



University Master in Aquaculture

**Feeding ecology of a demersal predator, the black anglerfish
(*Lophius budegassa* Spinola, 1807) in the northwestern
Mediterranean Sea**

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Abstract

Feeding activity is an important tool to study the relationships of marine organisms and the energy transfer between trophic levels in marine communities. Knowledge of the diet composition lets us to preserve diversity and improve the management of the marine resources for fisheries. The aim of this study was study the trophic ecology of a commercial species, *Lophius budegassa* in the NW Mediterranean Sea, for this purpose we studied differences in diet between two sizes (size 1: <30 cm and size 2: ≥30 cm) and throughout the year. These differences are determined with two methodologies: stomach content analysis through various indexes (%FO, %N, %W, IRI and %IRI) and stable isotope analysis of nitrogen and carbon in muscle samples of our individuals to perform Bayesian isotopic mixing models with isotopic values of the potential preys. Variation in the diet of *L. budegassa* in the NW Mediterranean Sea was related to different factors as size and season. Our results show that *L. budegassa* is an opportunistic predator with preference by fishes and lesser extent to crustaceans, although small proportion of molluscs and echinoderms were also found in some stomachs. We found differences in the diet between sizes, in particular, size 2 individuals included more crustaceans and less fishes in the diet than size 1, this was confirmed with both methodologies studied. Regarding seasonal changes in diet, we not found differences between seasons, nor in stomach contents nor with isotopic mixing models, being in all the seasons more important fishes than crustaceans as preys. This study shows the importance to use complementary approaches for diet studies as isotopic mixing models in addition to traditional methodologies as stomach content analysis to improve knowledge about the food web and their fluxes.

1. Introduction

The information about the feeding ecology of marine organisms is pivotal to understand its ecological role in the ecosystem. This knowledge is also crucial for commercial species to an efficient management of marine resources; also to implement ecosystem-based approaches to understand and predict changes in the ecosystem due to anthropogenic impact or environmental changes (Coll *et al.* 2009). In addition, diet information also play a key role to understand different ecological issues such, for example, the resources partitioning and the structure of fish communities (e.g., Macpherson 1981, Harmelin-Vivien *et al.* 1989), the differences in prey selection between species (e.g., Kohler and Ney 1982, Stergiou and Karpouzi 2002) and the ontogenetic shifts in diet (e.g., Stergiou and Fourtouni 1991, Labropoulou and Eleftheriou 1997). Moreover, the quality and quantity of food are some of the most important factors directly affecting growth, maturation and mortality of fish (Wootton 1990).

However, the trophic habits are not constant at specific level and, for example, the size of the individuals (Stergiou and Karpouzi 2002) or seasonal changes that control food availability (Preciado *et al.* 2006) could also affect the feeding strategies of the species.

Although a high amount of information about the feeding ecology of marine species has been published (see review in Stergiou and Karpouzi 2002), the information for some particular species is scarce. This is the case of the black anglerfish (*Lophius budegassa* Spinola, 1807) a predator species highly exploited due to its great economic value in the Western Mediterranean (Leonart and Maynou 2003).

They are characterized by the dorso-ventrally compressed morphology, a wide mouth, thin skin, an absence of scales and swimbladder, and a first dorsal ray modified which server as a lure (Fariña *et al.* 2008) for attracts potential prey and ambush them (Pereda *et al.* 1984, Laurenson and Priede 2005). *Lophius budegassa* is a demersal fish distributed along the Mediterranean Sea and northeastern Atlantic (Caruso 1986), inhabiting sandy, rocky and muddy bottoms on the continental shelf and upper slope to 800 m depth (Negzaoui-Garali *et al.* 2008).

Due to their economic value, during the last decades a specific *Lophius* spp. fishery industry has been developed in the Mediterranean (Hislop *et al.* 2001). As a clearly example, only in the Catalan Sea (NW Mediterranean Sea), more than 6,000 tons of *Lophius* spp. were landed during the last 10 years, being a highly exploited species in this region (Colmenero *et al.* 2013).

In comparison to other commercial species, the biological information of this species in the Mediterranean Sea is scarce, in particular in relation to its feeding ecology. The majority of studies related with *Lophius* spp. have been performed in the Atlantic Ocean and concern mainly on the reproduction (Duarte *et al.* 2001), growth (Duarte *et al.* 1997, Landa *et al.* 2001) and geographic and bathymetric distribution (Caruso 1985, Azevedo and Pereda 1994). Despite its particular predatory method and its commercial value, few studies have been realized related with their feeding behaviour. Among them Olaso *et al.* (1982) and Velasco *et al.* (2001) in the Atlantic coast of Spain, Azevedo (1996) in the Atlantic coast of Portugal and Preciado *et al.* (2006) in the Cantabrian Sea. In the Mediterranean Sea, the knowledge of the *Lophius* spp. biology is more limited (e.g. Tsimenidis and Ondrias 1980, Ungaro *et al.* 2002). The few published studies conducted in the Adriatic Sea (northeastern Mediterranean, Stagioni *et al.* 2013), in the Tunisian coast (central Mediterranean, Negzaoui-Garali *et al.* 2008) and Gulf of Castellammare (southern Mediterranean, Badalamenti *et al.* 2002) indicated that *Lophius budegassa* prey mainly on fishes followed by crustaceans and cephalopods. Also, Stagioni *et al.* (2013) and Negzaoui-Garali *et al.* (2008) found that the diet of the species change in relation to the size of the individuals and Negzaoui-Garali *et al.* (2008) found also seasonal changes in the diet. Ecological models developed in the same area of our study (Coll *et al.* 2006) used diet data of *Lophius* spp. from other regions due to the lack of information on this subject in the

Northwestern Mediterranean. Their results indicated that this species has an important ecological role in the region as top predator.

The study of the diet of marine fish has commonly relied on stomach content analysis (Hyslop 1980). Although this methodology allows high levels of taxonomic resolution, stomach content could overestimate prey that are difficult to digest, such as squid beaks or fish otoliths (Hyslop 1980). Also, for species such *Lophius* spp. that show ambush behaviour in the trawling nets sometimes is hard to separate the prey really consumed from the prey ambushed in the net. All of that reinforces the importance to have complementary analysis to a better understanding of the trophic ecology of marine organisms. The use of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) has been used as complementary tools to study the diet of marine predators during the last decades (Ramos and Gonzalez-Solis 2012, Shiffman *et al.* 2012). This approach is based on the fact that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are transformed from dietary sources to consumers in a predictable manner reflecting the food assimilated by the consumer in the tissue analyzed (Kelly 2000). Particularly, nitrogen isotopes indicate the trophic position of the individual since exhibit stepwise enrichment with upper trophic levels, whereas carbon isotopes varies among primary producers depending on photosynthetic pathway (C3 or C4 plants) showing the original sources of dietary carbon (Layman *et al.* 2012).

Moreover, by combining stable isotope values of consumers with those from their potential prey, isotopic mixing models can be applied to obtain estimates of the relative contribution of each prey to the diet of the consumer (e.g. by using the Stable Isotope Analysis in R [SIAR] isotopic mixing model; Parnell *et al.* 2010). Although outcomes of stomach content analysis and isotopic mixing models should be interpreted with caution, their combination is valuable to a better understanding of the feeding ecology of organisms (Ferraton *et al.* 2007, Caut *et al.* 2013, Navarro *et al.* 2014).

Aims of the present study

In the present study our main objective was to describe the feeding ecology of *L. budegassa* in the Catalan Sea (NW Mediterranean Sea). Specifically, we aimed to determine the effect of the size and the seasons in the diet of this species. For this, we combined the analysis of stomach contents and stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$).

2. Material and methods

2.1. Study area and sampling procedure

The study area was located along the continental shelf of the Catalan Sea associated to the Ebro river delta (NW Mediterranean Sea). In total, 150 individuals of different body sizes were used for analyses (Table 1, Fig. 1) from catches performed between 48 to 446 m depth.

Catches were from commercial otter bottom trawl in the Tarragona harbor throughout 2012 and during two oceanographic surveys conducted in the study area in 2013. With all the samples we have covered spring, summer, autumn and winter seasons. The oceanographic

surveys were performed on board the B/O *Ángeles Alvariño* (IEO) and the fish samples collected with a bottom trawl type GOV (Standard haul of MEDITS program, Bertrand *et al.* 2002).

Table 1. Method of analysis (SCA: stomach content analysis; SIA: stable isotopes analysis), size, number of individuals, total length (mean \pm standard deviation), depth and geographic position of *L. budegassa* collected in the Catalan Sea (NW Mediterranean Sea) between 2012-2013.

	Analysis	Size	N	TL (cm)	Depth (m)	Geographic position
Spring	SCA	Size1	5	20.86 \pm 8.03	74-429	40°33N 1°23E - 40°58N 1°31E
		Size2	3	37.73 \pm 13.22	364-429	40°35N 1°22E - 40°58N 1°31E
	SIA	Size1	10	16.13 \pm 10.45	364-429	40°35N 1°22.E - 40°58N 1°31E
		Size2	10	34.92 \pm 6.81	364-429	40°35N 1°22E - 40°58N 1°31E
Summer	SCA	Size1	19	13.14 \pm 10.75	55-446	40°02N 0°52E - 41°06N 1°34E
		Size2	21	38.95 \pm 5.78	65-312	39°55N 0°29E - 41°06N 1°34E
	SIA	Size1	30	14.76 \pm 7.00	55-446	40°02N 0°52E - 41°06N 1°34E
		Size2	21	38.03 \pm 5.84	65-312	39°55N 0°29E - 41°06N 1°34E
Autumn	SCA	Size1	8	16.41 \pm 4.47	152-391	40°33N 1°22E - 41°01N 1°21E
		Size2	5	41.02 \pm 6.84	318	40°33N 1°22E - 40°33N 1°22E
	SIA	Size1	15	19.56 \pm 6.29	152-391	40°33N 1°22E - 41°01N 1°21E
		Size2	10	37.57 \pm 6.18	318	40°33N 1°22E - 40°33N 1°22E
Winter	SCA	Size1	16	19.31 \pm 4.39	48-215	40°02N 0°59E - 41°10N 2°0E
		Size2	7	33.81 \pm 3.28	72-250	40°02N 0°59E - 41°06N 1°34E
	SIA	Size1	22	19.41 \pm 4.83	48-215	40°02N 0°59E - 41°10N 2°0E
		Size2	17	37.48 \pm 7.30	60-250	41°08N 1°53E - 40°13N 1°14E

Individuals collected from fishing harbors were carried in a fridge to the laboratory the same day. Samples collected during the oceanographic surveys were dissected immediately on board after each haul. In all cases, each individual was measured in total length (TL, in cm) and weighted (to the nearest 0.1 g). The stomachs were extracted and a small portion of muscle (without skin) of each individual was taken; both were frozen at -20°C until their later stomach content and stable isotopic determination.

2.2. Stomach content analysis

Similar to previous studies with the species (e.g. Maravelias and Papaconstantinou 2003) and according with the size our recollected specimens, individuals were distributed in size ranges in order to analyze possible differences in the feeding pattern and for the purposes of this study were divided into two size ranges: size 1 (TL<30 cm) and size 2 (TL \geq 30 cm). From each stomach the total dry weight of the content was scored. Stomachs that contained only fresh prey or any items presumably eaten in the net were excluded from analysis. The preys

were identified to the lowest possible taxonomic level, counted and weighted for subsequent calculation of trophic indexes. Each prey was weighted after removal of surface water.

Highly digested fishes were the main component in the stomach content so the better structure to identify the prey item were the otoliths (bony structures of the inner ear with variable morphology among species), hence, identification of fish prey was based almost totally on their examination (Tuset *et al.* 2008, Aforo 2014). Regarding crustaceans, determination was realized by distinctive characters such as cephalothorax and pleon, pereopods and rostrum morphology.

The vacuity index (%VI = (empty stomachs / total stomachs) x100) was determined. Three trophic indexes have been used:

- Frequency of occurrence (%FO = (num. stomachs containing 1 prey category / num. stomachs containing food) x100).
- Numeric prey abundance (%N = (num. individuals of 1 prey category / total num. of prey of all categories) x100).
- Wet weight (%W = (dry weight of 1 prey category / total dry weight of prey of all categories) x100).

Following Pinkas *et al.* (1971) an index of the relative importance of each prey was also calculated as: IRI = %FO x (%N + %W); and also %IRI expressed as: %IRI = (IRI / \sum IRI) x100 (Rosecchi and Nouaze 1987).

To determine the diet diversity, Shannon-Wiener index was calculated as $H' = -\sum p_{ij} \log p_{ij}$ where p_{ij} is the proportion of prey category i in the j the predator.

2.3. Stable isotope analyses

All muscle samples were lyophilized and subsequently 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana (www.ebd.csic.es/lie/index.html). Samples were combusted at 1020 °C using a continuous flow isotope ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyzer interfaced with a Delta V Advantage mass spectrometer which applies international standards, run each 9 samples; LIE-CV and LIE-PA, previously normalized with the international standards IAEA-CH-3, IAEACH-6, IAEA-N-1 and IAEA-N-2. Stable isotope ratios were expressed in the standard δ -notation (‰) relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Based on laboratory standards, the measurement error (standard deviation) was ± 0.1 and ± 0.2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. As the C:N ratio was always lower than 3.5 ‰, no correction of the $\delta^{13}\text{C}$ values was required to account for the presence of lipids in muscle samples (Logan *et al.* 2008).

2.4. Isotopic mixing models

To estimate the composition of the diet of *L. budegassa* SIAR Bayesian isotopic mixing model were used (Stable Isotope Analysis in R, SIAR 4.2; Parnell *et al.* 2010) based on their muscle isotopic values and those of their potential prey. Based on the dietary importance of the prey species found in the stomach content generated in the present study we used two main prey groups in the SIAR model (Fish: $\delta^{15}\text{N}$ (‰)= 8.72 ± 0.65 , $\delta^{13}\text{C}$ (‰)=- 19.51 ± 0.46 . Crustacean: $\delta^{15}\text{N}$ (‰)= 8.22 ± 0.66 , $\delta^{13}\text{C}$ (‰)=- 18.21 ± 0.67).

We used isotopic values of the potential prey from a isotopic library containing 128 demersal and pelagic species collected in the Catalan Sea during 2013 (IsoLibrary; ECOTRANS Project, unpublished data).

2.5. Statistical analyses

Chi-Square tests were performed to determine differences in the vacuity index between sizes and seasons.

PERMANOVA analyses were used to test the differences in diet between sizes and seasons of the stomach content.

A correlation was made to determine if there was relation between the body size and the stable isotopes.

T-Student were realized to determine interaction between season and size for both isotopes, after check the normality with Kolmogorov-Smirnov test (K-S test: $p>0.05$). Two ways ANOVA test were conducted for evaluate possible differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between sizes and seasons. Tukey post-hoc tests were used to examine between which seasons the isotopic values differ.

To examine if there were differences in depth distribution between sizes, normality was assessed using a Kolmogorov-Smirnov test. Data for both sizes were not normally distributed (size 1: $d_{85}=0.22$, $p<0.0001$; size 2: $d_{85}=0.24$, $p<0.0001$), consequently the Mann-Whitney test was used to evaluate possible differences in depth between sizes.

3. Results

In total 153 stomachs were dissected, of these 84 contained digested preys in greater or lesser extent (48 for size 1 and 36 for size 2), the rest of stomachs dissected were empty or contain undigested preys that presumably eaten in the net. Isotopic analysis of 135 individuals was made (77 individuals for size 1 and 58 for size 2). Hence, data of 150 individuals were used in this study (for 127 individuals we used both methods).

The body size of the total sampled individuals ranged from 5 to 53 cm (mean= 26.2 cm, SD= 12.0 cm) with a higher frequency (65.84%) for individuals from 15 to 37 cm size (Fig. 1).

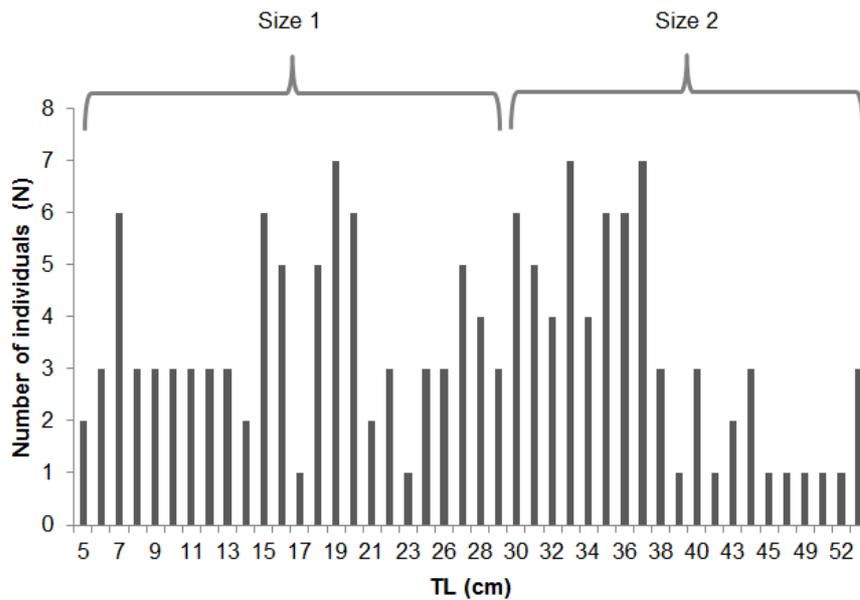


Fig. 1. Length distribution (total length in cm and number) of total samples of *L. budegassa* (N=150) used in this study. NW Mediterranean Sea.

3.1. Stomach content results

The 45.1% of all stomachs analyzed were completely empty. In overall, we found 35 different species from four prey categories (Osteichthyes, Crustacea, Mollusca and Echinodermata; Table 2) and the diversity index was $H'=3.23$. Osteichthyes were the most important prey group contributing 95.24% by occurrence, 57.93% by number, 90.49% by weight and 87.34% by %IRI (Table 2). At specific level, *Ophidion barbatum*, *Gadiculus argenteus*, *Trisopterus minutus*, *Gaidropsarus biscayensis*, *Lesueurigobius friesii*, *Gobius niger* and *Merluccius merluccius* were the most consumed prey of Osteichthyes. Crustacea was the second prey group contributing 44.05% by occurrence, 36.55% by number, 9.40% by weight and 12.51% by %IRI (Table 2). *Solenocera membranacea* was the main crustacean species with 8.56% by %IRI, followed by *Alpheus glaber*, *Processa* sp., *Parapenaeus longirostris* and *Pleisonika* sp. Mollusca and Echinodermata groups were found in very low importance, represented 0.1% and 0.05% by %IRI, respectively (Table 2).

Table 2. Diet composition of *Lophius budegassa* from the NW Mediterranean Sea (%FO. percentage of prey occurrence frequency; %N. percentage of numeric prey abundance; %W. percentage of wet weight abundance; IRI. index of relative importance; %IRI. percentage of modified index of relative importance of prey items).

Prey item	All individuals				Size 1 (TL < 30 cm; N=48)				Size 2 (TL ≥ 30 cm; N=36)			
	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI
OSTEICHTHYES	95.24	57.93	90.49	87.34	100.00	64.94	92.73	93.19	88.89	50.00	89.70	78.35
<i>Anguiliforme unid*</i>	1.19	0.69	0.76	0.14					2.78	1.47	1.03	0.38
<i>Conger conger</i>	3.57	2.07	1.40	0.98	4.17	2.60	0.95	0.96	2.78	1.47	1.57	0.46
<i>Gnathophis mystax</i>	2.38	1.38	4.16	1.05	2.08	1.30	9.67	1.48	2.78	1.47	2.21	0.56
<i>Ophichthus rufus</i>	1.19	0.69	0.07	0.07					2.78	1.47	0.09	0.24
<i>Gadiculus argenteus</i>	8.33	5.52	5.09	7.01	2.08	1.30	8.86	1.37	16.67	10.29	3.75	12.91
<i>Micromesistius poutassou</i>	1.19	0.69	5.26	0.56					2.78	1.47	7.13	1.32
<i>Trisopterus minutus</i>	7.14	4.14	7.46	6.57	8.33	5.19	8.95	7.64	5.56	2.94	6.93	3.02
<i>Gaidropsarus biscayensis</i>	2.38	1.38	30.92	6.10	2.08	2.60	12.27	2.01	2.78	4.41	0.32	0.73
<i>Molva dypterygia</i>	1.19	0.69	0.42	0.10					2.78	1.47	6.44	1.21
<i>Merluccius merluccius</i>	3.57	2.07	4.75	1.93					8.33	1.47	41.87	19.91
<i>Echiodon dentatus</i>	2.38	1.38	0.85	0.42					5.56	2.94	1.15	1.25
<i>Ophidion barbatum</i>	7.14	4.83	10.10	8.45	4.17	1.30	0.68	0.54	11.11	7.35	9.33	10.22
<i>Callionymus lyra</i>	1.19	0.69	0.33	0.10					2.78	1.47	0.45	0.29
<i>Callionymus maculatus</i>	2.38	1.38	0.20	0.30	4.17	2.60	0.77	0.91				
<i>Callionymus</i> sp.	3.57	2.07	0.16	0.63	6.25	3.90	0.61	1.83				
<i>Cepola macrophthalma</i>	2.38	1.38	5.17	1.24	2.08	1.30	18.42	2.66	2.78	1.47	0.48	0.30
<i>Deltentosteus quadrimaculatus</i>	3.57	2.07	0.26	0.66	6.25	3.90	0.98	1.98				
<i>Gobius niger</i>	5.95	4.83	1.07	2.78	8.33	7.79	12.09	10.73	2.78	1.47	1.08	0.39
<i>Lesueurigobius friesii</i>	7.14	4.14	3.96	4.58	12.50	7.79	4.08	9.62				
<i>Lithognathus mormyrus</i>	1.19	0.69	1.55	0.21					2.78	1.47	2.10	0.55
<i>Lepidopus caudatus</i>	1.19	0.69	0.08	0.07	2.08	1.30	0.32	0.22				
<i>Arnoglossus laterna</i>	2.38	1.38	1.99	0.64	4.17	2.60	7.59	2.75				
<i>Arnoglossus</i> sp.	1.19	0.69	0.56	0.12	2.08	1.30	2.13	0.46				
<i>Citharus linguatula</i>	1.19	0.69	0.56	0.12					2.78	1.47	0.76	0.34
<i>Lepidorhombus boscii</i>	1.19	0.69	0.77	0.14	2.08	1.30	2.94	0.57				

<i>Microchirus variegatus</i>	1.19	0.69	1.40	0.20					2.78	1.47	1.89	0.51
<i>Microchirus</i> sp.	1.19	0.69	0.09	0.07	2.08	1.30	0.35	0.22				
Osteichthyes unid*	16.67	9.66	1.11	14.22	25.00	15.58	1.05	26.95	5.56	2.94	1.13	1.25
CRUSTACEA	44.05	36.55	9.40	12.51	31.25	28.57	7.11	6.59	61.11	45.59	10.21	21.51
Amphipoda												
Gammaridea (<i>Rhachotropis</i> sp.)	1.19	1.38	0.00	0.13					2.78	2.94	0.00	0.45
Euphausiacea												
Euphasia unid*	1.19	4.83	0.02	0.46					2.78	10.29	0.03	1.58
Decapoda												
<i>Alpheus glaber</i>	7.14	4.14	0.37	2.55	10.42	6.49	1.12	5.14	2.78	1.47	0.10	0.24
<i>Parapenaeus longirostris</i>	1.19	0.69	1.65	0.22					2.78	1.47	2.23	0.57
<i>Pleisonika</i> sp.	1.19	0.69	0.35	0.10					2.78	1.47	0.48	0.30
<i>Processa</i> sp.	3.57	2.76	0.20	0.84	4.17	2.60	0.15	0.74	2.78	2.94	0.22	0.48
<i>Solenocera membranacea</i>	10.71	6.21	3.87	8.56	2.08	1.30	3.16	0.60	22.22	11.76	4.12	19.46
Natantia unid*	17.86	15.86	2.94	26.62	14.58	18.18	2.69	19.72	22.22	13.24	3.03	19.92
MOLLUSCA	4.76	3.45	0.06	0.10	6.25	5.19	0.15	0.20	2.78	1.47	0.03	0.03
Veneroida												
<i>Tellina</i> sp.	1.19	0.69	0.01	0.07	2.08	1.30	0.02	0.18				
Gastropoda												
<i>Turritella</i> sp.	1.19	1.38	0.02	0.13	2.08	2.60	0.06	0.36				
Gastropoda unid*	2.38	1.38	0.04	0.27	2.08	1.30	0.07	0.18	2.78	1.47	0.03	0.23
ECHINODERMATA	3.57	2.07	0.05	0.05	2.08	1.30	0.00	0.02	5.56	2.94	0.07	0.11
Echinodermata unid*	3.57	2.07	0.05	0.60	2.08	1.30	0.00	0.18	5.56	2.94	0.07	0.92

*unid. prey taxa not determined.

In relation to the size, we did not find differences in the vacuity index ($\chi^2=0.629$, $p=0.428$) between the two sizes analyzed. Shannon diversity index was $H'=2.83$ for size 1 individuals and $H'=2.99$ for size 2.

Regarding depth distribution, Mann-Whitney U test was used and not found differences in depth distribution between sizes ($U_{150}=2534$ $p=0.38$).

The diet composition of our stomachs between sizes showed significant differences (PERMANOVA, $pseudo-F=1.87$, $p=0.01$).

Osteichthyes for size 1 presented 93.19% by %IRI and 78.35% for size 2 individuals. For size 1 individuals the most abundant prey among fishes was *Gobius niger* (%IRI=10.73), followed by *Lesuerigobius friesii* (%IRI=9.62%) and *Trisopterus minutus* (%IRI=7.64%; Table 2). In contrast, for size 2 individuals the demersal fish *Merluccius merluccius* was the most important found in the stomachs (%IRI=19.91%), followed by *Gadiculus argenteus* (%IRI=12.91%) and *Ophidion barbatum* (%IRI=10.22%; Table 2).

Crustaceans presented 6.59% by %IRI for size 1 individuals and 21.51% for size 2. At specific level, the prawn *Solenocera membranacea* was the most important prey for size 2 (%IRI=19.46%) and *Alpheus glaber* for size 1 (%IRI=5.14%; Table 2).

Between seasons, analyzing the both sizes together, we found differences in the vacuity index ($\chi^2=8.071$, $p=0.045$): spring was the season with higher proportion of empty stomachs (66.7%) in comparison with the rest of the seasons (39.8-49.8%). Shannon diversity index was similar in all seasons ranging from 2.18 as the lesser value in autumn to 2.83 as the highest value in summer.

Regarding diet composition of our stomach contents, differences between spring, summer, autumn and winter were not found (PERMANOVA, $pseudo-F=1.15$, $p=0.17$).

In all seasons, the main preys were osteichthyes, followed by crustaceans (Fig. 2). Molluscs and echinoderms groups have a low importance whatever the season.

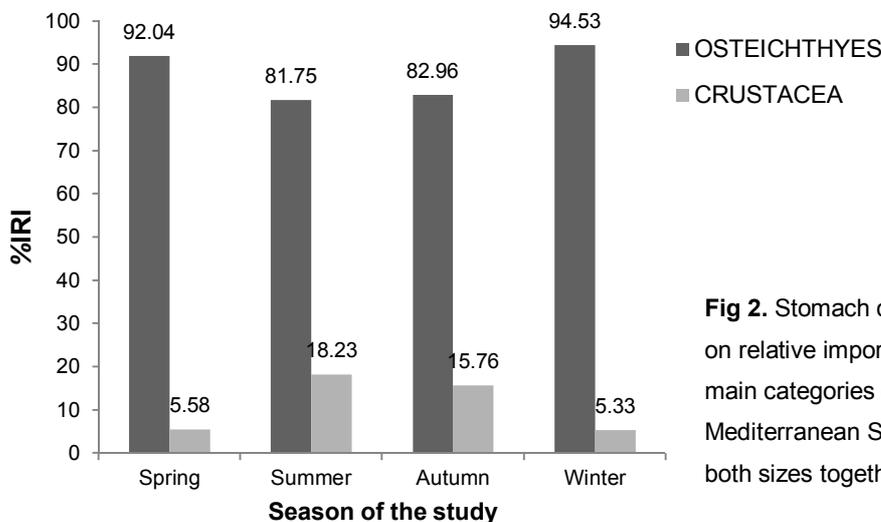


Fig 2. Stomach content analyses based on relative importance index (%IRI) of main categories of prey species. NW Mediterranean Sea between seasons for both sizes together.

3.2. Stable isotopes results

Stable isotopes of carbon and nitrogen were correlated with the body size ($\delta^{15}\text{N}$: $R^2=0.77$, $p<0.0001$; $\delta^{13}\text{C}$: $R^2=0.72$, $p<0.0001$).

In overall, size 2 individuals showed higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than size 1 individuals along all the seasons ($\delta^{15}\text{N}$: $F_{3,134}=54.91$, $p<0.0001$; $\delta^{13}\text{C}$: $F_{3,134}=7.67$, $p<0.0001$; Table 3, Fig.3).

Table 3. Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of *L. budegassa* muscle tissue between sizes. NW Mediterranean Sea.

	N	Mean \pm S.D.	Minimum	Maximum
$\delta^{15}\text{N}$ (‰)				
Size 1	77	9.93 \pm 1.05	6.69	11.87
Size 2	58	11.26 \pm 0.54	9.86	11.26
$\delta^{13}\text{C}$ (‰)				
Size 1	77	-18.75 \pm 0.46	-19.67	-18.75
Size 2	58	-18.22 \pm 0.34	-18.83	-18.22

Due to the interaction between size and season was significantly for both isotopes ($p<0.05$), we analyzed the seasonal differences in the isotopic values for each size separately.

Size 1 individuals showed differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between seasons ($\delta^{15}\text{N}$: $F_{3,76}=6.93$, $p<0.0001$; $\delta^{13}\text{C}$: $F_{3,76}=8.31$, $p<0.0001$). Post-hoc tests indicated that size 1 individuals had lower $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values during spring and summer than during autumn and winter (Fig. 3). For size 2 individuals we also found differences for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between seasons ($\delta^{15}\text{N}$: $F_{3,54}=3.26$, $p=0.03$; $\delta^{13}\text{C}$: $F_{3,54}=5.60$, $p=0.002$). Post-hoc tests indicated that size 2 individuals had higher $\delta^{13}\text{C}$ values during spring and summer than during autumn and winter, and higher $\delta^{15}\text{N}$ during summer than during the other seasons (Fig. 3).

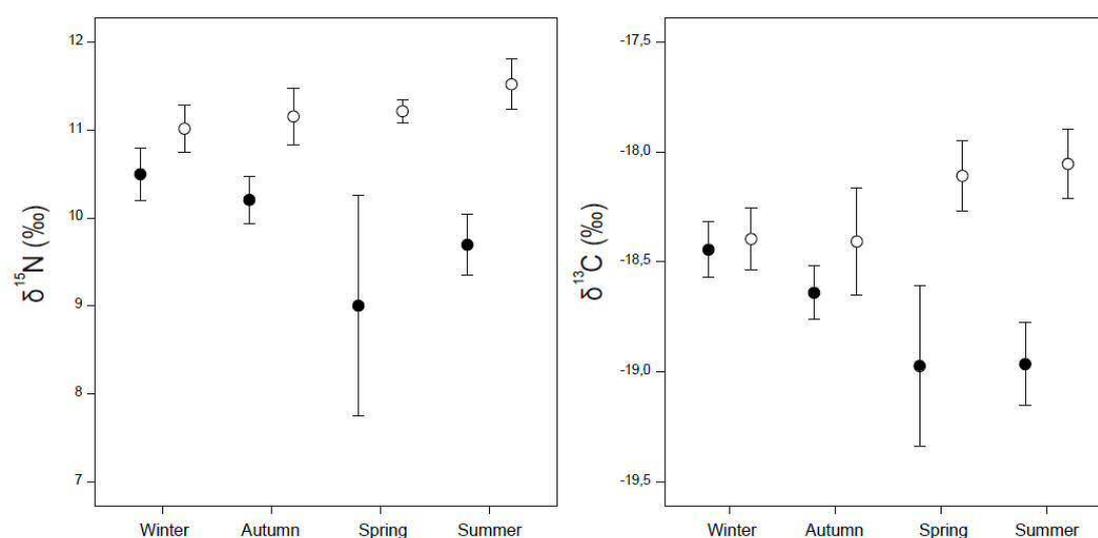


Fig. 3. Mean and 95% CI of N and C isotopic values for size 1 (black circles) and size 2 (white circles) of *Lophius budegassa* throughout the seasons. NW Mediterranean Sea.

In coincidence with the stomach content's results, the proportion of each potential prey estimated by SIAR models revealed that fish was the main prey in the diet, independently of the size and the season; ranging from 74% in the size 2 in summer to 96% for size 1 in the same season (Fig. 4). The importance of crustaceans was clearly low for both sizes groups and for all the seasons, ranging from 3% in the size 1 individuals in summer to 25% for the size 2 also in summer (Fig. 4).

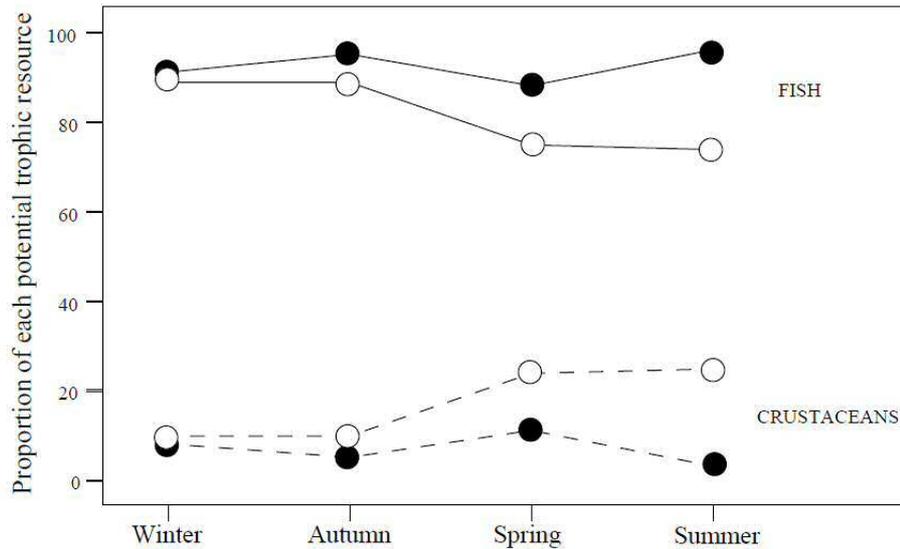


Fig. 4. Mean percentage of the contribution of fish and crustaceans in the diet of size 1 (black circles) and size 2 (white circles) individuals of *Lophius budegassa* throughout the seasons estimated from Bayesian mixing models (SIAR).

4. Discussion

The present study indicates that *Lophius budegassa* is a predator, especially of fishes (osteichthyans) followed by crustaceans, although small proportion of molluscs and echinoderms were also found in some stomachs. Variation in the diet of *L. budegassa* in the NW Mediterranean Sea was related to different factors as size and season. Our results about feeding incidence show that the proportion of individuals with empty stomachs was similar to that found by Stagioni *et al.* (2013) in the Adriatic Sea and higher to that found by Negzaoui-Garali *et al.* (2008) in the Tunisian coasts. These differences on stomach vacuity of *L. budegassa* of Tunisian waters could be due to that those waters are less exploited and hence there are more available preys. However it could be also due to that the catches in Tunisian waters were performed near the coast, at less depth what decreases the effects of decompression and hence the regurgitation, therefore, reduces the number of empty stomachs found (Azevedo 1996, Stagioni *et al.* 2013).

The empty stomachs are found at a variable rate through the year, this suggest differences in the feeding intensity depending on the season (Crozier 1985, Armstrong *et al.*

1996). In our samples, spring was the season with higher proportion of empty stomachs, other authors as Crozier (1985) found also more empty stomachs in spring and summer in other species of lophiiformes (*L. piscatorius*), in the northern Irish Sea. Instead, Negzaoui-Garali et al. (2008) found higher proportion of empty stomachs in winter in Tunisian coasts and Preciado et al. (2006) reported the highest percentage of empty stomachs in autumn in Cantabrian Sea. Since *L. budegassa* is an opportunistic predator, the seasonal variations in diet and the feeding intensity are usually due to availability or abundance of the preys (Laurenson and Priede 2005). In our case, we found changes in the availability of the potential preys in the sampling area, with a minor abundance of the main preys of *L. budegassa* as *Solenocera membranacea*, *Lesueurigobius friesii* and *Alpheus glaber*, among others, in winter and spring (IsoLibrary; ECOTRANS Project, unpublished data). Those were the seasons with lesser preys in the stomach content and, hence, minor feeding activity that was reflected in a higher proportion of empty stomachs.

The clear importance of fishes in the diet of our study is in agreement with previous studies performed in other Mediterranean areas (Tunisian coast: Negzaoui-Garali et al. 2008; Adriatic Sea: Stagioni et al. 2013). The wide spectrum of fish species found in the stomachs indicates that *L. budegassa* is an opportunistic predator. This opportunistic behavior is shown in the stomach contents since the most common preys are also the most abundant in the study area (Bruno et al. 2001). So species such as *Gadiculus argenteus* and *Trisopterus minutus* presented a high importance in the diet due to their availability and abundance in the NW Mediterranean (Cohen et al. 1990).

The feeding behavior of *L. budegassa* indicates that it is an important predator within the food web of NW Mediterranean sharing trophic level with other predators like dolphins, large pelagic fishes and adult hake (Coll et al. 2006, Navarro et al. 2013). Regarding the habitat, *L. budegassa* shares habitat with other benthic species such *Chimaera monstrosa*, *Conger conger* or *Trachyrincus scabrus* (Macpherson 1981) who live also on rocky and sandy bottoms. Focusing in the feeding habits, other fishes as *Merluccius merluccius* present a similar diet, showing common preys such as *Alpheus glaber*, *Solenocera membranacea* or *Gaidropsarus biscayensis*, among others (Cartes et al. 2004, Carpentieri et al. 2005). In studies about diet of *L. budegassa* in the Mediterranean Sea, were found species as *Gadiculus argenteus*, *Ophidion barbatum*, *Lesueurigobius friesii*, *Solenocera membranacea* or *Alpheus glaber* although with less importance that in our results. Instead, species such as *Gobius niger* that found in our stomach contents, not found in the others studies performed in the Mediterranean although its presence is common in Mediterranean Sea (Froese and Pauly 2014)

The great variability in the weight of the preys found in the stomachs indicate that *L. budegassa* presents a feeding strategy based on an indiscriminate hunting of ambush according to Macpherson (1983). A quarter of our stomachs dissected presented several preys inside with different stages of digestion contrary to Macpherson (1983) who found a single prey item (or very rarely two) by stomach with the same digestion stage in *L. upsicephalus*; this could suggest that *L. budegassa* is a more active feeder than *L. upsicephalus*.

For our study we considered that is important combine stomach content and isotope stable analysis because both methods integrate and reflect different time scale. Our results confirm the importance of stable isotopes analysis as complementary technique, since stomach content integrate a short period of time before sampling that could be considered as snapshot. Instead, stable isotope values reflect all the food assimilated by the fish even months before sampling (MacAvoy et al. 2001).

Our stomach content results show differences in the diet between sizes. In particular, size 2 individuals included more crustaceans and less fishes in the diet than size 1, although the main preys for both sizes are fishes. This pattern was consistent with previous studies (Preciado et al. 2006; Stagioni et al. 2013).

The stable isotopes analysis of our individuals supports the ontogenetic diet changes in *L. budegassa* based on the significant differences in the nitrogen isotopes values between both sizes analysed for *L. budegassa*. These differences could be due to variability in nitrogen values of the different species of prey or to the increase of size, since this study and previous works demonstrated a positive relation between $\delta^{15}\text{N}$ in muscle and fish size, due to nitrogen isotope reflects the trophic level of organisms, which generally increases with the fish size (e.g. De Niro and Epstein 1981). We also observed a slight increase of carbon isotopes with an increase in total length, which could indicate differences in organic sources at the base of the food chains (De Niro and Epstein 1978). Also, the differences in preferences of fish prey in the stomach content indicated differences on habitat distribution by sizes. Size 1 individuals feeds mainly on small benthic fish such as *Gobius niger*, *Lesuerigobius friesii* or *Arnoglossus laterna*, that are more frequent in shallow waters, while, size 2 individuals feed on deep-water fish species such as *Merluccius merluccius*, *Gadiculus argenteus* and *Ophidion barbatum*. However, we also found some coastal species like *Gobius niger* as preys of size 2, but in low proportion; as well, *Gadiculus argenteus* and *Ophidion barbatum* are found in the size 1 lesser extent. These results suggest that it could be a depth distribution between sizes as was found by Maravelias and Papaconstantinou (2003) in the northeastern Aegean Sea, although we did not found in our samples such size-related distribution according to the depth. This species according with Maravelias and Papaconstantinou (2003) presents bathymetric movements but vertical migration is unlikely due to the feeding strategy. Bathymetric movements and the deep-strata distribution between sizes-classes could explain the ontogenetic changes reported in our samples.

Other possible explanation to diet shifts could be the horizontal displacement since its observed that *Lophius* spp. travel longest distances (reaching up to 408 km in the northeast Atlantic according to Landa et al. 2008), thought the goal of this movements is not clear; this prove that *Lophius* spp. is a good swimmer contrary to Wheeler (1969) who described them as weak swimmers.

Regarding the seasonal changes, others authors as Preciado *et al.* (2006) and Negzaoui-Garali *et al.* (2008) described seasonal changes in the diet of *L. budegassa* but our stomach contents results do not show significant differences in the feeding habits between seasons, in agreement also with the isotopic mixing model results. On the contrary, we found differences of diet from nitrogen isotopic analysis of individuals that show significant differences throughout the year. That could be due to the variability in nitrogen value of the prey items as it depends on the species and their size. The mean size of the small individuals is lower in spring and summer, this could influence in the lower isotopic values observed in these seasons.

According to Landa *et al.* (2008), other Lophiiformes species, display seasonal onshore-offshore movements in response to prey availability, thermal conditions or spawning period. These movements could contribute to seasonal variation in diet found in other studies and could be reflected in our results of carbon isotopes that show differences in the distribution according to season. Variation of the nitrogen values corroborate that *L. budegassa* is an opportunistic predator. Spring shows the lower nitrogen values in our individuals (mean=10.11 \pm 1.66), according with stomach contents that shows also lesser proportion of preys together with previous season.

Finally, from our study arise the importance of the application of isotopic mixing models using the information obtained from stomach contents about main preys and isotope ratios from *L. budegassa* individuals. These results confirm the great importance of fishes and to lesser extent, crustaceans in the diet of this species.

5. Conclusions

L. budegassa is an important predator within the food web of the Catalan Sea (NW Mediterranean). The stomach content results and isotopic mixing models indicated that this species is a predator with preference for fishes. It also presents ontogenetic shifts in their diet, since large individuals display higher proportion of crustaceans. Instead, seasonal differences were not found nor in stomach contents nor with isotopic mixing models.

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