Trophic niche segregation between sympatric chondrichthyes

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September 2012

Abstract

Understanding how marine predators are structured and how they interact is a scientific challenge. Nowadays, to learn about trophic niche segregation, ecologists use two complementary methodologies, stomach content and isotopic analysis.

The aim of this study was to examine the trophic ecology of three chondrichthyans (velvet belly lanternshark, blackmouth catshark and rabbitfish) that are common on discards of the Gulf of Lion (NW Mediterranean). For each species, we examined the diet of each species and the potential differences in diet between sexes, depths and individuals. We combined two methodologies; the analysis of stomach content (%N and %FO) and the analysis of stable isotope to perform Bayesian isotopic mixing models (combining isotopic values of predators and prey). We also calculated the trophic levels of each species.

Our results showed a clear segregation between rabbitfish and the other two species. Rabbitfish showed a diet mainly composed by crabs followed by fish while velvet belly lanternshark and blackmouth catsharks were composed mainly by shrimps followed by crabs. In addition we found depth-related variations in the dietary habits probably related to natural variations in food availability and morphological effects. There were significant differences in the trophic level between species. At individual level, our results revealed some shifts in individual diets and in some cases individuals from one species had greater similarity in diet with individuals of other species than with individuals of the same species.

This study illustrates the importance of using complementary approaches, which will allow more efficient monitoring of food web changes. Furthermore, individual isotopic models open new opportunities for study individual specialization.

Resum

Entendre com els depredadors marins estan estructurats i com interactuen és un repte científic. Avui en dia, per estudiar la segregació tròfica, els ecòlegs utilitzen dues metodologies complementàries, el contingut estomacal i l'anàlisi isotòpic.

L'objectiu d'aquest estudi va ser examinar l'ecologia tròfica de tres condrictis (Negret, Moixina i Guilla) que són comuns en els descarts del Golf de Lleó (NO Mediterrani). Per a cada espècie, es va analitzar la dieta i les possibles diferències de la dieta entre els sexes, les profunditats i els individus. Hem combinat dues metodologies, l'anàlisi de contingut estomacal (% N i % FO) i l'anàlisi d'isòtops estables per realitzar models bayesians de mescla isotòpica (combinant els valors isotòpics de depredadors i preses). També es van calcular els nivells tròfics de cada espècie.

Els nostres resultats van mostrar una clara segregació entre quimeres i les altres dues espècies. La quimera mostrava una dieta composta principalment per crancs i peixos, mentre que la dieta del negret i la moixina estaven formades principalment per gambes i crancs. A més trobem variacions de la dieta relacionats amb la profunditat probablement degut a variacions naturals en la disponibilitat d'aliments i degut a efectes morfològics. No hi va haver diferències significatives del nivell tròfic entre les espècies. A nivell individual, els resultats van revelar alguns canvis en les dietes individuals i en alguns casos els individus d'una espècie presentaven una major similitud en la dieta amb individus d'altres espècies que amb els individus de la mateixa espècie.

Aquest estudi il·lustra la importància d'utilitzar mètodes complementaris, la qual cosa permetrà un seguiment més eficaç dels canvis en les xarxes tròfiques. D'altra banda, els models isotòpics individuals obrin noves oportunitats per estudiar la especialització individual.

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1. INTRODUCTION

There is current concern about the oceans health and the effects of an increased fishing intensity (Pauly et al. 2003). To move towards an effective management of fishing activities, it is pivotal to understand and quantify the interactions between the components of marine ecosystems (Cury et al. 2005).The Mediterranean Sea is an example of a region with a high fishing intensity and habitat modification (Bianchi & Morri 2000; Coll et al. 2010). For this reason, an important step to preserve marine biodiversity in the Mediterranean is to understand the role of marine species in the ecosystems, thus advancing on our knowledge on population dynamics, the trophic ecology of each species and the ecological interactions between them, and their trophic position in the food web.

There is little doubt that marine predators, like chondrichthyans, play a pivotal role on the structure of marine ecosystems worldwide via top-down control within the food-webs (Heithaus et al. 2008). Reductions in chondrichthyan populations or changes in their feeding habits could provoke trophic cascades, affecting the structure and functioning of entire marine ecosystems (Valls et al. 2011, Shiffman & Gallagher 2012, Preti et al. 2012). There are clearly evidences indicating that Mediterranean chondrichthyans populations have declined and are declining due to human exploitation of fisheries, habitat loss and pollution (Stevens 2000, Massutí & Moranta 2003, Sion & Bozzano 2004, Ferretti et al. 2008, Coll et al. in press). Furthermore, because chondrichthyans have low fecundity and slow developing between immature and mature states, these organisms have reduced capacity to recover after reductions in their populations (Stevens 2000, Dulvy & Reynolds 2002, Baum et al. 2003, Lucifora et al. 2009, Worm et al. 2009). However, basic biological information is scarce. For this reason, the dynamics of potential trophic cascade effects cannot be accurately understood in many ecosystems (Cortes 1999, Heithaus et al. 2008, Ajemian et al. 2012).

Mediterranean chondrichthyans include 45 species of sharks, 34 batoid species and an unique species of chimaera classified as a near threatened

species in the UICN Red List (Malak et al. 2011).The commercial value of chondrichthyans is very low compared to that of fishes, crustaceans or cephalopods in the Mediterranean Sea due to they are mainly a component of by-catch that is discarded back to the sea (Cavanagh & Gibson 2007). During the last decades, in the north-western Mediterranean many chondrichthyans have been accidentally captured by fisheries (mainly trawlers and seine-pursers), in special in association to the spectacular increase in the fishing intensity and efficiency (Walker et al. 2005). Between the chondrichthyans, several species are commonly captured such as; various elasmobranch like, small-spotted catshark (*Scyliorhinus canicula),* blackmouth catshark (*Galeus melastomus*) and velvet belly lanternshark (*Etmopterus spinax*) and one holocephali, rabbitfish (*Chimaera monstrosa*). From an ecological point of view, these demersal predators could play an important role as predators in the Mediterranean demersal ecosystem, in special after the strong decline of large pelagic sharks Ferreti et al. 2008, 2011, Coll et al. in press).

Blackmouth catshark and velvet belly lanternshark are two small elasmobranch, both distributed through the Mediterranean and eastern Atlantic Ocean (Carrasson et al. 1992,Sánchez et al. 2005,Fanelli et al. 2009, Valls et al. 2011). In comparison with others Mediterranean sharks, both blackmouth catshark and velvet belly lanternshark are considered abundant demersal sharks on the middle slopes of the western Mediterranean (Carrasson et al. 1992, Fanelli et al. 2009). Previous trophic studies conducted in the Mediterranean indicated contrasting patterns; while some studies suggested that these species segregate their trophic habits (Carrasson et al. 1992, Cortes 1999, Stergiou & Karpouzi 2002, Fanelli et al. 2009), others suggest clearly overlap in their diet (Valls et al. 2011). In the case of the rabbitfish, there is a clearly lack of knowledge about its biology and feeding ecology in the Mediterranean Sea. Only Macpherson et al. (1980) report information of the diet of this holocephalan from samples collected during 1977-1978 in the western Mediterranean Sea. However, there are no studies that examine simultaneously the diet of these three abundant species from the same area, information that can provide, in addition to their diet, insights of the potential trophic overlap or segregation between these species. Similarly, there is no information about the

potential differences between sexes or depths in these species. Sexual differences in diet are described for marine predators with marked sexual dimorphism such as sharks or rays. This may serve as a mechanism to reduce intraspecific competition for food between males and females (Wearmouth & Sims 2008). Similarly, differences in feeding strategies between different depths have also been reported for sharks (Carrasson et al. 1992). This pattern is usually explained by variation in the relative abundance of prey availability due to differences in the biological and environmental factors between depths (Carrasson et al. 1992). These studies are essential to correctly quantify the trophic position of these organisms in marine food webs.

Diet and trophic ecology of fish have been traditionally studied using stomach content analysis (Cortes 1999, Stergiou & Karpouzi 2002). This technique is useful because it provides a detailed quantification of prey items for each predator. However, this technique has some limitations since stomach content represent only part of what an animal has eaten recently, may be skewed due to differences in the digestibility of prey and it is necessary an appreciable high number of samples to an accurate description of the diet (MacNeil et al. 2005). During the last years the use of stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) have been increasingly used as an effective tool to study trophic segregation among species and feeding habitats, complementing the stomach content information (Navarro et al. 2009, Hussey et al. 2011). This approach is based on the fact that δ^{15}N and δ^{13}C values are transformed from dietary sources to consumers in a predictable manner. Nitrogen isotopic values show a predictable increase in the isotopic ratio throughout the trophic levels, i.e., typically from 2.5‰ to 5‰ (Kelly 2000). Carbon isotopic values show little change with trophic transfers, but are useful indicator of the dietary source of carbon (Kelly 2000). An interesting peculiarity of this approach is that, depending on the turnover of the analyzed tissue, the trophic information integrates different periods of feeding events (from days to several months). For example, stable isotopic values of stingrays muscle integrate the information during a period of 98 days (MacNeil et al. 2005) and in sandbar shark it is calculated to be approximately of 80-100 days for muscle (Logan & Lutcavage 2010).

Moreover, by combining stable isotope values for consumers and their potential prey, isotopic mixing models can be applied to obtain estimates of the relative contribution of each prey item to the diet of the consumer (e.g. stable isotope analysis in R [SIAR] isotopic mixing model,(Parnell et al. 2010)) Furthermore, recent studies have shown the importance of using different resources among individuals, which may have important ecological implications and stable isotope analysis can be used to detect this individual variability (Bolnick et al. 2003, Matich et al. 2011, Shiffman & Gallagher 2012). However, stable isotope analyses do not provide the same taxonomic resolution that stomach content analysis can generates (Shiffman & Gallagher 2012).

Objectives of the study

The general aim of the present study was to provide new insights on the feeding ecology of three species of chondrichthyes (blackmouth catshark, velvet belly lanternshark and rabbitfish) living in sympatry in the Gulf of Lion, Northwestern Mediterranean Sea.

Specifically we aimed at examining (i) the diet of each species, (ii) the potential differences in the diet between sexes, (iii) between two depths, and (iv) the individual differences in the diet.

To determine the diet we combined the use of the analysis of stomach content and the analysis of stable isotope values as complementary methodologies. With the stomach content analysis we described the dietary composition of each species, and looked at differences between species, sex and depth by characterizing the frequency and number of different prey. With the stable isotope values, in addition to the quantification of isotopic values of $\delta^{15}N$ and $\delta^{13}C$, we used Bayesian isotopic mixing models (combining isotopic values of predators and prey) to estimate the dietary habits of each individual of the three species analyzed. By using isotopic $\delta^{15}N$ values we also calculated the trophic level of species.

2. MATERIAL AND METHODS

2.1. Study area and sampling procedures

The study was conducted in the Gulf of Lions (Figure 1), one of the most productive areas of the northwestern Mediterranean Sea, mainly due to the discharge of nutrients and particulate matter of the Rhône River (Salat 1996). River inputs are transported from the Rhône River to the North Catalan Sea (Denis & Grenz 2003, Bautista-Vega et al. 2008), where the shelf becomes narrow with submarine canyons. These nutrients are transported by the general water mass circulation, the cyclonic Northern Current and the Liguro-Provençal-Catalan front (Abelló et al. 2002). Samples were collected by Spanish bottom trawl fishing vessels that developed their activity in fishing grounds located in international waters of the Gulf of Lions. Since this area is of difficult access to both the French and the Spanish fishing fleet, it is thought that the area has been moderately (Massutí et al. 2008). This is a marine area with submarine canyons, structures that have rich habitats and of a high ecological importance. In terms of biomass, although our study species are not the targets of the fisheries, chondrichthyes appear in high numbers in the fishing operation (Massutí et al. 2008).
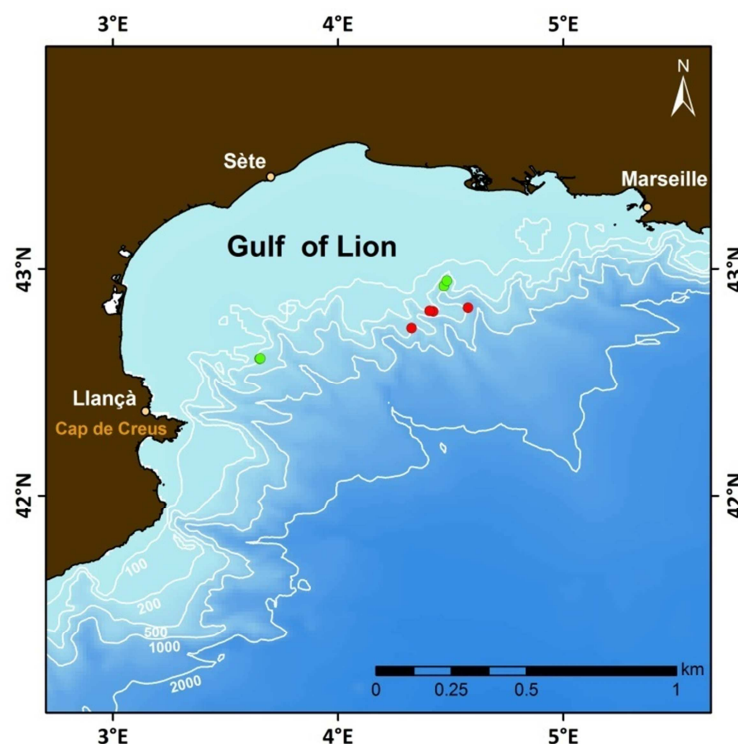
Figure 1. Map of the sampling area. Dots are the positions of the trawlers, and red and green colors correspond to upper slope (300-500 m) where the fishing fleet is targeting the Norway Lobster (*Nephrops norvegicus*) and middle slope (500-750 m) directed to capture the red shrimp (*Aristeus antennatus*), respectively.

Specimens of blackmouth catshark (n=43), velvet belly lanternshark (n=39) and rabbitfish (n=35) were captured during seven days in summer 2011 (from June to September) by the trawling fishing boats based at Port de la Selva and Llançà harbour (Figure 1). The trawling operations were distributed in two bathymetrical strata, upper slope (300-500 m) and middle slope (500-750 m) where the fishing fleet is targeting the Norway Lobster (*Nephrops norvegicus*) and red shrimp (*Aristeus antennatus*), respectively. Between 10 and 12 adults (males and females) of each species of similar size were collected in both upper and middle slope. The sizes (body length) of the collected specimens were; >45 cm for blackmouth catshark; >20 cm for velvet belly lanternshark; and >54 cm for rabbitfish. Each specimen was separated and immediately frozen in the boat after being captured and were stored frozen at -20ºC until their diet, morphological measures and isotopic sampling were performed in the laboratory.

2.2. Morphological measures

For each individual, the sex, body length (cm), and body mass (g) were recorded. In the case of the body length we measured each individual from the anus to the end point of the head. We also recorded the maturity-state (immature or mature) of each individual. In the case of males, the maturity-state was determined according to the degree of calcification of the clasper and the degree of testes development and reproductive ducts. In the case of females, the maturity-state was determined by the condition of uteri, oviducal glands and ovarian follicles (following the Stehmann (2002) methodology).

2.3. Stomach content analysis

The stomach of all specimens was extracted after dissection. Each stomach was weighted in a digital balance and its gut contents were extracted. Prey items found in each stomach were carefully separated, dried on paper towels and identified Prey items were classified as cephalopod, pleocyemata that was called crabs, dendrobranchiota that was called shrimps, molluscs and fish

items. Individuals of each identified group were counted. Whenever fragments were found, the number of individuals was registered as the smallest number to not overestimate the occurrence of a particular prey.

The diet of each species was quantified using two trophic indexes: 1) frequency of occurrence (%FO), which is the percentage of stomachs with a specific type of prey in relation to the total number of stomach containing food, and 2) numerical composition (%N), expressed as the percentage contribution of each prey to the whole content. The vacuity index (%V) that is the percentage of empty stomachs was also calculated.

2.4. Stable isotope analyses

A small portion of dorsal muscle (without skin and cartilage) was extracted from each individual chondrichthyan. We also collected a small portion of muscle of different preys (crabs, shrimps, cephalopods and fish) found in the stomachs without evidence of digestion. In the case of cephalopods, to obtain more samples we also collected the beaks. All samples were freeze-dried, powdered and 0.9-1.0 mg of each sample was packed into tin capsules. Stable isotope analyses were performed at the Laboratory of Stable Isotopes at the *Estación Biológica de Doñana* (www.ebd.csic.es/lie/index.html). All samples were combusted at 1020ºC using a continuous flow isotope-ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyzer interfaced with Delta V Advantage mass spectrometer.

All isotope abundances are expressed in δ-notation as parts per thousand (‰) deviation from the IAEA standard AIR ($\delta^{15}N$) and VPDB ($\delta^{13}C$). Based on laboratory standards, the measurement error was ±0.2 and ±0.1for $\delta^{15}N$ and $\delta^{13}C$, respectively. A total of 117 samples of chondrichthyan individuals and 36 samples of chondrichthyan prey tissue were analyzed for ^{13}C and ^{15}N.

Since cephalopods mandibles (beaks) are depleted in $\delta^{15}N$ when compared with muscle tissues, the $\delta^{15}N$ values of beaks were adjusted by adding 3.5‰ to their $\delta^{15}N$ values (following recommendations from Cherel et al. 2009). Since

the ratio C:N was low than 4‰ in all cases, we did not correct the δ^{13}C values for the effect of lipids (following Logan & Lutcavage 2010 recommendations).

2.5. Isotopic mixing model

To estimate the diet composition of each species at individual level we applied the Bayesian model available in SIAR 4.1.3(Stable Isotope Analysis in R 2012) (Parnell et al. 2012). This model runs under the free software R environment (R Development Core Team 2009). The model allows the inclusion of sources of uncertainty in the data. In particular, the variability in the isotope signatures (mean and standard deviation) of potential prey species can be incorporated into the model (Parnell et al. 2012). We used the isotopic discrimination factors of 0.90±0.33‰ for δ^{13}C and 2.29±0.22‰ for δ^{15}N estimated by Hussey et al. (2010) for shark species.

2.6. Trophic level

We estimate the trophic level (TL) of each individual of chondrichthyan by using the equation: $TL_{consumer} = TL_{basal} + (\delta^{15}N_{consumer} - \delta^{15}N_{prey}) / \Delta\delta^{15}N$. $TL_{consumer}$ refers to the mean TL of each individual. $\delta^{15}N_{prey}$ was the isotopic values of microplankton ($\delta^{15}N_{prey}$= 4.95‰; TL_{basal} =1.5) collected in the Gulf of Lion during the summer of 2010 by Costalago et al. (2012). The isotopic discrimination factor for N ($\Delta\delta^{15}N$) was the same used with the isotopic mixing model (Hussey et al. 2010).

2.7. Statistical analyses

To examine the morphological differences in body length and body mass between species, sexes and depths (upper and middle slope), ANOVA tests, including "species", "sex", "depth" and their interaction as categorical variables were applied. We normalized all variables using log-transformation before ANOVA tests. The factor species had three levels (velvet belly lanternshark,

blackmouth catshark, and rabbitfish), the factor sex had two levels (female and male) and the factor depth had two levels (300-500 and 500-750 m.).

In the case of the dietary composition (analyzed using SIAR isotopic model and stomach content data) the effects of species, sex and depth and their interaction were tested with two-way semi-parametric permutation multivariate analyses of variance tests (PERMANOVA test) on the Euclidean distance matrix. PERMANOVA allows for the analysis of complex designs (multiple factors and their interaction) without the constraints of multivariate normality, homoscedasticity, and having a greater number of variables than sampling units of traditional ANOVA tests. The method calculates a pseudo-F statistic directly analogous to the traditional F-statistic for multifactorial univariate ANOVA models but uses permutation procedures to obtain p-values for each term in the model (Anderson et al. 2008).

All results are shown as means ± standard deviation except when otherwise indicated. Statistical analyses were carried out using SPSS-18, and PRIMER-E 6 software. Significance level for all tests was adopted at $p < 0.05$.

3. RESULTS

The sample was composed of 16 males and 23 females of velvet belly lanternshark of which 36 were immature and 3 mature, 22 males and 21 females of blackmouth catshark of which 8 were immature and 29 mature, 13 males and 22 females of rabbitfishes of which 11 were immature and 24 mature (Table 1).

Table 1 Sample size (n) mean ± standard deviation of body mass, body length, $\delta^{15}N$, $\delta^{13}C$, and trophic level (TL) values of velvet belly lanternshark, blackmouth catshark and rabbitfish from the Gulf of Lion (NW Mediterranean) by sex and depth factors.

|		n	Body mass (g)	Body length (cm)	$\delta^{13}C$ ($^0/_{00}$)	$\delta^{15}N$ ($^0/_{00}$)	TL
Velvet belly lanternshark							
Upper slope	Males	10	36.45±8.44	12.03±1.10	-19.28±0.88	8.01±0.44	2.84±0.19
	Females	10	30.05±9.60	11.39±1.23	-18.96±0.33	7.72±0.17	2.71±0.08
Middle slope	Males	6	50.25±35.50	13.62±2.97	-18.69±0.50	8.76±0.53	3.16±0.23
	Females	13	99.23±86.05	16.27±4.37	-18.65±0.99	8.99±0.61	3.27±0.27
Blackmouth catshark							
Upper slope	Males	10	291.90±65.90	20.76±1.60	-19.28±0.88	8.19±0.39	3.18±0.17
	Females	10	409.40±91.55	23.16±1.30	-18.96±0.33	8.88±0.43	3.21±0.19
Middle slope	Males	12	313.83±43.13	21.44±0.87	-18.69±0.50	8.93±0.24	3.24±0.11
	Females	11	455.60±136.64	23.96±2.43	-18.65±0.99	9.24±0.31	3.37±0.13
Rabbitfish							
Upper slope	Males	7	611.14±95.55	20.51±1.64	-17.04±0.76	10.80±0.54	4.05±0.23
	Females	8	877.38±315.66	23.10±3.22	-16.89±0.52	10.58±0.42	3.96±0.18
Middle slope	Males	6	696.67±92.18	21.65±1.27	-16.22±0.35	11.80±0.25	4.49±0.11
	Females	14	828.64±316.00	22.38±3.06	-16.58±0.52	11.25±0.48	4.25±0.21

3.1. Morphological differences

Body mass and body length was significantly different between the individuals of the three species (ANOVA test; Body mass, $F=401.60$ $p<0.001$. Body length, $F=127.31$, $p<0.001$) although blackmouth catshark and rabbitfish differences in body length were no significant and the velvet belly lanternshark was the smallest species. Due to these differences between species in the morphological variables we compared the effect of depth and sex for each species separately (see Table 2). Rabbitfish did not have morphological differences. Only Velvet belly lanternshark body length and body mass showed significant differences between depths. Individuals of velvet belly lanternshark were larger and heavier at middle slope. Blackmouth catshark presented significant differences of body length and body mass between sexes, where females were larger and heavier, but results did not show differences between depths (Table 2).

Table 2 Summary of the ANOVA tests examining differences in body length and body mass values between sexes and depth of velvet belly lanternshark, blackmouth catshark and rabbitfish from the Gulf of Lion (NW Mediterranean).

Parameter		Effect	$F_{(df)}$	P
Velvet belly lanternshark				
		Depth	$8.96_{(1,39)}$	**0.005**
	Body length	Sex	$0.52_{(1,39)}$	0.48
		Depth*sex	$2.24_{(1,39)}$	0.14
		Depth	$13.26_{(1,39)}$	**0.001**
	Body mass	Sex	$1.70_{(1,39)}$	0.20
		Depth*sex	$6.81_{(1,39)}$	**0.01**
Blackmouth catshark				
		Depth	$2.13_{(1.43)}$	0.15
	Body length	Sex	$23.69_{(1,43)}$	**<0.001**
		Depth*sex	$0.005_{(1,43)}$	0.94
		Depth	$1.52_{(1,43)}$	0.22
	Body mass	Sex	$22.64_{(1.43)}$	**<0.001**
		Depth*sex	$0.001_{(1,43)}$	0.98
Rabbitfish				
		Depth	$0.07_{(1,35)}$	0.79
	Body length	Sex	$2.50_{(1,35)}$	0.12
		Depth*sex	$0.98_{(1,35)}$	0.33
		Depth	$0.09_{(1,35)}$	0.76
	Body mass	Sex	$2,75_{(1,35)}$	0.11
		Depth*sex	$0.60_{(1,35)}$	0.44

3.2. Isotopic and trophic level comparison

Because $\delta^{13}C$ and $\delta^{15}N$ did not differ between sexes in any species (all p>0.05, ANOVA tests), the effect of the sex was not taken in account in the subsequence comparisons.
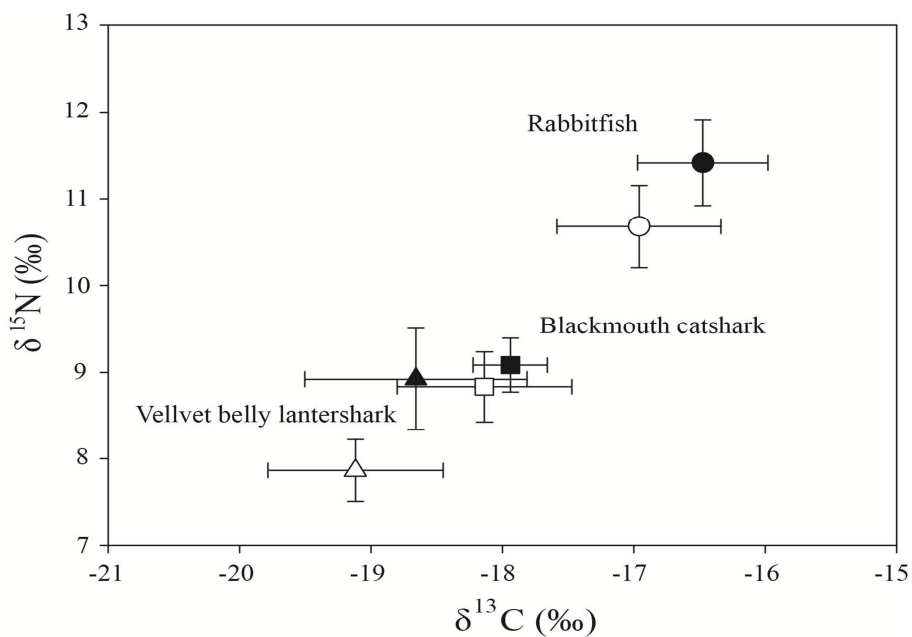
Figure 2. Mean stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) of velvet belly lanternshark (triangle), blackmouth catshark (square) and rabbitfish (circle) from the Gulf of Lion (NW Mediterranean). The depth range is represented by empty symbols for the upper slope (300-500 m) and by solid symbols for middle slope (500-750 m).

$\delta^{15}N$ and $\delta^{13}C$ values showed significant differences between the three species and between the two depths (Table 3). Between species, rabbitfish showed higher $\delta^{15}N$ and $\delta^{13}C$ values than velvet belly lanternshark and blackmouth catshark (Figure 2).The interaction between species and depth for the $\delta^{15}N$valueswas significant, due to the less pronounced differences between the two depths in blackmouth catshark than in the other two species (Figure 2). In the middle slope the mean values of $\delta^{13}C$ and δ15N were higher than in the upper slope (Table 1 and 3).

Table 3 Summary of the ANOVA tests examining differences δ^{15}N and δ^{13}C values, between velvet belly lanternshark (VBL), blackmouth catshark (BC) and rabbitfish (RB) from the Gulf of Lion (NW Mediterranean).

Parameter	Effect	Pseudo-F$_{(df)}$	P	Post-hoc
δ^{15}N	Species	364.11 $_{(2,116)}$	**0.001**	BC x VBL xRB
	Depth	68.58$_{(1,116)}$	**0.001**	
	Species*depth	8.65$_{(2,116)}$	**0.001**	
δ^{13}C	Species	115.77 $_{(2, 116)}$	**0.001**	BC x VBL xRB
	Depth	11.12 $_{(1, 116)}$	**0.003**	
	Species*depth	0.68$_{(2, 116)}$	0.51	

Symbols designating species combination in Tukey post-hoc test summaries are: *VBL* Velvet belly lanternshark ; *BC* Blackmouth catshark ; *RB* Rabbitfish .Pairs of means differing significantly (P = 0.05) by Tukey test are linked with an '*x*'.

Trophic level (TL) values estimated using δ^{15}N values were higher for rabbitfish (TL= 4.17±0.26) followed by blackmouth catshark (TL=3.25±0.16) and velvet belly lanternshark (TL=2.99±0.31).

3.3. Isotopic mixing model

In overall, the proportion of each potential prey for the three species estimated by SIAR models differed between species (Pseudo-F=128.05, p=0.001) and depths (Pseudo-F=28.62, p=0.001) (Table 4 and 5). In particular, the diet of velvet belly lanternshark was composed mainly by shrimps (67.63±10.56; 37.18±12.89) followed by crabs (13.23±3.35; 30.23±7.27) (Table 5, Figure 5).

Table 4. Sample size (n) and mean and standard deviation of δ^{15}N and δ^{13}C values of potential prey groups for velvet belly lanternshark, blackmouth catshark and rabbitfish.

Prey group	n	δ^{15}N (‰)	δ^{13}C (‰)
Fish	9	8.61±0.93	-19.50±0.80
Cephalopod	10	7.50±0.81	-19.00±0.61
Crabs	3	7.18±0.52	-16.08±1.80
Shrimps	7	7.82±0.66	-19.39±0.90

Blackmouth catshark showed a diet mainly composed by crabs (34.65±7.83; 39.15±3.37) and by shrimps (38.10±9.19; 30.54±5.90). Rabbitfish showed a diet mainly composed by crabs (75.65±26.19; 43.04±3.84) followed by fish (14.27±14.58; 39.22±9.24). Although PERMANOVA tests indicated that the diet differed between depths, this difference was only evident in the velvet belly lanternshark (Table 5). Velvet belly lanternshark showed more proportion of cephalopods in its diet at upper-slope (63.07%) than at middle-slope depth (31.83%).

Table 5. Mean ± standard deviation of diet composition of velvet belly lanternshark (VBL), blackmouth catshark (BC) and rabbitfish (RB) from the Gulf of Lion (NW Mediterranean) estimated with SIAR model.

	n	Fish (%)	Shrimps (%)	Crabs (%)	Cephalopods (%)
Velvet belly lanternshark					
Upper slope	20	9.75±4.37	67.63±10.56	13.23±3.35	9.39±5.29
Middle slope	19	14.74±4.54	37.18±12.89	30.23±7.27	17.83±5.32
Blackmouth catshark					
Upper slope	20	12.20±2.54	38.10±9.19	34.65±7.83	15.05±3.46
Middle slope	23	13.09±1.24	30.54±5.90	39.15±3.37	17.23±2.07
Rabbitfish					
Upper slope	15	14.27±14.58	4.76±7.22	75.65±26.19	4.93±7.34
Middle slope	20	39.22±9.24	8.93±3.70	43.04±3.84	8.81±3.90

According to the Bayesian mixed model most of individuals of rabbitfish sampled at middle-slope had a different dietary composition in comparison with the other two species and the other depth (Figure 3). That is, few individuals at middle-slope were mix with individuals of rabbitfish at 500-700 that were closer to those of blackmouth catshark at upper-slope and middle-slope and velvet belly lanternshark at middle-slope. Individuals of velvet belly lanternshark at upper-slope were mostly grouped with a differentiated trophic niche. Accordingly, cluster analyses revealed 4 differentiated groups of individuals of the three species (Figure 4).
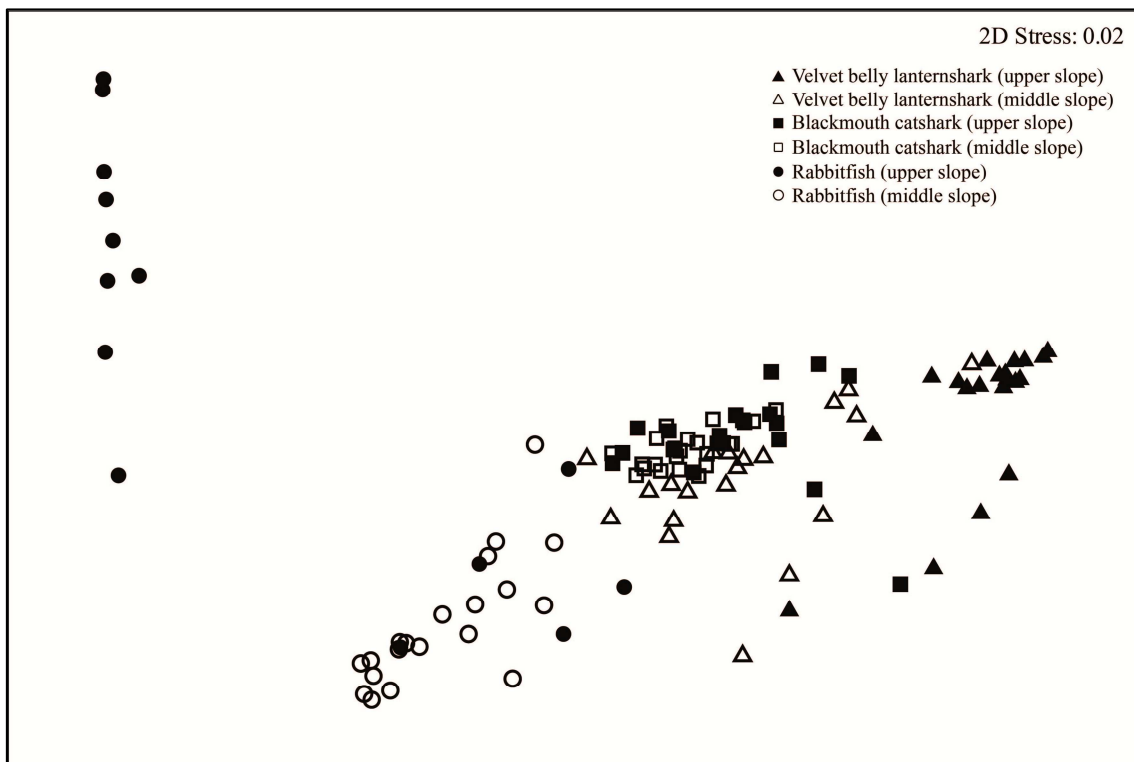
Figure 3. Non-metric Multi-Dimensional Scaling (MDS) ordination results of velvet belly lanternshark (triangle), blackmouth catshark (square) and rabbitfish (circle) from the Gulf of Lion (NW Mediterranean).

Figure 4. Dendrogram of group-averaged cluster analysis of Euclidean distance similarities based on diet estimated with the SIAR isotopic mixing model for velvet belly lanternshark, blackmouth catshark and rabbitfish captured in different depths in the Gulf of Lion (NW Mediterranean).
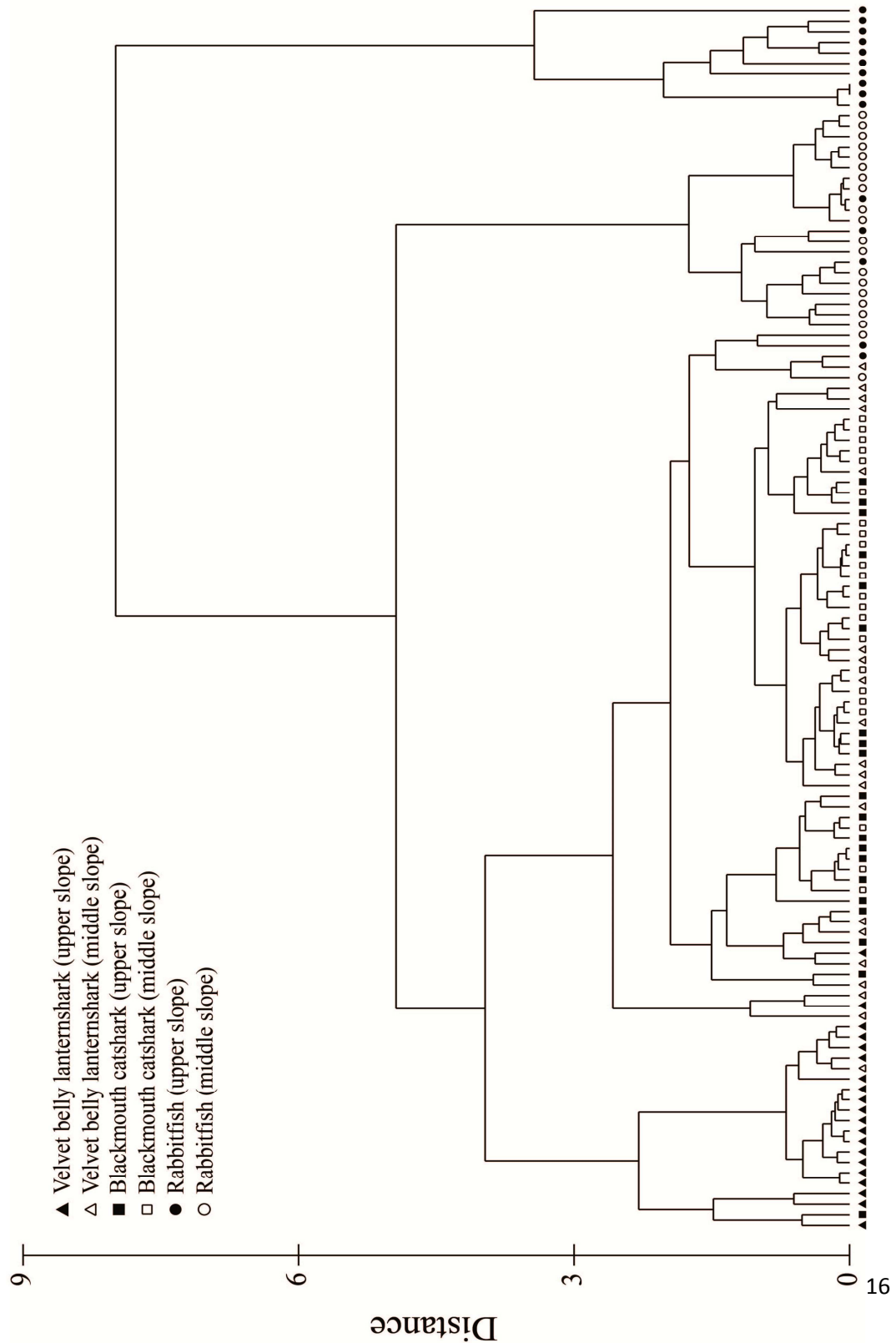
3.4. Stomach content

Of all individuals examined, 46.15% of the stomachs (20 individuals) of velvet belly lanternshark, 4.65% of blackmouth catshark, and 0%of rabbitfish were empty. The diet composition of these three species (%FO and %N) did not differ between sexes (%FO; Pseudo-F=1.03 p=0.38. %N; Pseudo-F=1.35, p=0.23). However, we found significant differences in the diet estimated with the stomach content between species (Table 7). In particular, the diet of blackmouth catshark was composed mainly of cephalopods in both depths, and rabbitfish fed preferably on crabs (Table 6). Although we had fewer stomachs to analysed from the velvet belly lanternshark, its diet was compose mainly by shrimps at the middle slope and by fish and shrimps at upper slope (Table 6).
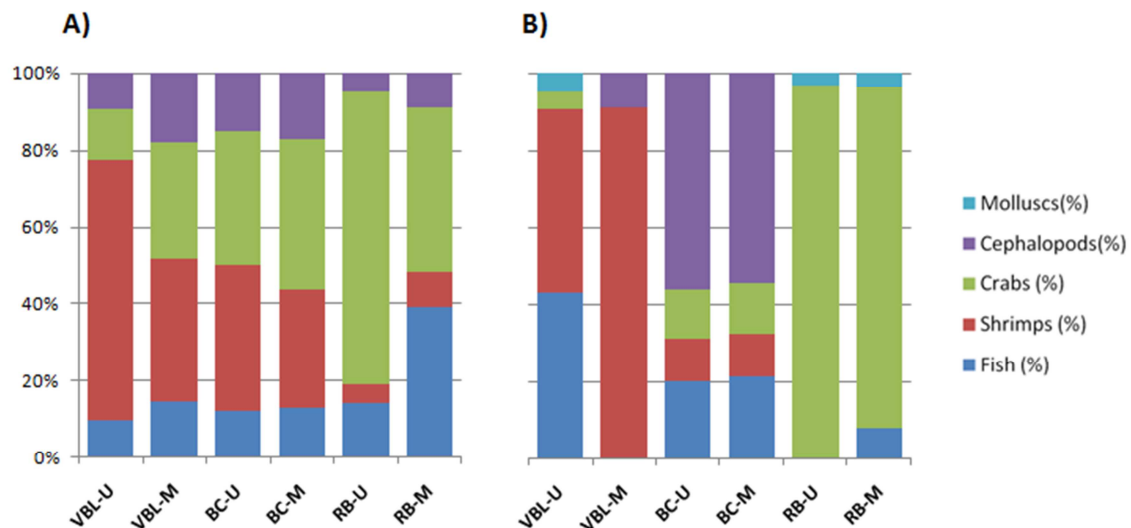
Table 6.Diet composition of 39 velvet belly lanternshark, 43 blackmouth catshark and 35 rabbitfish expressed as percentage frequency of occurrence (%FO) and percentage by number (%N).

PREY		Velvet belly lanternshark		Blackmouth catshark		Rabbitfish	
		300-500	500-750	300-500	500-750	300-500	500-750
Crabs	%FO	1.92±6.93	-	10.42±25.20	9.92±17.20	96.67±12.91	70.42±39.78
	%N	1.92±6.93	-	9.33±24.53	8.57±16.45	99.05±3.69	76.99±37.26
Shrimps	%FO	19.23±38.40	62.50±35.36	9.17±17.29	8.33±22.82	-	-
	%N	19.23±38.40	64.58±35.00	7.25±14.26	9.84±25.70	-	-
Cephalopod	%FO	-	6.25±17.68	47.08±40.40	40.87±35.64	-	-
	%N	-	4.17±11.79	47.42±41.42	42.78±37.41	-	-
Fish	%FO	17.31±37.34	-	16.67±22.13	15.87±26.99	-	6.25±15.97
	%N	17.31±37.34	-	17.92±25.11	14.05±25.48	-	2.17±5.35
Mollusc	%FO	1.92±6.93	-	-	-	3.33±12.91	
	%N	1.92±6.93	-	-	-	0.95±3.69	
Unidentified	%FO	44.23±48.04	31.25±37.20	16.67±31.88	15.48±27.92	-	10.42±25.20
	%N	44.23±48.04	31.25±37.20	18.08±33.58	15.24±28.84	-	8.54±23.43

Table 7 Summary of the MANOVA tests examining differences in %FOand %Nvalues, between velvet belly lanternshark (VBL), blackmouth catshark (BC) and rabbitfish (RB) from the Gulf of Lion (NW Mediterranean).

Parameter	Effect	*Pseudo-F$_{(df)}$*	P	Post-hoc
%FO	Species	32.52 $_{(2,116)}$	**0.001**	BC x VBL xRB
	Depth	0.81 $_{(1,116)}$	0.52	
	Species*depth	1.78$_{(2,116)}$	0.06	
%N	Species	35.97 $_{(2, 116)}$	**0.001**	BC x VBL xRB
	Depth	0.99 $_{(1, 116)}$	0.40	
	Species*depth	1.52$_{(2, 116)}$	0.13	

Symbols designating species combination in Tukey post-hoc test summaries are: *VBL* Velvet belly lanternshark ; *BC* Blackmouth catshark ; *RB* Rabbitfish .Pairs of means differing significantly (*P* = 0.05) by Tukey test are linked with an '*x*'.

Figure 5. Representation of mean diet composition (percentage) estimated by SIAR (A) and stomach content analysis %FO (B) of velvet belly lanternshark at the upper slope (VBL-U) and middle slope (VBL-M), blackmouth catshark at the upper slope (BC-U) and middle slope (BC-M) and rabbitfish at the upper slope (RB-U) and middle slope (RB-M).

4. DISCUSSION

To improve the knowledge of species ecology is important to obtain information on diet and trophic position of the species. Here, we report new information of the dietary habits (diet composition and trophic level) of three chondrichthyans coexisting in the same area of the north-western Mediterranean Sea. These differences are assessed between species, sex and depth. To our knowledge this is the first study in the western Mediterranean dealing with the trophic ecology of these species (velvet belly lanternshark, blackmouth catshark and rabbitfish) by combining data from stomach content and isotopic analyses together. Other studies analyse trophic velvet belly lanternshark and blackmouth catshark but only with stomach content analysis (Polunin et al. 2001, Valls et al. 2011). Moreover, there is a lack of information for the rabbitfish despite this species is classified as Near Threatened by the UICN list (Dagit et al. 2007).

4.1. Interspecific differences in diet

Although some qualitative differences in the prey groups between methods have been found, both stomach content and stable isotope analyses indicated a clearly trophic segregation between rabbitfish, velvet belly lanternshark and blackmouth catshark. Rabbitfish showed a diet mainly composed by crabs followed by fish. In other studies diets of chimaeras seem to prey similar groups, crustacean and small fishes (Carrier et al. 2004). The only report about the diet of rabbitfish in the western Mediterranean reported that the main prey for rabbitfish was equinoderms and crabs (MacPherson 1980).

The diet of velvet belly lanternshark was composed mainly by shrimps followed by crabs. Our results differ from what is reported in the literature, because teleost and cephalopods had been previously described as the most consumed prey by this species(Carrasson et al. 1992, Neiva, et al. 2006). However, Coelho et al. (2009) reported that crustaceans were the dominant prey group, in line with results of our study. Blackmouth catshark had a similar diet to velvet belly lanternshark with a high consumption of shrimps and crabs, whereas

fishes and cephalopods were secondary preys. These results indicated that these two shark species have the same trophic role in the ecosystem and were similar to the results reported previously in the Mediterranean Sea(Carrasson et al. 1992, Olaso et al. 2004, Valls et al. 2011). However, we found qualitative differences in relation to the importance of cephalopods in the diet of blackmouth catshark between stomach content and isotopic mixing model. In particular, stomach content analysis suggested that the main prey were cephalopods. This difference could be explained because cephalopods are overestimated in the stomach content analyses since they are retained in the stomach easily since they beaks are difficulty to digest.

Interspecific differences in diet could be related to body-size differences or different foraging abilities as well. For example, it has been described that larger shark species include in their diet mainly cephalopods and teleost (Cherel et al. 2009). In other studies it has been shown a corresponding increase in $\delta^{15}N$ with size of the animal for several marine taxa (Cherel et al. 2009, Newsome et al. 2009, Hussey et al. 2011, Borrell et al. 2011).Cortes (1999) reported that the trophic level of shark and body size was correlated. These changes due to size are expected because at the same time other modifications of the animals take place, like changes in size of the swimming speed and in consequence with changes in habitat utilization, energy requirements and capacity to forage. All these changes allow larger species to feed on more divers prey (Wetherbee & Cortés 2004, Borrell et al. 2011). Furthermore, in some cases differences between sexes explained differences in diet, but this was not our case. Accordingly, it is possible that segregation of rabbitfish from the other species was due to the bigger of our captured individual of rabbitfish.

4.2. Depth-related differences in diet

In addition to interspecific differences in diet, we also found depth-related variations in the dietary habits, probably related to natural variations in the food availability with depth (Carrasson et al. 1992). For example, SIAR results indicated that velvet belly lanternshark consumed mainly shrimps at the upper

slope and shrimps and crabs at middle slope. The stomach content analyses corroborate that the main consumed prey were shrimps at least in the middle slope, but due to the high vacuity (46.15%) of the stomach, data from stomach content of velvet belly lanternshark was not fully representative. Similar to the velvet belly lanternshark, the diet of the rabbitfish was different between both depths. The isotopic mixing model show that at the upper slope the diet was basically based on crabs and at middle slope it had a more diverse diet with an important percentage of fish. In this case, the Bayesian model coincided with results in stomach content analysis. It is relevant to notice that differences between depths that were found in rabbitfish were due to a higher consumption of fish with depth?.The difference in the diet between depths probably was related to a different availability of potential preys. In the case of velvet belly lanternshark, in addition to the potential effect of the depth-variations in the food availability, morphological-related effect could be involved since the specimens of velvet belly lanternsharks from the middle slope were smaller (in body mass and size) than the upper slope ones.

4.3. Trophic level differences between species

Comparing trophic positions of chondrichthyans we will be able to better understand the ecological role of each species and how fisheries exploitation affects them (Cortes 1999, Shiffman & Gallagher 2012). Traditionally, trophic level has been obtained from stomach content analysis (TL-SCA), but this method provides insight of the last meal. Instead, trophic level calculated from SIA (TL-SIA) provides insights of the diet strategy of a period of months. Moreover, TL-SIA examines assimilated material unlike TL-SCA (Boyle et al. 2012). The TL-SIA that we obtained for velvet belly lanternshark, blackmouth catshark and rabbitfish were 2.99±0.31, 3.25±0.16, and 4.17±0.26, respectively. These values differ from the trophic levels reported in previous studies such as Velvet belly lanternshark TL= 3.8±0.5, blackmouth catshark TL=4.2±0.6, and rabbitfish TL=3.5±0.6 (Carrasson et al. 1992, Froese & Pauly 2012). These differences could be explained by differences in the feeding habits between areas of study or because the two methods have a different integrative periods (Hussey et al. 2011). Should be considered the fact that all individuals used on

the analyses were adults or at least the longest range of individuals that was possible. Therefore, the TL-SIA values could be overestimated because juvenile individuals were not included. However, TL-SCA of velvet belly lanternshark and blackmouth catshark were smaller than TL-SIA and it was expected that TL increase with total length of the sharks.

Comparison of TL-SIA between species was also significant. Because in our sampling velvet belly lanternshark presented a smaller size than the other two species, differences between TL of rabbitfish and velvet belly lanternshark may be due to differences of length between species. Moreover individuals of velvet belly lanternshark were mostly immature unlike the other species. Differences of TL-SIA between blackmouth catshark and rabbitfish should be directly related to the differences showed in the diet between both species since all individuals of study were adults.

4.4. Individual differences

In a previous analyses the overall diet between species, sex and depths were compared, ignoring the behaviors and habits of individuals (Matich et al. 2011). There is increasing evidence that individual specialization is important in predators due to the near position to the top of the food webs due to they tend to incorporate energy from a wider range of prey but a generalist diet of a species may be a collection of individual-level trophic specialists (Matich et al. 2011). At individual level, our results revealed some interesting differences between individuals of the three species analyzed. In the case of the rabbitfish, despite overall this species is segregate in diet from the other two species, some individuals of rabbitfish had a similar diet to velvet belly lanternshark, most of them were rabbitfish individuals captured at middle slope. However, there was a clear diet overlap between most of individuals of velvet belly lanternshark at middle slope and blackmouth catshark (both depths). Finally individuals of velvet belly lanternshark at upper slope form differentiated group. Considering individual variation in trophic studies is important to determine the real impact of one predator in its ecosystem, special when clearly individual segregation in feeding strategies exists (Matich et al. 2011, Shiffman &

Gallagher 2012).These also poses new challenges to food-web modeling methodologies.

5. CONCLUSIONS

In summary, in this study we describe the diet of three chondrichthyans, velvet belly lanternshark, blackmouth catshark and rabbitfish from the Gulf of Lion (north-western Mediterranean Sea). The results revealed that the rabbitfish was segregated in terms of diet from the other two sharks. Moreover, probably due to variations in food availability, the species showed depth-related differences in the diet. From a methodological point of view, we illustrate and emphasize the utility of the use of two complementary approaches, stomach content and isotopic analyses, in diet studies of marine predators. This strategy can allow faster and more efficient monitoring of food web changes over long periods of time. Furthermore, the use of individual isotopic models allows examining the individual variation in the feeding habits between individuals, opening new opportunities for the study of the individual-specialization in trophic ecology studies.We suggest that more comparative studies of SIA and SCA should be conducted. Moreover, due to the amount of seasonal variability in the Gulf of Lion, it is recommended to integrate this variability in further studies.

5. ACKNOWLEDGEMENT

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