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Feeding habits of the short-finned squid *Illex coindetii* in the western Mediterranean Sea using combined stomach content and isotopic analysis

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The ommastrephid squid, Illex coindetii*, is one of the most abundant cephalopods in the Mediterranean Sea and an important predator in the ecosystem. In the present study, we examined the diet habits of* I. coindetii *in the north-western Mediterranean Sea by combining two complementary approaches: stomach content and stable isotopic analyses. Specifically, we examined whether the diet differed between sizes and seasons. Stomach content results indicated that the diet of* I. coindetii *was composed of 35 prey items including four major groups; namely the crustaceans* Pasiphaea sivado*, Amphipods, squid of the Order Teuthida, and pelagic and mesopelagic fish. Differences were found among different ontogenetic sizes: juvenile individuals fed mainly on crustaceans (%IRI = 77.59), whereas adult individuals fed on a wider range of prey items, including the shrimp* P. sivado *(%IRI = 33.21), the amphipod* Anchylomera blossevillei *(%IRI = 0.91), the decapod* Plesionika *sp. (%IRI = 0.19), the carangid* Trachurus trachurus *(%IRI = 0.34) and some Myctophids species (%IRI = 0.21). Differences were also found between seasons in the year. In winter, crustaceans were the main prey items, whereas in summer the diversity of prey was higher, including fish, crustaceans and molluscs. Similar to the stomach contents, stable isotopic results indicated differences among sizes. $\delta^{15}N$ values were higher in adult squids than in juveniles because they fed on prey at higher trophic levels. In conclusion, this study indicates that feeding habits of* I. coindetii *vary seasonally and ontogenetically. These feeding variations may be associated with trophic competence scenarios based on size, and also with the availability and abundance of prey throughout the year.*

Keywords: *Illex coindetii*, isotopic mixing model, stable isotope analysis, stomach content analysis, trophic ecology, Western Mediterranean

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INTRODUCTION

Cephalopods play an important role in marine ecosystems (Rodhouse & Nigmatullin, 1996; Coll *et al*., 2013a). The reduction in abundance of large marine predators such as sharks, mammals, large fish and seabirds is altering ecosystems, promoting the expansion and increase of the biomass and abundance of cephalopods, increasing their importance in marine ecosystems (Caddy & Rodhouse, 1998; Rosas-Luis *et al*., 2008; Coll *et al*., 2013a; Navarro *et al*., 2013). This is the case for *Illex coindetii* (Vèrany, 1839), an Ommastrephid squid widely distributed throughout the Mediterranean Sea, eastern Atlantic and western Atlantic (Nesis, 1987). An increase in its abundance in the Mediterranean during the last few decades, and its importance as both prey and predator, have made *I. coindetii* an important organism in the ecosystem (Rosas-Luis *et al*., 2014). Thus, knowledge of its trophic ecology is important in understanding the interaction between *I. coindetii* and other species in the ecosystem (Clarke & Kristensen, 1980; Rosas-Luis *et al*., 2014). Different diet patterns of *I. coindetii* were found in previous studies in the Mediterranean Sea. Although Sánchez (1982), Sánchez *et al.* (1998) and Petric *et al.* (2011) indicated that fish was the main prey for adult *I. coindetii*, followed by crustaceans and molluscs, recently Rosas-Luis *et al.* (2014) indicated that the diet of adult squid was composed mainly by crustaceans followed by fish. These results suggest an apparent change in the main trophic habits of *I. coindetii* over the last three decades, making clear the necessity to confirm this trend with additional studies and more specific analyses. Furthermore, there is no information about the diet habits of small-sized juveniles, which may be important in order to understand the trends and variability of feeding behaviour of this species.

The study of the feeding ecology of marine predators has traditionally been based on stomach content analysis (Stergiou & Karpouzi, 2001). Although this analysis permits high levels of taxonomic resolution, cephalopods often have

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empty stomachs and it is sometimes difficult to identify stomach contents, because squid macerate their prey with their beaks (Castro & Hernández-García, 1995). The use of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) has been applied as an alternative and complementary tool to study the feeding ecology and trophic position of predators including squid (Cherel & Hobson, 2005, 2009; Stowasser *et al.*, 2006; Guerra *et al.*, 2010; Ruiz-Cooley *et al.*, 2010; Navarro *et al.*, 2013). This approach is based on the fact that $\delta^{15}N$ and $\delta^{13}C$ values are transformed from dietary sources to consumers in a predictable manner (Kelly, 2000) and indicate the diet of the consumer over a longer time period (i.e. from several weeks to months; Ruiz-Cooley *et al.*, 2006). $\delta^{15}N$ values show a stepwise enrichment between 2–5‰ with each trophic level and are reliable indicators of the consumer's trophic position (Layman *et al.*, 2012). $\delta^{13}C$ values show little change due to trophic transfer, but are useful indicators of dietary sources of carbon (Layman *et al.*, 2012). Moreover, with the use of isotopic mixing models we can estimate the relative contribution of each prey item to the diet of the consumer. These models combine the stable isotope values for consumers with those of their potential prey (e.g. Stable Isotope Analysis in R [SIAR] isotopic mixing model; Parnell *et al.*, 2010). While caution is needed when interpreting the outcomes of both types of analyses and they may not be directly comparable, their combination is highly useful to better understanding the trophic ecology of marine predators (Navarro *et al.*, 2014; Albo-Puigserver *et al.*, 2015).

In the present study, we examined the diet habits of *I. coindetii* in the Catalan Sea (north-western Mediterranean Sea; Figure 1) by combining stomach content and isotopic analyses. Specifically, we examined whether the diet differs between ontogenetic sizes (juveniles and adults) and seasons (summer and winter). Our study provides new insights into the ecological role of this species within the north-western Mediterranean ecosystem, providing new data on how this abundant cephalopod exploits available resources.

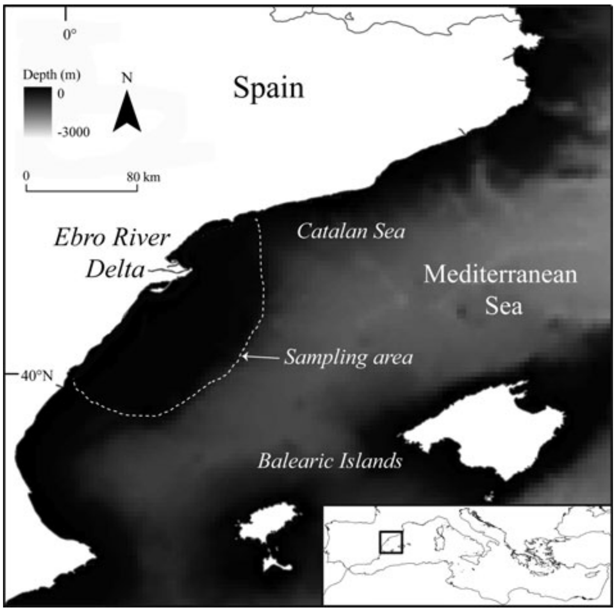

Fig. 1. Map of the study area (north-western Mediterranean Sea), indicating the area where squids were collected.

MATERIALS AND METHODS

Study area and sampling procedures

Samples were obtained from the continental shelf and slopes associated with the Ebro River delta (north-western Mediterranean Sea; Figure 1) during winter and summer of 2013. This area is highly productive due to the contributions of organic matter at the mouth of the Ebro River, and the effect of the northern current along the continental slope (Salat, 1996). A total of 292 individuals were sampled in this area during two experimental fishing cruises as well as from commercial demersal trawlers. After the catch, we measured the mantle length (ML), total weight, sex and maturity of each individual. Moreover, a small portion of flesh from the mantle from 62 individuals was collected (24 samples were from winter and 38 from summer of 2013) and stored at −20°C until their isotopic determination. Based on the size at first maturity (following Jereb & Ragonese, 1995), we grouped all individuals into two groups: adult individuals (11–22 cm mantle length) and juvenile individuals (4–10 cm mantle length).

Stomach content analysis

The stomach of all individuals was extracted after dissection. Each stomach was weighed in a digital balance at a precision of 0.001 g to calculate the stomach content weight (SCW) and fullness weight index (FWI) following the Rasero *et al.* (1996) equation: FWI = (SCW × 100)/(BW-SCW), with BW being the body weight.

Stomach content identification was determined under a binocular microscope (60–120×). All prey items were weighed to the nearest 0.001 g. The otoliths of fish, the beaks of cephalopods and the exoskeletons of crustaceans were used to identify the prey, following different reference classification guides (Clarke, 1986; Zariquiey-Álvarez, 1986; Boshi *et al.*, 1992; Smale, 1996; Tuset *et al.*, 2008). We did not measure the length of the prey items.

Frequency of occurrence (%FO) and numeric (%N) and gravimetric (%W) methods were used to quantify the diet. %FO was calculated as the percentage of stomachs with a certain prey relative to the total number of stomachs analysed; %N is the number of individuals of a certain prey relative to the total number of individual prey items; %W is defined as the weight of a certain prey relative to the total weight of the stomachs (Cailliet, 1976). Index of relative importance (IRI): IRI = (%N + %W)*(%FO) (Pinkas *et al.*, 1971) was calculated for each season (winter and summer) and ontogenetic-size (juveniles and adults). To allow comparisons between groups, the IRI was expressed as a percentage ($\%IRI_i = 100 \cdot IRI_i / \sum_{i=1}^{n} IRI_I$; Cortés, 1997).

Stable isotope analysis and isotopic mixing model

All fresh samples collected randomly from the capture individuals were subsequently freeze-dried and powdered, and 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratorio de Isótopos Estables of the Estación Biológica de Doñana

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(LIE.EBD, Spain; www.ebd.csic.es/lie/index.html). All samples were combusted at 1020°C using a continuous flow isotope-ratio mass spectrometry system by means of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The isotopic composition is reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite (δ^{13}C) and atmospheric N_2 (δ^{15}N). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of ±0.1 and ±0.2‰ for δ^{13}C and δ^{15}N, respectively. The standards used were: EBD-23 (cow horn, internal standard), LIE-BB (whale baleen, internal standard) and LIE-PA (feathers of Razorbill, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna). Since C:N values were lower than 3.5, we did not correct the δ^{13}C for the effect of lipids (following Logan & Lutcavage, 2010).

To estimate the potential contribution of the different prey groups to the diet of *I. coindetii*, we adopted a Bayesian multi-source isotopic mixing model (SIAR 4.2; Parnell & Jackson, 2013). The SIAR model estimates the potential contribution of each prey in the diet based on their isotopic values and those of the potential prey. This model runs under the free software R (R Development Core Team 2009) and allows the inclusion of sources of uncertainty in the data, in particular the variability in the stable isotope ratios of the predator and the potential prey (Parnell *et al.*, 2010). To run the model SIAR, values of the potential prey were taken from a reference isotopic library that contains up to 128 species collected in the area of study during 2013 (IsoLibrary; ECOTRANS project; Barría *et al.*, 2015). Main potential prey groups were selected according to the stomach information. Thus, three main groups were selected: crustaceans ($\delta^{15}N = 6.19 \pm 0.25$‰; $\delta^{13}C = -19.47 \pm 0.27$ ‰), cephalopods ($\delta^{15}N = 9.7 \pm 1.44$‰; $\delta^{13}C = -18.79 \pm 0.67$‰) and fish ($\delta^{15}N = 8.12 \pm 0.30$‰; $\delta^{13}C = -19.53 \pm 0.58$‰). Diet tissue discrimination factors of 2.14‰ for δ^{15}N and 0.30‰ for δ^{13}C were used following Caut *et al.* (2009).

Statistical analysis

Differences in %W, δ^{15}N and δ^{13}C between adult and juvenile individuals and seasons were tested using two-way semi-parametric permutation multivariate analyses of variance tests (PERMANOVA test) on the Euclidean distance matrix (Anderson *et al.*, 2008). In the case of a significant result, pairwise tests were carried out. PERMANOVA allows for the analysis of complex designs (multiple factors and their interaction) without the constraints of multivariate normality, homoscedasticity and greater numbers of variables than sampling units, as in traditional ANOVA tests. The method calculates a pseudo-F-statistic directly analogous to the traditional *F*-statistic for multifactorial univariate ANOVA models, using permutation procedures to obtain *P* values for each term in the model (Anderson *et al.*, 2008). PERMANOVA tests were performed with the PRIMER-E 6 software (Anderson *et al.*, 2008). Significance level for all tests was adopted at $P < 0.05$.

RESULTS

Stomach content results

Of the 292 individuals analysed, 172 stomachs were empty. Overall, the stomach contents of the remaining 120 individuals of *Illex coindetii* were composed mainly by crustaceans (%IRI = 56.65), followed by fish (%IRI = 33.12) and molluscs (%IRI = 9.80) (Table 1). Cnidarians and ctenophores were also found in the stomachs but in a very low proportions (%IRI < 1) (Table 1). At a specific level, the most important crustacean species were the shrimp *Pasiphaea sivado* (Risso, 1816), the amphipod *Anchylomera blossevilleii* (Milne Edwards, 1830), and the pandalid *Plesionika* sp. (Spence Bate, 1888) (Table 1). In relation to fish, the most important species were the clupeid *Sprattus sprattus* (Linnaeus, 1758) and the perciform *Trachurus trachurus* (Linnaeus, 1758) (Table 1). Within the molluscs group, the cephalopod order Teuthida was the most abundant, and the most important cephalopod species were the squid *Histioteuthis* sp. (d'Orbigny, 1841) and species of the clade Thecosomata (Table 1).

Based on the %W, the diet of *I. coindetii* differed between adult and juvenile individuals (Table 3). Juveniles showed a diet composed mainly of crustaceans (%IRI = 77.59), followed by molluscs (mostly squid; %IRI = 15.01) and fish (%IRI = 4.92). In contrast, adults showed a diet composed mainly of fish (%IRI = 55.14), followed by crustaceans (%IRI = 39.31) and molluscs (%IRI = 5.41) (Table 1).

The PERMANOVA tests showed differences between juveniles between summer and winter seasons ($P < 0.01$; Table 3). During winter, juveniles fed mainly on crustaceans (%IRI = 99.09) with the shrimp *P. sivado* (IRI% = 74.06) as the most important crustacean prey. The second group was fish (%IRI = 0.33), followed by the mollusc groups. In summer, molluscs were the most important group for juveniles (%IRI = 62.26) followed by fish (%IRI = 16.67) and crustaceans (IRI% = 12.10) (Figure 2A). In contrast to juveniles, adults showed similar diets between seasons ($P = 0.52$) (Table 3). During winter, adults fed on crustaceans (%IRI = 52. 35) followed closely by fish (%IRI = 41. 96) and molluscs (%IRI = 5.33). During summer, adults fed mainly on fish (%IRI = 69.12) followed by crustaceans (%IRI 25.64) and molluscs (%IRI = 5.24).

Isotopic results

δ^{15}N values differed significantly between adults and juveniles, and between winter and summer (Tables 2 and 3) and δ^{15}N values of juveniles differed significantly between winter and summer (Figure 3). In contrast, δ^{13}C values did not differ between seasons but did differ between adults and juveniles (Tables 2 and 3; Figure 3). SIAR outputs indicated a high contribution of crustaceans in juveniles (mean contribution = 94.6%) during winter, followed by fish (3.9%) and molluscs (1.4%) (Figure 2B). In summer, the mean contribution of crustaceans to the total diet of juvenile individuals represented 71.8% followed by fish (23.5%) and molluscs (4.6%). For adult individuals, the importance of the different prey groups between summer and winter was similar. The mean contribution of crustaceans in summer and winter, respectively, was 45.9 and 47.9% (Figure 2B). The group of fishes represented 40.2% in summer and 19.8% in winter. Finally, molluscs

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Table 1. Percentage of number (%N), frequency of occurrence (%FO), weight (%W) and relative importance (%IRI) of the different prey identified in the stomach contents of *Illex coindetii* individuals collected during 2013 in the North-western Mediterranean.

Prey	**%N**			**%W**			**%FO**			**%IRI**		
	Juveniles	**Adults**	**Both**	**Juveniles**	**Adults**	**Both**	**Juveniles**	**Adults**	**Both**	**Juveniles**	**Adults**	**Both**
Actinopterygii	11.36	33.06	**23.92**	15.12	46.03	**42.97**	14.92	60.38	**35.00**	4.92	55.15	**33.12**
Mycthophidae												
Diaphus sp.	1.14		0.48	10.80		1.07	1.50		0.83	0.317		0.05
Benthosema glaciale		1.65	0.96		0.89	0.81		1.89	0.83		0.14	0.06
Unidentified		0.83	0.48		0.50	0.45		1.89	0.83		0.07	0.03
Stomiidae												
Chauliodus sloani	1.14	0.83	0.96	0.20	0.32	0.31	1.50	1.89	1.67	0.18	0.06	0.08
Stemoptychidae												
Maurolicus muelleri		1.65	0.96		0.52	0.47		1.89	0.83		0.12	0.05
Parapelididae												
Arctozenus risso		0.83	0.48		1.21	1.09		1.89	0.83		0.10	0.05
Spariadae												
Pagellus sp.		0.83	0.48		1.57	1.41		1.89	0.83		0.11	0.06
Carangidae												
Trachurus trachurus		1.65	0.96		1.84	1.66		3.77	1.67		0.34	0.18
Clupeidae												
Sprattus sprattus		2.48	1.44		1.99	1.79		3.77	1.67		0.46	0.22
Engraulidae												
Engraulis encrasicolus		0.83	0.48		1.57	1.41		1.89	0.83		0.11	0.06
Unidentified fish	9.09	21.49	16.27	4.12	35.63	32.50	11.94	43.40	25.83	11.73	61.13	50.80
Mollusca	19.31	10.74	**14.35**	40.03	13.83	**16.43**	25.37	18.87	**22.50**	15.01	5.42	**9.80**
Cephalopoda												
Ommastrephidae												
Illex coindetii	1.14	0.83	0.96	0.14	0.36	0.34	1.49	1.89	1.67	0.18	0.07	0.09
Todaropsis eblanae		0.83	0.48		0.06	0.05		1.89	0.83		0.05	0.02
Histioteuthidae												
Histoteuthis sp.	1.14	0.83	0.96	0.80	9.62	8.75	1.49	1.89	1.67	0.19	0.40	0.09
Loliginidae												
Loligo forbessii		1.65	0.96		0.37	0.34		1.89	0.83		0.12	0.02
Loligo sp.	1.14		0.48	1.63		0.16	1.49		0.83	0.20		0.02
Theuthida												
Unidentified	11.36	1.65	5.74	35.81	1.12	4.56	14.92	3.77	10.00	22.32	0.29	4.15
Sepiidae												
Sepia elegans		0.83	0.48		1.32	1.19			0.83			0.06
Unidentified Cephalopoda		1.65	0.96		0.43	0.39		3.77	1.67		0.24	0.09
Gastropoda												
Thecosomata	4.54	0.83	2.39	1.66	0.30	0.43	5.97	1.89	4.17	2.91	0.06	0.47
Unidentified Bivalvia		1.65	0.96		0.24	0.22		1.89	0.83		0.11	0.04
Crustacea	56.81	52.06	**54.07**	42.21	39.93	**40.15**	49.25	33.96	**42.50**	77.59	39.31	**56.65**
Decapoda												
Pasiphaea multidentata	1.14		0.48	2.02		0.20	1.49		0.83	0.19		0.02
Pasiphaea sivado	25	39.67	33.49	12.21	31.67	29.75	11.94	16.98	14.17	32.36	33.21	36.12
Eualus sp.	1.14		0.48	1.10		0.11	1.49		0.83	0.19		0.02
Ligur sp.	2.27		0.96	1.13		0.11	2.98		1.67	0.74		0.07
Plesionika sp.	6.81	2.48	4.31	12.15	0.96	2.07	5.97	1.89	4.17	4.87	0.19	1.07
Parapenaeus longirostris	1.14		0.48	1.32		0.13	1.49		0.83	0.19		0.02
Unidentified Penaeidae	1.14		0.48	0.91		0.09	1.49		0.83	0.19		0.02
Thysanoessa		0.83	0.48		0.79	0.71		1.89	0.83		0.08	0.04
Amphipoda												
Anchylomera blossevillei	3.41	3.30	3.35	3.15	2.54	2.60	4.48	5.66	5.00	1.71	0.91	1.20
Phrosina semilunata		0.83	0.48		1.97	1.79		1.89	0.83		0.12	0.08
Phrosina sedentaria	2.27	0.83	1.44	1.66	0.99	1.06	1.49	0.09	1.67	0.37		0.17
Unidentified	5.68	0.83	2.87	1.86	0.40	0.49	7.46	1.89	5.00	4.53	0.06	1.20
Unidentified Crustacea	6.82	3.31	4.78	4.70	0.64	1.04	8.95	7.55	8.33	6.73	0.94	1.96
Cnidaria	1.14		0.48	0.03		0.01	1.49		0.83	0.18		0.02
Ctenophora		3.30	1.91		1.89	0.18		0.20	0.83		0.22	0.07
Algae												
Phaeophyta	1.14		0.48	0.95		0.09	1.49		0.83	0.19		0.02
Unidentified algae	2.72		0.96	0.08		0.01	2.98		1.67	0.71		0.06
Organic matter	7.95	0.83	3.83	1.56	0.01	0.17	10.44	1.89	6.67	8.80	0.05	1.07
N full stomachs	67	53	120									

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Table 2. Number of samples (n), mean and standard deviation of δ^{15}N, δ^{13}C and C:N ratio values of *Illex coindetii* by ontogenetic stage (juveniles and adults) and season.

	n	δ^{15}N(‰)	δ^{13}C (‰)	C:N
Juveniles				
Winter	12	7.83 ± 0.23	−18.43 ± 0.17	3.33 ± 0.13
Summer	29	8.97 ± 0.79	−19.22 ± 0.16	3.39 ± 0.06
Adults				
Winter	12	9.66 ± 0.72	−18.64 ± 0.55	3.31 ± 0.04
Summer	9	9.58 ± 0.20	−18.53 ± 0.41	3.44 ± 0.09

represented 13.7% in summer and 32.3% in winter (Figure 2B).

DISCUSSION

In this study, the trophic ecology of *Illex coindetii* was analysed using two complementary methods, stomach content and stable isotope analysis. The use of both methodologies offers an integrated perspective of the diet of *I. coindetii* at different temporal scales. Stomach content analysis allows the determination of the main prey up to the species level (Sánchez, 1982; Castro & Hernández-García, 1995; Petric *et al.*, 2011; Rosas-Luis and Sánchez 2014; Rosas-Luis *et al.*, 2014). However, this method only allows for information on the most recent prey ingested. Additionally, in the case of squid, difficulties in finding soft prey in stomachs and the high proportion of empty stomachs indicate the limitations of this methodology. Despite its use mostly over the last decade, stable isotope analysis has great potential for trophic ecology studies in cephalopods as it offers information about the diet at a larger temporal scale (~1 month for mantle tissue; Takai *et al.*, 2000; Cherel & Hobson, 2005; Navarro *et al.*, 2013).

The results of the present study highlight the importance of crustaceans in the diet of *I. coindetii*, in particular the decapods *Pasiphaea sivado* and *Anchylomera blossevillei*. These results could suggest that crustaceans have been increasing in importance as a prey item for *I. coindetii* in recent years, since being reported as a secondary prey in older studies (Sánchez, 1982; Castro & Hernández-García, 1995; Sánchez *et al.*, 1998) to becoming the most important prey in the feeding habits of this species in the western Mediterranean (Rosas-Luis *et al.*, 2014). This apparent change in the diet was confirmed with both stomach content and stable isotopic results. Probably, this change of feeding behaviour of *I. coindetii* can be related to a reduction in the abundance of pelagic fish due to the increase in the fishing pressure in the Northwestern Mediterranean during the last decade

Table 3. PERMANOVA test results on the presence of significant differences of %W, δ^{15}N and δ^{13}C values between seasons (summer and winter) and ontogenetic stages (juvenile and adult) of *Illex coindetii* from the NW Mediterranean.

Parameter	Factor	df	Pseudo-F	*P* (perm)
%W	Season	1,116	2.79	0.001*
	Maturity	1,116	5.19	<0.001*
	Season × Maturity	1,116	1.61	0.05
δ^{15}N	Season	1,58	4.01	0.05
	Maturity	1,58	26.54	<0.001*
	Season × Maturity	1,58	9.86	0.004*
δ^{13}C	Season	1,58	0.06	0.8
	Maturity	1,58	59.52	<0.001*
	Season × Maturity	1,58	0.55	0.46

*Statistical significance, *P*(perm) <0.05.

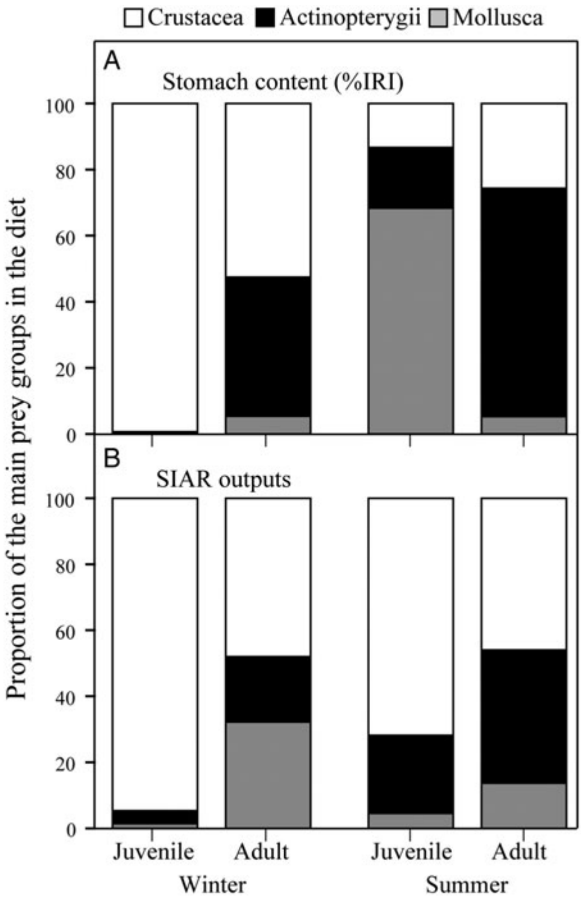

Fig. 2. Mean proportional contributions of fish (Actinopterygii), crustaceans (Crustacea) and molluscs (Mollusca) to the diets of *Illex coindetii* in relation to the size and season based on (A) stomach content analysis and (B) SIAR models.

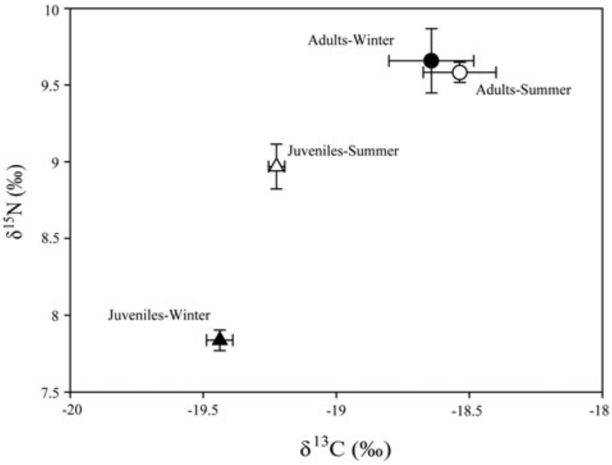

Fig. 3. Mean and standard deviation of δ^{15}N and δ^{13}C values of *Illex coindetii* by size and season.

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(Coll *et al.*, 2013b). Fish was the group second in importance in the diet of *I. coindetii*, including species such as *Sprattus sprattus*, not reported previously in the diet of *I. coindetii*, and *Trachurus trachurus* (Sánchez, 1982; Castro & Hernández-García, 1995; Sánchez *et al.*, 1998; Petric *et al.*, 2011; Rosas *et al.*, 2014). Similar to previous studies, we found that cephalopods are present in the diet of *I. coindetii* (e.g. Sánchez, 1982; Castro & Hernández-García, 1995; Rosas-Luis *et al.*, 2014). In particular, cephalopods of the genera *Histioteuthis* and the clade Thecosomata were the most abundant cephalopods found in the stomachs. However, the presence of other cephalopods was very low, confirming the low incidence of this feeding behaviour in *I. coindetii* (Sánchez, 1982; Sánchez *et al.*, 1998; Rosas-Luis *et al.*, 2014).

Both stomach content and isotopic mixing models indicated differences in the feeding habits between juveniles and adults. The importance of fish was greater for adults than juveniles, whereas crustaceans were more important for juveniles, especially during winter. These diet differences may be related to the ontogenetic development of the beak of squids as the individual grows (Castro & Hernández-García, 1995), allowing the larger adult individuals to prey upon the larger species such as fish (Costalago *et al.*, 2012; Pereira *et al.*, 2014).

Crustaceans were the most important prey during winter. This result may be due to the *I. coindetii* migration during winter when this squid is found in deeper waters (Omori, 1974), and preying on crustaceans such as *P. sivado* (Sánchez & Martín, 1993; Castro & Hernández-García, 1995). On the contrary, in summer, *I. coindetii* moves to shallower waters (Sánchez *et al.*, 1998) where it feeds on a wide range of prey represented in the higher number of molluscs and fish prey identified as a consequence of an increase in the availability of prey in shallow waters.

Regarding seasonal differences, according to the stomach content analysis, molluscs were the most important prey for juveniles of *I. coindetii* in summer, but the SIAR analysis results did not reflect this preference. Results of the stomach content analysis agree with those of Sánchez (1982), Sánchez *et al.* (1998), Petric *et al.* (2011) and Rosas-Luis *et al.* (2014) and are related to the generalist feeding behaviour of small-sized squid and the abundance of this prey during summer. However, as suggested by Keller *et al.* (2012), hard structures (such as beaks) are difficult to digest and may accumulate in the stomachs, promoting an overestimation of their importance in the diet of juveniles of *I. coindetii*. Thus, the large increase of molluscs in the diet of juveniles in summer indicated by the stomach contents should be interpreted with caution. According to the isotope values, the higher values of δ^{15}N found in juveniles during summer are probably related to a slightly greater presence of fish and molluscs than in winter.

Furthermore, regarding the bathymetric distribution of prey, this study agrees with Sánchez *et al.* (1998) as juveniles and adults share the same bathymetric range during the year and, moreover, confirms that although *I. coindetii* comes very close to the seabed during its daily vertical migrations, it never seeks food there but feeds mainly on prey swimming off the bottom.

In summary, based on stomach and stable isotopic results, we confirmed the wide range of prey in the diet of *I. coindetii*, with the importance of crustaceans of the genus *Pasiphaea* in their feeding habits, and the change in diet between seasons and between juveniles and adults. These results provide new insights into the ecological role of this species in the Mediterranean ecosystem and, from a technical perspective, the use of both stomach content and isotopic analyses present new opportunities to examine the trophic ecology of other Mediterranean cephalopods.

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