials (valine) were run at the start and end of the sample run, and after every nine samples. Standard deviations of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ to standard reference materials were 0.49 ‰ and 0.02 ‰, respectively. Standard reproducibilities for replicate samples ($n = 3$) of individual prey species and monk seal fur were 0.1 ‰ and 0.02 ‰ for $^{15}N/^{14}N$ and $^{13}C/^{12}C$, respectively. We did not correct $\delta^{13}C$ values for the Suess effect, as the change in isotope values from the mid-1990s to 2008 was negligible.

2.3. *Data analyses*

We used a Kolmogorov–Smirnov test and a Levene's test to check the assumptions that isotope values for seals and their foods are normally distributed and variances are equal between sample groups, respectively. We then used either Student's t-tests or Mann–Whitney U tests to compare isotope values between seal age classes (i.e. juvenile vs adult), sexes, coastal, and pelagic prey species, and to test predictions that $\delta^{13}C$ values for seal hair are similar to their coastal foods and elevated in $\delta^{13}C$ relative to their pelagic foods (with all foods corrected for isotopic discrimination). To compare isotope values of seals to their prey, we added mean discrimination factors ($\Delta^{13}C = 2.8 \pm 0.5$ ‰; $\Delta^{15}N = 2.7 \pm 0.4$ ‰) for seal (Harp seals *Pagophilus groenlandicus*, Common seals *Phoca vitulina*, Ringed seals *Phoca hispida*) hair to the isotope values of each food sample

[32,58]. We set all significance tests to $\alpha = 0.05$ and carried out these analyses using the SPSS (ver. 15).

We also used the stable isotope mixing model, IsotopeR [59] to estimate the proportions ($\bar{x}$, 95 % CI) of coastal and pelagic foods in seal diets. We used all discrimination-corrected food data to conduct this analysis. We applied discrimination factor error to each source ($\delta^{13}C = 0.5$ ‰; $\delta^{15}N = 0.4$ ‰; i.e. the SD of mean discrimination factors described above) as well as measurement error to each sample ($\delta^{13}C$ and $\delta^{15}N = 0.2$ ‰) to more accurately estimate dietary parameters [59].

3. Results

Monk seal $\delta^{13}C$ and $\delta^{15}N$ values for hair ranged from −16.4 to −13.8 ‰ and 8.2–13.6 ‰, respectively (Table 1). We found no differences in isotope values between seal age classes ($\delta^{13}C$: $t = 1.196$, $P = .245$; $\delta^{15}N$: $t = 1.731$, $P = .1$) or between sexes ($\delta^{13}C$: $t = 0.497$, $P = .625$; $\delta^{15}N$: $t = 0.017$, $P = .987$) (Table 1).

We found that isotope values for prey muscle were different between coastal and pelagic prey ($\delta^{13}C$: $W = 37.000$ $P < .001$; $\delta^{15}N$: $t = 2.780$; $P = .007$; Table 2, Figure 2). As predicted, we found that $\delta^{13}C$ values for seal hair were similar ($W = 687.000$ $P = .797$) to their known coastal foods and elevated in $\delta^{13}C$ relative to their known pelagic foods (when all foods were corrected for isotopic discrimination) ($\delta^{13}C$: $t = 7.410$, $P < .001$; Figure 2).

Table 1. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values derived from the hair of deceased Mediterranean monk seals from Greece, 1996–2008.

Sampling date	Sex	Reproductive state	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
02/02/1996	Female	Adult	−14.0	9.6
24/09/1997	Male	Adult	−14.4	10.4
06/05/1998	Male	Adult	−15.8	9.9
22/08/1999	Female	Adult	−14.2	9.6
15/04/1999	Female	Adult	−15.3	8.2
02/06/1999	Female	Adult	−15.6	9.3
10/08/2000	Female	Adult	−16.4	10.8
21/02/2000	Male	Juvenile	−14.7	9.4
20/04/2000	Male	Juvenile	−15.2	9.5
07/05/2002	Male	Adult	−15.1	11.3
24/01/2002	Male	Juvenile	−14.8	8.2
28/11/2002	Male	Juvenile	−15.3	9.4
18/05/2003	Male	Adult	−15.3	10.6
12/10/2004	Female	Adult	−14.7	10.9
17/12/2005	Male	Adult	−14.7	9.1
01/06/2005	Unknown	Adult	−14.9	10.2
19/05/2006	Female	Adult	−14.5	8.3
27/01/2006	Male	Adult	−15.5	11.6
31/05/2007	Female	Adult	−14.4	9.9
19/09/2007	Female	Adult	−15.1	9.5
03/07/2007	Male	Adult	−13.8	11.0
27/02/2008	Female	Adult	−14.2	10.8
04/09/2008	Female	Adult	−14.7	13.6
Mean ± SD			−14.8 ± 0.6	10.0 ± 1.2
Female ($N = 11$)				
Mean ± SD			−14.8 ± 0.7	10.0 ± 1.4
Male ($N = 11$)				
Mean ± SD			−14.9 ± 0.5	10.0 ± 1.0
Adult ($N = 19$)				
Mean ± SD			−14.8 ± 0.6	10.2 ± 1.2
Juvenile ($N = 4$)				
Mean			−15.0 ± 0.2	9.1 ± 0.6

Table 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values derived from the muscle of known Mediterranean monk seal prey from Greece in 2008. The means and standard deviations (SD) of isotope values are provided when more than one sample was measured.

Class	Family	Species	Group	*N*	$\delta^{13}C$ (‰)	SD	$\delta^{15}N$ (‰)	SD
Actinopterygii	Citharidae	*Citharus linguatula*	Coastal	1	−15.1		14.4	
Actinopterygii	Congridae	*Conger conger*	Coastal	1	−14.9		12.1	
Actinopterygii	Gobiidae	*Gobius geniporus*	Coastal	1	−13.6		14.9	
Actinopterygii	Labridae	*Coris julis*	Coastal	1	−15.9		13.6	
Actinopterygii	Mullidae	*Mullus barbatus*	Coastal	5	−15.1	0.7	13.2	0.9
Actinopterygii	Mullidae	*Mullus surmuletus*	Coastal	4	−15.2	1.2	10.5	2.7
Actinopterygii	Phycidae	*Phycis blennoides*	Coastal	1	−15.2		15.3	
Actinopterygii	Phycidae	*Phycis sp.*	Coastal	1	−15.8		9.7	
Actinopterygii	Scorpaenidae	*Scorpaena porcus*	Coastal	1	−14.5		9.8	
Actinopterygii	Scorpaenidae	*Scorpaena scrofa*	Coastal	1	−15.3		13.6	
Actinopterygii	Scorpaenidae	*Scorpaena notata*	Coastal	1	−14.7		14.6	
Actinopterygii	Serranidae	*Serranus hepatus*	Coastal	1	−14.6		14.6	
Actinopterygii	Serranidae	*Serranus cabrilla*	Coastal	2	−16.0	0.2	11.1	0.9
Actinopterygii	Sparidae	*Diplodus annularis*	Coastal	1	−13.6		10.1	
Actinopterygii	Sparidae	*Pagellus acarne*	Coastal	1	−15.9		14.3	
Actinopterygii	Sparidae	*Pagellus erythrynus*	Coastal	1	−13.3		13.5	
Actinopterygii	Sparidae	*Oblada melanura*	Coastal	1	−15.7		11.0	
Actinopterygii	Sparidae	*Sarpa salpa*	Coastal	1	−16.8		11.9	
Actinopterygii	Sparidae	*Pagrus pagrus*	Coastal	3	−12.7	0.7	15.3	0.6
Actinopterygii	Sparidae	*Boops boops*	Coastal	5	−16.0	0.4	11.1	2.6
Actinopterygii	Sparidae	*Diplodus sargus sargus*	Coastal	4	−13.8	1.3	10.6	0.3
Cephalopoda	Loliginidae	*Loligo vulgaris*	Coastal	4	−14.2	0.4	16.9	0.4
Cephalopoda	Octopodidae	*Eledone moschata*	Coastal	5	−15.1	0.5	11.5	2.4
Cephalopoda	Octopodidae	*Octopus vulgaris*	Coastal	4	−12.3	0.3	12.8	1.0
Cephalopoda	Sepiidae	*Sepia officinalis*	Coastal	4	−15.0	0.4	9.8	0.4
Malacostraca	Penaeidae	*Penaeus kerathurus*	Coastal	4	−15.7	0.3	11.2	0.2
Malacostraca	n.a	Crab	Coastal	3	−15.0	0.1	12.8	0.2
Actinopterygii	Centracanthidae	*Spicara maena*	Pelagic	2	−17.2	0.5	9.7	0.6
Actinopterygii	Carangidae	*Trachurus trachurus*	Pelagic	4	−16.2	0.2	11.7	0.5
Actinopterygii	Centracanthidae	*Spicara smaris*	Pelagic	4	−16.4	0.1	8.4	0.5
Actinopterygii	Centracanthidae	*Spicara flexuosa*	Pelagic	1	−15.9		13.2	
Actinopterygii ($\bar{x}$, 25 species)				49	−18.0	1.2	9.1	2.2
Cephalopoda ($\bar{x}$, 4 species)				17	−17.0	1.2	9.9	2.9
Malacostraca ($\bar{x}$, 2 species)				7	−18.1	0.4	9.1	0.8
Coastal species ($\bar{x}$, 27 species)				62	−17.5	1.2	9.6	2.3
Pelagic species ($\bar{x}$, 4 species)				11	−19.2	0.4	7.6	1.7

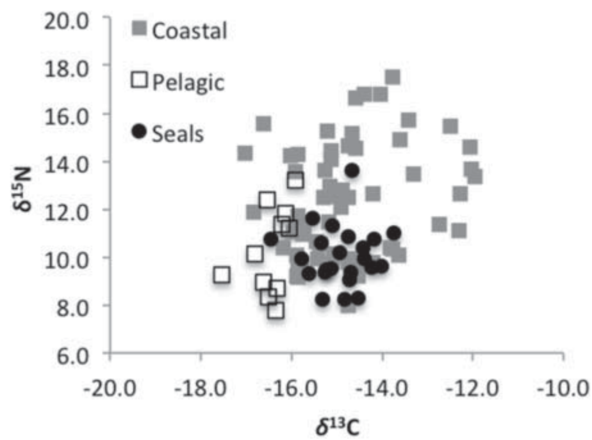

Figure 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of Mediterranean monk seals (1996–2008) and their known coastal and pelagic prey items sampled in Greece in 2008. All prey were corrected for isotopic discrimination (see text for details).

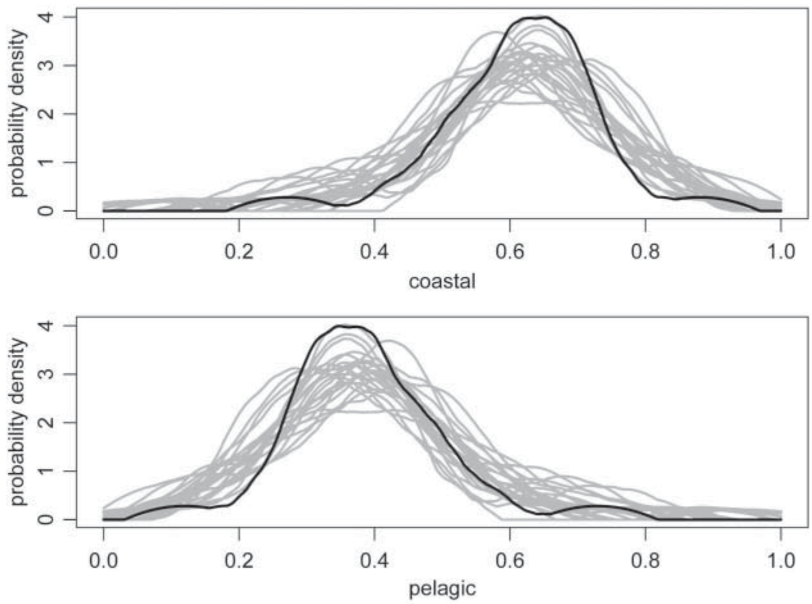

Figure 3. Proportional contributions (expressed as marginal posterior distributions) of coastal and pelagic prey in seal diets estimated at the population (black) and individual-level (gray) by IsotopeR [59].

IsotopeR estimated, on average, that 61 % (CI = 39–79 %) and 39 % (CI = 21–61 %) of seal diets were derived from coastal and pelagic sources, respectively (Figure 3).

4. Discussion

Results from this study confirm that Mediterranean monk seals from Greece foraged primarily for coastal prey before they died. In particular, the results of our δ^{13}C analysis indicate that monk seals commonly forage in benthic and reef-associated habitats along the coast throughout the year. We also found that nitrogen isotopes values varied (range of ∼5 ‰) among seals in the eastern Mediterranean Sea, suggesting a diverse diet derived from multiple trophic levels. These results are in accordance with the current information available about the species in the eastern Mediterranean Sea [10,13,21]. This information, however, is limited and originated mainly from studies of stomach content analysis of dead individuals, which have fundamental methodological biases.

Stable isotopes have been used in past studies to investigate intrapopulation niche variation and foraging strategies [60–62]. Differences in the ecological niches and foraging strategies between both sexes and age classes have been well documented in several pinniped species: southern elephant seals (*Mirounga leonina*) [63,64], Antarctic fur seals (*Arctocephalus gazella*) [65], and northern fur seals (*Callorhinus ursinus*) [66]. In this study, we found no significant differences in δ^{15}N and δ^{13}C values between juveniles and adults and between females and males; similar isotope values among seals indicate that seals forage for similar prey, albeit in different relative proportions (the latter causing the observed variation in their isotope values) [67].

The foraging strategies of pinnipeds are controlled by a number of factors, such as differences in physiology [68,69], energetic requirements, development, learning, or prey preferences [67]. The complex, yet generally similar diets of seals, as suggested by isotopic analysis, implicate two main factors that may play a significant role in their foraging behaviour. First, the marine environment throughout Greece, when compared to the oceanic environments most pinnipeds live and breed in, is relatively uniform in its bathymetry and diversity of species. Second, Mediterranean monk seals, especially breeding females and their pups spend a large portion of the year on land (due to their extended lactation period) in suitable coastal caves [12,13]. This may last up to five months and is the longest of any phocid species [70,71]. During this time, nursing females are likely restricted by duration, and therefore distance, of their foraging trips by their pups' fasting abilities [72]. Due to the mating system and the social structure of Mediterranean monk seal populations several juveniles and the dominant adult males that also use these suitable caves [73] are likely to forage in areas close to the suitable pupping caves as well. The use of these coastal caves in the eastern Mediterranean Sea occurs mainly during the pupping season in autumn and winter [13,74], and little is known about habitat use and the activity patterns of the species on land or at sea during the rest of the year. Hair from monk seals in Greece will likely reflect the diet of the species during the limited time when the new fur is grown and therefore is not suitable for seasonal dietary comparisons. In order to test more comprehensively for seasonal differences in monk seal diets in Greece, we recommend a longitudinal diet analysis of seal vibrissae or the collection and analysis of a variety of metabolic tissues with different isotopic turnover rates [32]. In particular, isotope values from segments of vibrissae could be compared among seals to understand their seasonal diets.

Foraging behaviour of seals is controlled largely by the diving capabilities and physiology of individuals, which in turn are related to the size of the animal [68,69]. Despite marked differences in their external appearances [75], differences in size between female and male adult Mediterranean monk seals are small [73], which may also partially explain their similar diets. If this hypothesis were true, however, we should have observed a significant difference in diet between juvenile and adult seals. Interestingly, data from diving studies on rehabilitated juvenile monk seals indicate that they too are capable of diving to depths of 200 m [16], a depth that includes most of the major prey identified during stomach content analysis [20,21]. Although we cannot disregard the possibility of adult monk seals being capable of diving to greater depths, the available dive and foraging data in the eastern Mediterranean Sea indicate that this is not necessary for the species as all energetic requirements appear to be provided by foraging in areas shallower than 200 m in the highly productive sub-littoral zone.

5. Conclusions – management and conservation implications

SIA was used for the first time in the eastern Mediterranean Sea to investigate the diets of Mediterranean monk seals. Although it is currently impossible to reconstruct the diets of seals by taxa using carbon and nitrogen isotope data (because some prey of different taxa have similar isotopic signatures [76]), we were able to test predictions related to their general foraging patterns. Stable isotope values derived from monk seal hair are consistent with a diet composed primarily of coastal prey in the eastern Mediterranean Sea. In addition, the results of the study indicated that adult female and male, and juvenile and adult monk seals have similar diets. These findings have two important implications for this critically endangered species. First, the increased use of marine coastal areas, in combination with the intensive use of coastal caves, dictate the need for a wide-ranging and systematic protection of the coastal ecosystems through the creation of a functional network of marine protected areas. As a coastal consumer, Mediterranean monk seals have negative interactions with coastal fishermen [77]. This calls for the implementation of effective management measures to mitigate such interactions. The effective protection of coastal habitats and the proper mitigation of human–seal conflicts have been identified in the 'National Strategy and Action Plan for the Monk Seal in Greece 2009–2015' and the 'Action Plan for the mitigation of the negative effects of monk seals–fisheries interactions in Greece' as among the top conservation priorities for the protection of Mediterranean monk seals in Greece [57,78]. Considering the critically endangered status of the Mediterranean monk seal these conservation priorities should be implemented as soon as possible.

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References

[1] Dehn L-A, Sheffield GG, Follmann EH, Duffy LK, Thomas DL, O'Hara TM. Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biol. 2007;30:167–181.
[2] Crawford K, McDonald RA, Bearhop S. Applications of stable isotope techniques to the ecology of mammals. Mamm Rev. 2008;38:87–107.
[3] Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE, Costa DP. Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. Oecologia. 2012;169:395–406.
[4] Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. The ecology of individuals: incidence and implications of individual specialization. Am Nat. 2003;161:1–28.
[5] International Union for Conservation of Nature. IUCN Red List of Threatened Species. Version 2010.3; 2010.
[6] Johnson WM, Karamanlidis AA, Dendrinos P, de Larrinoa PF, Gazo M, Gonzalez LM, Güçlüsoy H, Pires R, Schnellmann M. Monk seal fact files. Biology, behaviour, status and conservation of the Mediterranean monk seal, *Monachus monachus*. The Monachus Guardian; 2006. [cited 2014 June 17]. Available from: http://www.monachus.org.
[7] Pires R, Neves HC, Karamanlidis AA. The critically endangered Mediterranean monk seal *Monachus monachus* in the archipelago of Madeira: priorities for conservation. Oryx. 2008;42:278–285.
[8] MOm. Status of the population of the Mediterranean monk seal (*Monachus monachus*) in Greece. Athens: MOm/Hellenic Society for the Study and Protection of the Monk seal; 2007.
[9] Martínez-Jauregui M, Tavecchia G, Cedenilla MA, Coulson T, Fernández de Larrinoa P, Muñoz M, González LM. Population resilience of the Mediterranean monk seal *Monachus monachus* at Cabo Blanco peninsula. Mar Ecol Prog Ser. 2012;461:273–281.
[10] Adamantopoulou S, Androukaki E, Kotomatas S. The distribution of the Mediterranean monk seal in Greece based on an information network. Vol. 1, Contributions to the Zoogeography and Ecology of the Eastern Mediterranean Region; 1999. p. 399–404.
[11] Güçlüsoy H, Kiraç CO, Veryeri NO, Savas Y. Status of the Mediterranean monk seal, *Monachus monachus* (Hermann, 1779) in the coastal waters of Turkey. EU J Fish Aquat Sci. 2004;21:201–210.
[12] Guçu AC, Guçu G, Orek H. Habitat use and preliminary demographic evaluation of the critically endangered Mediterranean monk seal (*Monachus monachus*) in the Cilician Basin (Eastern Mediterranean). Biol Conserv. 2004;116:417–431.
[13] Dendrinos P, Karamanlidis AA, Kotomatas S, Legakis A, Tounta E, Matthiopoulos J. Pupping habitat use in the Mediterranean monk seal: a long-term study. Mar Mamm Sci. 2007;23:615–628.
[14] Karamanlidis AA, Paravas V, Trillmich F, Dendrinos P. First observations of parturition and postpartum behavior in the Mediterranean monk seal (*Monachus monachus*) in the Eastern Mediterranean. Aquat Mamm. 2010;36:27–32.
[15] Adamantopoulou S, Androukaki E, Dendrinos P, Kotomatas S, Paravas V, Psaradellis M, Tounta E, Karamanlidis AA. Movements of Mediterranean monk seals (*Monachus monachus*) in the Eastern Mediterranean sea. Aquat Mamm. 2011;37:256–261.
[16] Dendrinos P, Karamanlidis AA, Androukaki E, McConnell BJ. Diving development and behavior of a rehabilitated Mediterranean monk seal (*Monachus monachus*). Mar Mamm Sci. 2007;23:387–397.
[17] Marchessaux D. Recherches sur la Biologie, l'Ecologie et le Statut du Phoque Moine, *Monachus monachus*. Marseille: GIS Posidonie Publ.; 1989.
[18] Neves HC. Preliminary findings on the feeding strategy of the Monk Seal *Monachus monachus* (Pinnipedia: Monachinae) on the Desertas islands. Boletim do Museum Municipal do Funchal. 1998;Suppl. No. 5:263–271.
[19] Salman A, Bilecenoglu M, Güçlüsoy H. Stomach contents of two Mediterranean monk seals (*Monachus monachus*) from the Aegean Sea, Turkey. J Mar Biol Assoc UK. 2001;81:719–720.
[20] Karamanlidis AA, Kallianiotis A, Psaradellis M, Adamantopoulou S. Stomach contents of a subadult Mediterranean monk seal (*Monachus monachus*) from the Aegean Sea. Aquat Mamm. 2011;37:280–283.
[21] Pierce GJ, Hernandez-Milian G, Santos MB, Dendrinos P, Psaradellis M, Tounta E, Androukaki E, Edridge A. Diet of the Monk seal (*Monachus monachus*) in Greek waters. Aquat Mamm. 2011;37:284–297.

[22] Cebrian D, Fatsea H, Mytilineou C. Some data on biometry and stomach content of a Mediterranean Monk Seal found in Santorini Island (Greece). Rapport Commission International pour l'exploration scientifique de la Mer Mediterranée. 1990;32:237.
[23] Burns JM, Trumble SJ, Castellini MA, Testa JW. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biol. 1998;19:272–282.
[24] Murie DJ, Lavigne DM. Interpretation of otoliths in stomach contents analyses of phocid seals: quantifying fish consumption. Can J Zool. 1986;64:1152–1157.
[25] Gales NJ, Cheal AJ. Estimating diet composition of the Australian sea lion (*Neophoca cinerea*) from scat analysis – an unreliable technique. Wildl Res. 1992;19:447–468.
[26] Bowen WD. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can J Fish Aquat Sci. 2000;57:898–905.
[27] Sheffield G, Fay FH, Feder H, Kelly BP. Laboratory digestion of prey and interpretation of walrus stomach contents. Mar Mamm Sci. 2001;17:310–330.
[28] Pierce GJ, Boyle PR. A review of methods for diet analysis in piscivorous marine mammals. Ocean Mar Biol Ann Rev. 1991;29:409–486.
[29] Hobson KA, Sease JL, Merrick RL, Piatt JF. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Mar Mamm Sci. 1997;13:114–132.
[30] Aurioles-Gamboa D, Newsome SD, Salazar-Pico S, Koch PL. Stable isotope differences between sea lions (*Zalophus*) from the Gulf of California and Galapagos Islands. J Mammal. 2009;90:1410–1420.
[31] Zhao LY, Schell DM. Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. Mar Ecol Prog Ser. 2004;281:267–273.
[32] Hobson KA, Schell DM, Renouf D, Noseworthy E. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci. 1996;53:528–533.
[33] Kelly JF. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool. 2000;78:1–27.
[34] Kurle CM. Stable isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. Can J Zool. 2002;80:902–909.
[35] Zhao LY, Shell DM, Castellini MA. Dietary macronutrients influence ^{13}C and ^{15}N signatures of pinnipeds: captive feeding studies with harbour seals (*Phoca vitulina*). Comp Biochem Physiol A: Mol Integr Physiol. 2006;143: 469–478.
[36] Hopkins JB, Koch PL, Ferguson JM, Kalinowski ST. The changing anthropogenic diets of American black bears over the past century in Yosemite National Park. Front Ecol Environ. 2014;12:107–114.
[37] Burton RK, Koch PL. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia. 1999;119:578–585.
[38] Hirons AC, Schell DM, Finney BP. Temporal records of δ^{13}C and δ^{15}N in North Pacific pinnipeds: inferences regarding environmental change and diet. Oecologia. 2001;129:591–601.
[39] Kurle CM, Worthy GAJ. Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. Mar Ecol Prog Ser. 2002;236:289–300.
[40] Zhao LY, Castellini MA, Mau TL, Trumble SJ. Trophic interactions of Antarctic seals as determined by stable isotope signatures. Polar Biol. 2004;27:368–373.
[41] Newsome SD, Etnier MA, Aurioles-Gamboa D. Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Mar Mamm Sci. 2006;22:556–572.
[42] Wada E, Mizutani H, Minagawa M. The use of stable isotopes for food web analysis. Crit Rev Food Sci Nutr. 1991;30:361–371.
[43] Hobson KA, Welch HE. Determination of trophic relationships of pinnipeds in Alaska marine food web using δ^{13}C and δ^{15}N analysis. Mar Ecol Prog Ser. 1992;84:9–18.
[44] Gannes LZ, Martinez del Rio C, Koch P. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comp Biochem Phys A. 1998;119:725–737.
[45] Vander Zanden MJ, Rasmussen JB. Variation in δ^{15}N and δ^{15}C trophic fractionation: Implications for aquatic food web studies. Limnol Oceanogr. 2001;46:2061–2066.
[46] Rubenstein DR, Hobson KA. From birds to butterflies: animal movements patterns and stable isotopes. Trends Ecol Evol. 2004;19:256–263.
[47] Cardona L, Revelles M, Sales M, Aguilar A, Borrell A. Meadows of the seagrass *Posidonia oceanica* are a relevant source of organic matter for adjoining ecosystems. Mar Ecol Prog Ser. 2007;335:123–131.
[48] Michener RH, Kaufman L. Stable isotope ratios as tracers in marine food webs: an update. In: Michener RH, Lajtha K, editors. Stable isotopes in ecology and environmental science. Oxford: Wiley-Blackwell; 2007. p. 238–282.
[49] Pinela AM, Borrell A, Cardona L, Aguilar A. Stable isotope analysis reveals habitat partitioning among marine mammals off the NW African coast and unique trophic niches for two globally threatened species. Mar Ecol Prog Ser. 2010;416:295–306.
[50] Giménez J, Gómez-Campos E, Borrell A, Cardona L, Aguilar A. Isotopic evidence of limited exchange between Mediterranean and eastern North Atlantic fin whales. Rapid Commun Mass Spectrom. 2013;27:1801–1806.
[51] Gazo M, Lydersen C, Aguilar A. Diving behaviour of Mediterranean monk seal pups during lactation and post weaning. Mar Ecol Prog Ser. 2006;308:303–309.
[52] Aurioles D, Koch PL, Le Boeuf BJ. Differences in foraging location of mexican and california elephant seals: evidence from stable isotopes in pups. Mar Mamm Sci. 2006;22:326–338.

[53] Badosa E, Pastor T, Gazo M, Aguilar A. Moult in the Mediterranean monk seal from Cap Blanc, western Sahara. Afr Zool. 2006;41:183–192.

[54] Jenkins SG, Partridge ST, Stephenson TR, Farley SD, Robbins CT. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. Oecologia. 2001;129:336–341.

[55] Murphy S, Spradlin TR, Mackey B, McVee J, Androukaki E, Tounta E, Karamanlidis AA, Dendrinos P, Joseph E, Lockyer C, Matthiopoulos J. Age determination, growth and age-related mortality of Mediterranean monk seals (*Monachus monachus*). Endang Spec Res. 2011;16:149–163.

[56] Davenport SR, Bax NJ. A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. Can J Fish Aquat Sci. 2002;59:514–530.

[57] Action Plan for the mitigation of the negative effects of monk seal – fisheries interactions in Greece. Athens, Greece: MOm, WWF Greece, Fisheries Research Institute; 2009.

[58] Lesage V, Hammill OM, Kovacs MK. Diet tissue fractionation of stable carbon and nitrogen isotopes in phocid seals. Mar Mamm Sci. 2002;185:182–193.

[59] Hopkins JB, Ferguson JM. Estimating the diets of animals using stable isotopes and a comprehensive bayesian mixing model. PLoS ONE. 2012;7:e28478.

[60] Araujo MS, Bolnick DI, Machado G, Giaretta AA, dos Reis SF. Using δ^{13}C stable isotopes to quantify individual level diet variation. Oecologia. 2007;152:643–654.

[61] Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H. Determining trophic level niche width: a novel approach using stable isotope analysis. J Anim Ecol. 2004;73:1007–1012.

[62] Jaeger A, Connan M, Richard P, Cherel Y. Use of stable isotopes to quantify seasonal changes of trophic niche and levels of population and individual specialisation in seabirds. Mar Ecol Prog Ser. 2010;401:269–277.

[63] Bailleul F, Authier M, Ducatez S, Roquet F, Charassin JB, Cherel Y, Guinet C. Looking at the unseen: combining animal bio-logging and stable isotopes to reveal a shift in the ecological niche of a deep diving predator. Ecography. 2010;33:709–719.

[64] Newland C, Field IC, Nichols PD, Bradshaw CJA, Hindell MA. Blubber fatty acid profiles indicate dietary resource partitioning juvenile southern between adult and juvenile southern elephant seals. Mar Ecol Prog Ser. 2009;384: 303–312.

[65] Cherel Y, Kernaléguen L, Richard P, Guinet C. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. Biol Lett. 2009;5:830–832.

[66] Zeppelin TK, Orr AJ. Stable isotope and scat analyses indicate diet and habitat partitioning in northern fur seals *Callorhinus ursinus* across the eastern Pacific. Mar Ecol Prog Ser. 2010;409:241–253.

[67] Orr AJ, Newsome SD, Laake JL, VanBlaricom GR, DeLong RL. Ontogenetic dietary information of the California sea lion (*Zalophus californianus*) assessed using stable isotope analysis. Mar Mamm Sci. 2012;28:714–732.

[68] Burns JM. The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. Can J Zool–Rev Can Zool. 1999;77:737–747.

[69] Horning M, Trillmich F. Development of hemoglobin, hematocrit, and erythrocyte values in Galapagos fur seals. Mar Mamm Sci. 1997;13:100–113.

[70] Aguilar A, Cappozzo LH, Gazo M, Pastor T, Forcada J, Grau E. Lactation and mother-pup behaviour in the Mediterranean monk seal Monachus monachus: an unusual pattern for a phocid. J Mar Biol Assoc UK. 2007;87:93–99.

[71] Dendrinos P. Contribution to the study of the Mediterranean monk seal's (*Monachus monachus*) ecology and biology at the island complex of Northern Sporades. Greece: National and Kapodistrian University of Athens; 2011.

[72] Melin SR, DeLong RL, Thomason JR, VanBlaricom GR. Attendance patterns of California sea lion (*Zalophus californianus*) females and pups during the nonbreeding season at San Miguel Island. Mar Mamm Sci. 2000;16: 169–185.

[73] Pastor T, Cappozzo HL, Grau E, Amos W, Aguilar A. The mating system of the Mediterranean monk seal in the Western Sahara. Mar Mamm Sci. 2011;27:E302–E320.

[74] Dendrinos P, Tounta E, Kotomatas S, Kottas A. Recent data on the Mediterranean monk seal population of the Northern Sporades. Bios (Macedonia/Greece). 1994;2:11–16.

[75] Samaranch R, Gonzalez LM. Changes in morphology with age in Mediterranean monk seals (*Monachus monachus*). Mar Mamm Sci. 2000;16:141–157.

[76] Dehn L-A, Follmann EH, Thomas DL, Sheffield GG, Rosa C, Duffy LK, O'Hara TM. Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci Total Environ. 2006;362:103–123.

[77] Karamanlidis AA, Androukaki E, Adamantopoulou S, Chatzispyrou A, Johnson WM, Kotomatas S, Papadopoulos A, Paravas V, Paximadis G, Pires R, Tounta E, Dendrinos P. Assessing accidental entanglement as a threat to the Mediterranean monk seal *Monachus monachus*. End Spec Res. 2008;5:205–213.

[78] Notarbartolo di Sciara G, Adamantopoulou S, Androukaki E, Dendrinos P, Karamanlidis AA, Paravas V, Kotomatas S. National strategy and action plan for the conservation of the Mediterranean monk seal in Greece, 2009–2015. Athens: MOm; 2009.