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RESEARCH PAPER

New Information on Deep-Sea Cephalopod *Ancistroteuthis lichtensteinii* (Cephalopoda: Onychoteuthidae) in the Adriatic Sea

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Abstract

Specimens of *Ancistroteuthis lichtensteinii* were collected in the South Adriatic Pit during the deep-sea research performed in the South Adriatic Pit in 2010. They were caught at depths between 690 and 1.200 m with pelagic and bottom trawls. A total of 39 individuals were collected. The mantle length ranged from 13 to 129 mm, and body weight from 0.2 to 63.1 g. The length-weight relationship revealed a negative allometric growth. Morphological characteristics of upper and lower beak showed a positive correlation with mantle length and body weight. When compared to the body length, the highest growth rate in the sample was determined for the crest length and the lowest rate for the rostrum length, both for upper and lower beak. Statolith microstructure analysis showed that individual age ranged between 72 and 178 days and average age of the analysed specimens was 108 days. This study proved that juveniles of this rare species inhabit depths of the South Adriatic Sea. The general lack of information on *A. lichtensteinii* is not a result of its absence in the area but of the scarcity of investigations in depths of the South Adriatic Pit and use of inadequate sampling techniques for the pelagic species.

Keywords: Angel squid, distribution, age, length-weight relationship, beak morphometry.

Introduction

Ancistroteuthis lichtensteinii (Férussac, 1835), family Onychoteuthidae, is the only species of the genus *Ancistroteuthis*. The species, also known as the angel clubhook squid or just angel squid, inhabits open warm-temperate waters. Although it can be found in a wide depth range, from the surface to about 1270 m (Villanueva, 1992), it is generally considered as a mesopelagic species. It is distributed in the Atlantic Ocean, from the north-western Spain and the Straits of Gilbraltar to Angola on the east and in the Gulf of Mexico and south-western Atlantic on the west, and it is also distributed in the south-western Pacific and throughout the Mediterranean Sea (Roper & Jereb, 2010).

The biology and ecology of the species is poorly investigated. The majority of records of this species are from the Western Mediterranean (Vecchione, Young, & Tsuchiya, 2008). First records on *A. lichtensteinii* are related to the investigations of teutofauna in the western and central basin of the Mediterranean (Naef, 1921; Denger, 1926; Mangold, 1973). Since then, the species has been registered in several areas (Villanueva, 1992; Rasero, Gonzalez, & Guerra, 1993; Lefkaditou, Politou, & Papaconstantinou, 2000; Lefkaditou, Mytilineou, Maiorano, & D'Onghia, 2003; Gonzalez & Sanchez, 2002; D’Onghia *et al*., 2011; Quetglas *et al*., 2013), including the Adriatic Sea where its presence was mainly presumed based on findings of beaks in the stomachs of the predators *Xiphias gladius* and *Grampus griseus* (Gamulin-Brida & Ilijanić, 1972; Bello, 1990; Bello, 1991; Bello, 1996). The records outside the Mediterranean are very rare: the Gulf of Mexico (Voss, 1956), off West Africa (Adam, 1962), and the southwestern Pacific (Rancurel, 1970).

There is a lack of information on this generally poorly known species as it inhabits open-sea areas in a wide depth range but outside the continental shelf and is therefore less accessible for the fishing tools. It is distributed in the deep Southern Adriatic Pit which has been the least investigated area in the Adriatic Sea and this is the first time that a larger number of specimens were caught and available for more detailed analysis.

The aim of this study was to obtain new information and increase the knowledge on *A. lichtensteinii*, especially regarding the spatial distribution, depth range, beak morphometry, size and sexual composition of the caught population, length-weight relationships, age and growth of this deep-sea

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species in the Adriatic Sea.

Materials and Methods

A total of 39 individuals of *A. lichtensteinii* were caught during March, May and August 2010 in the frame of the expedition FAO AdriaMed Deep Sea Survey in the South Adriatic Pit (Table 1). All trawls were carried out on board of the research vessel “Andrea”, using bottom trawl net (May and August) and pelagic trawl net (March, May and August).

An experimental bottom trawl net, GOC 73, with large vertical opening (2-3 m) and 20 mm stretched mesh size at the cod-end (Bertrand, Gil de Sola, Papaconstantinou, Relini, & Souplet, 2002) was used for sampling of demersal cephalopods with the speed of trawling being 2.5 knots, duration 2.5 hours and the length of released rope between 2800 and 3000 m.

For sampling in the water column, the commercial pelagic trawl net with 16 mm stretched mesh size at the cod-end was used. The time of trawling with this fishing gear was between 30 and 170 min depending on depth, with the speed of trawling being 4 knots.

Stations were positioned in the South Adriatic Pit (41°09.53’ to 43°25.29’ N and 14°17.66’ to 18°14.35’ E), and randomly distributed taking into account the sea bottom topography and avoiding areas known to be repositories of explosive and toxic materials.

Ancistroteuthis lichtensteinii specimens were determined using characteristic diagnostic features as described in identification keys of Mangold & Boletzky (1987) and Nesis (1987).

The dorsal mantle length (ML) in millimetres, body weight (BW) to the nearest 0.01 g, sex and sexual maturity of all individuals were noted. Stages of sexual maturity were assessed based on macroscopic observation of gonads, reproductive outputs and other parts of the reproductive system (MEDITS, 2007).

Lower and upper beaks of the analysed individuals were preserved in small plastic tubes with water after laboratory examinations of each specimen. The beaks of two smallest individuals were isolated and preserved together with the buccal mass and this organic tissue was removed using sodium-hypochlorite solution prior to the analysis of the beaks. A total of 7 beak morphometric characters of the lower and upper beak were measured by digital caliper with the precision of 0.01 mm following Clarke (1962, 1986): hood length (HL), crest length (CL), rostral length (RL), wing length (WL), jaw angle width (JW), width of the lateral wall (Lwa) for the upper jaw and length of the baseline (BL) for the lower jaw. To estimate the morphometric relationships between body variables the allometric equation $Y=aX^b$ was used with DML and BW as independent variable and different beak dimensions as dependent variable. Linear regression analysis of the log-transformed data was used to obtain relationships of different beak measurements to dorsal mantle length (ML) and body weight (BW). When ML was used as the independent variable, values of the calculated variable b (relative growth rate), indicated positive allometry ($b>1$), negative allometry ($b<1$) or isometry ($b=1$) (Petrić, Ferri, Škeljo, & Krstulović Šifner, 2010; Ikica, Vuković, Đurović, Joksimović, & Krstulović Šifner, 2014).

The length-weight relationship was described using the equation $BW=aML^b$. Parameters a and b were calculated statistically by least squares regressions. A Student’s t-test was used to evaluate relative growth isometry for the obtained relationship. The P value of less than 0.05 was considered as being

Table 1. Position of stations (beginning of trawling), depth and number of individuals (N), of the species *A. lichtensteinii* sampled in the South Adriatic Pit in 2010

Position of stations		N	Depth (m)	Month
Latitude	Longitude			
Pelagic trawl				
41°50.220'N	17°45.490'E	3	1189	March
42°09.390'N	17°13.750'E	1	1023	March
41°59.080'N	17°32.060'E	4	1180	March
41°44.230'N	17°53.660'E	5	1194	March
42°04.720'N	17°22.480'E	8	1100	March
41°51.620'N	17°07.270'E	2	850	May
41°42.970'N	17°18.570'E	2	1060	May
41°57.067'N	16°55. 092 E	1	690	May
41°36.810'N	17°31.730'E	3	1133	May
42°00.431'N	17°44.143'E	2	1200	August
42°07.106'N	17°32.988'E	1	1180	August
41°54.973'N	17°46.500'E	1	1200	August
Bottom trawl				
41°55.395'N	17°45.647'E	1	1194	May
42°13.960'N	17°12.490'E	1	975	May
42°16.210'N	17°28.450'E	2	1111	May
42°14.920'N	17°15.790'E	2	1013	May

statistically significant.

Age in days was assessed based on the statolith microstructure. Statoliths were mounted, ground and polished on both sides, first concave and then convex, to ensure the clear reading of increment rings. The Crystal Bond^{TM} thermoplastic cement, melted on the hot plate and warmed at around 120 °C, was used to mount the statoliths. Once the cement hardened, lapping sheets of 30, 12, 5 and 0.05 µm were used for grinding and polishing of the statolith surface. Statoliths were prepared using the transmitting light microscope Olympus SZX10 and Olympus BX51 and growth rings, from the natal ring to the outer edge of the statolith, were counted using photographs under 200X and 400X magnification made by microscope camera Olympus DP25 and the computer program Cell^A. The number of increments (NI) was calculated for the whole sample with four independent countings for each statolith. Countings with variation of more than 10% were not taken into consideration. Assuming daily deposition of increments, number of rings (NI) corresponds with age of the individual expressed in number of days, and the hatch date (HD) can be estimated from number of increments and the date of capture (Arkhipkin & Nigmatullin, 1997).

Results

Geographical and Depth Distribution

Thirty-three (85%) specimens of *A. lichtensteinii* were caught in the water column by pelagic trawl, and 6 (15%) with bottom trawl net. Only 4 individuals were found at depth stratum 500-1000 m (i.e. between 690 and 975 m) and much higher abundances of this species were at depths over 1000 m, with 35 specimens caught at deepest parts of the South Adriatic Pit (i.e. between 1013 and 1200 m) (Figure. 1, Table 1).

Population Structure

Out of 39 analysed individuals in the sample, 24 were of undetermined sex, 10 were females and 5 were males. The mantle length of all individuals ranged between 13 and 129 mm and body weight between 0.2 and 63.1 g (Table 2). Due to the low number of specimens, differences in mean mantle length and body weight of males and females were not tested for significance. The smallest individual (13 mm ML) of *A. lichtensteini* was found in March and the largest in August 2010 (129 mm). Specimens of smaller sizes (10-80 mm ML) dominated, accounting for 90% of the whole sample (Figure. 2). All analysed individuals were sexually immature, including the largest male that measured 129 mm of the mantle length.

The most of individuals (76.9%) were caught at depths between 1100 and 1200 m and they had a very wide mantle length range (15-129 mm). Comparison of the size of animals (ML) and depth distribution revealed a weak positive correlation (r=0.1196).

Length-Weight Relationships

The following length-weight relationship was obtained for the whole sample (Figure. 3):

$$BW=0.0005 \times ML^{2.3672}, r=0.988$$

The species *A. lichtensteinii* showed a negative allometric growth, with estimated coefficient b, 2.3672, being significantly different from 3 (P<0.05).

Age and Growth

A total of 49 statoliths, extracted from 29 specimens with mantle length (ML) between 13 and 106 mm and body weight (BW) from 0.2 to 31.7 g were analysed. Estimated individual growth was between 72 and 178 days and the average age of examined specimens was 108.76 days (Table 3). The youngest (ML = 17 mm, 80 days) and the oldest (ML = 106 mm, 178 days) individuals were both caught in spring 2010.

Relationships between the mantle length (ML) and body weight (BW) and number of statolith increments (NI) are best described with the following equations:

$$ML = 0.8150 \times NI - 49.639, r = 0.898$$

$$BW = 0.0154 \times e^{0.0443 \times NI}, r = 0.837$$

Beak Morphometry

Upper and lower rostral length ranged from 0.56 to 3.43 mm (mean±SD; 1.49±0.80) and from 0.49 to 4.43 mm (1.35±0.85), respectively. Upper hood length was from 1.5 to 11.26 mm (4.35±2.36) while lower hood length was between 0.41 and 3.03 mm (1.37±0.66). Upper and lower crest length was between 2.4 and 15.11 mm (6.22±3.15) and from 1.04 to 5.84 mm (2.50±1.17), respectively. Upper jaw angle width was between 0.4 and 2.31 mm (1.04±0.50) and lower jaw length in the range between 0.45 and 2.86 mm (1.05±0.57). Upper and lower wing length ranged between 0.73 and 3.98 (1.64±0.81) and from 0.96 to 5.71 mm (2.27±1.20), respectively. Width of the lateral wall (ULWa) had range between 0.81 and 4.73 mm (2.09±0.96) and length of the baseline (LBL) was from 1.22 to 6.93 mm (2.99±1.42) (Table 4.). Due to the small number of individuals of determined sex, the regression analyses between upper and lower beak variables of *A. lichtensteinii* were done for the whole sample without comparison between males and females. A positive allometric growth of measured beak morphometric features in relation to the mantle length (ML) was found, except for the relationship between lower rostral length and mantle length (LRL/ML)

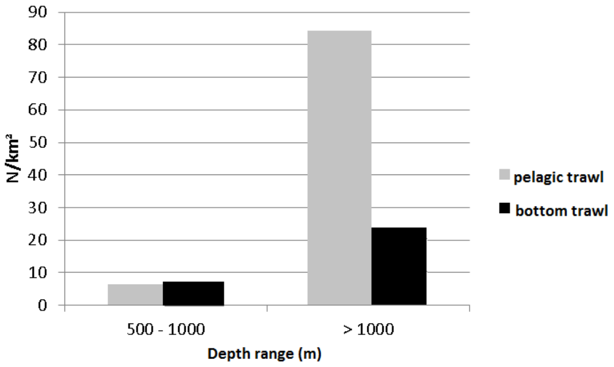

Figure 1. Abundance indices (N/km^2) for *A. lichtensteinii* sampled in the South Adriatic Sea during FAO AdriaMed 2010.

Table 2. Number (N), mantle length (ML) and weight (W) of females (♀), males (♂), undetermined individuals (und) and total sample (TOT) with mean value ($\bar{X}$), standard deviation (SD) and standard error (SE) of *A. lichtensteinii* in the South Adriatic Pit in 2010

N	ML (mm)	$\bar{X}$ ± SD	SE	W (g)	$\bar{X}$ ± SD	SE
♂ 5	48 - 129	79.20 ± 33.88	15.15	4.1 - 63.1	21.32 ± 24.88	11.13
♀ 10	28 - 106	62.70 ± 26.00	8.22	1.7 – 31.7	9.85 ± 8.83	2.79
und 24	13 - 69	29.10 ± 15.51	3.47	0.2 - 10.3	1.87 ± 2.73	0.61
TOT	13 - 129	45.86 ± 29.33	4.70	0.2 – 63.1	6.93 ± 12.07	1.93

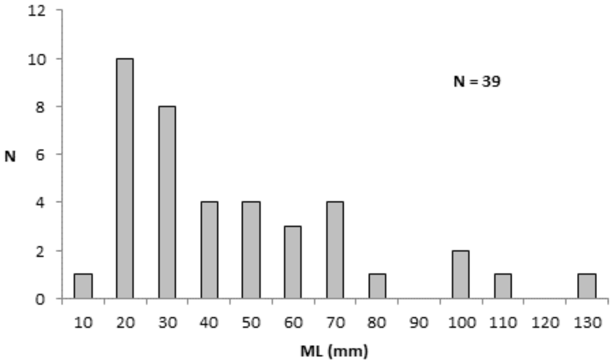

Figure 2. Length frequency distribution of *A. lichtensteinii* in the South Adriatic Pit during FAO AdriaMed Deep Sea Survey 2010.

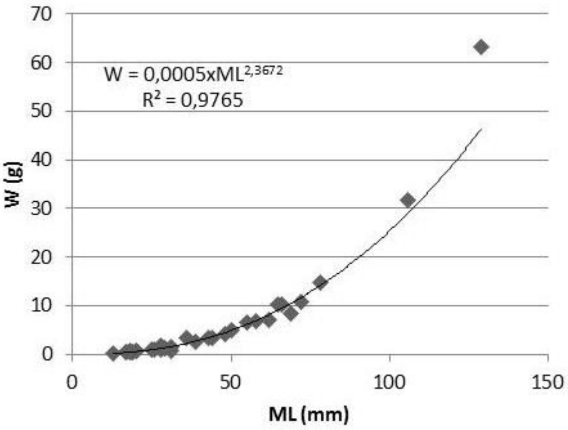

Figure 3. Length-weight relationships obtained for the whole sample of *A. lichtensteinii* in the South Adriatic Pit (W-body weight, ML-mantle length).

Table 3. Range of individual age in days estimated by counting increment rings of statoliths (NI), mantle length (ML) and body weight (BW) with mean values ($\bar{X}$) and standard deviations (SD) for the total sample (TOT) of *A. lichtensteinii* in the South Adriatic Pit

	NI (days)			ML (mm)			BW (g)		
	min	max	$\bar{X}$ ± SD	min	max	$\bar{X}$ ± SD	min	max	$\bar{X}$ ± SD
TOT	72	178	108.76 ± 23.65	13	106	38.21 ± 22.08	0.2	31.7	4.09 ± 6.27

Table 4. Range (mm), mean value ($\bar{X}$), standard deviation (SD) and standard error (SE) of the upper (U) and lower beak (L) morphometric characters (mm) of *A. lichtensteinii* from the South Adriatic Pit

Beak character		Min	Max	$\bar{X}$	SD	SE
UHL	Upper hood length	1.5	11.26	4.35	2.36	0.43
UCL	Upper crest length	2.4	15.11	6.22	3.15	0.58
URL	Upper rostral length	0.56	3.43	1.49	0.80	0.15
UWL	Upper wing length	0.73	3.98	1.64	0.81	0.15
UJW	Upper jaw angle width	0.4	2.31	1.04	0.50	0.09
ULWa	Width of the lateral wall	0.81	4.73	2.09	0.96	0.18
LHL	Lower hood length	0.41	3.03	1.37	0.66	0.12
LCL	Lover crest length	1.04	5.84	2.50	1.17	0.21
LRL	Lover rostral length	0.49	4.43	1.35	0.85	0.15
LWL	Lower wing length	0.96	5.71	2.27	1.20	0.22
LJW	Lower jaw angle width	0.45	2.86	1.05	0.57	0.10
LBL	Length of the base line	1.22	6.93	2.99	1.42	0.26

where an isometric growth was observed. The regressions between all upper and lower beak measurements and body weight (BW) showed allometric relationships (Table 5). The highest growth rate in the whole sample was found for the upper and lower crest length (CL) while the rostral length of both beaks (RL) showed the slowest growth when compared to the body size. The values of the correlation coefficient were very high for all calculated relationships; between 0.914 and 0.985, and from 0.927 to 0.960 for upper and lower beak, respectively.

The relationships between lower beak rostral length and body weight, and between lower rostral length and mantle length are presented by the equations:

$$\log BW = 0.196 + 2.424 \log LRL \quad (r = 0.955)$$

$$\log ML = 1.49 + 1.023 \log LRL \quad (r = 0.956)$$

Discussion

Ancistroteuthis lichtensteinii was caught at stations with depths between 690 to 1200 m which indicates that this species, at least in earlier life stages, inhabits deep waters of the south Adriatic Sea. Although in case of the deep-sea trawling it is difficult to assess the exact depth at which specimens were caught, the fact is that in the Adriatic Sea this species was never found neither in coastal areas nor at depths bellow 500 m (Krstulović Šifner *et al*., 2005; Krstulović Šifner *et al*., 2011). Previously only one specimen of the species was registered in the Adriatic Sea, without precise locality being indicated (Gamulin-Brida & Ilijanić, 1965; Gamulin-Brida & Ilijanić, 1972), while its beaks were found in the stomach contents of the large predators in the South Adriatic Sea (Bello, 1990, 1996). The species was completely absent from the samples of the Adriatic deep-sea survey in 2008 (pers. obs.) so it seems that its abundance in deep South Adriatic areas is highly variable and probably dependent on season, currents and availability of food.

The length-weight relationship obtained for *A. lichtensteinii* from the South Adriatic Pit indicated a negative allometric growth of the species (b=2.37). Merella, Quetglas, Alemany, and Carbonell (1997) also found a negative allometry (b=2.60) when analysing nine specimens of *A. lichtensteinii* from the Balearic Islands. As these specimens had mantle lengths between 50 and 125 cm, this relationship is related only to the growth of juvenile and sexually immature specimens as was the case in this study. The length-weight relationships calculated for some other members of the family Onychoteuthidae showed similar pattern, e.g. Jackson, George, and Buxton (1998) for *Onykia ingens* stated negative allometric growth, with higher average weight of females in mature specimens and similarly, Bigelow (1994) found that in species *Onychoteuthis borelijaponica* growth was negative allometric, with higher weight growth in females (b=2.915) when compared to males (b=2.596).

The life span of *A. lichtensteinii* was not possible to assess due to the lack of the mature specimens in the sample, but it was found that the oldest individual was approximately 6 months old (178 days) and its mantle length was only 106 mm.

Table 5. Relationships between lower and upper beak morphometric features and mantle length (ML) and body weight (BW) for the whole sample of the species *A. lichtensteinii*. (N – number of specimens; r – correlation coefficient; S – significance level of b compared to isometry (* P<0.05, ** P<0.01, ns – not significant)

Equation	N	r	S
Upper beak			
log ML = 0.92 + 1.124 log HL	30	0.978	**
log ML = 1.45 + 1.074 log RL	30	0.965	*
log ML = 0.68 + 1.201 log CL	30	0.979	**
log ML = 1.37 + 1.199 log WL	30	0.948	**
log ML = 1.25 + 1.168 log LWa	30	0.914	**
log ML = 1.61 + 1.195 log JW	30	0.979	**
log BW = -1.173 + 2.702 log HL	30	0.985	**
log BW = 0.101 + 2.585 log RL	30	0.973	**
log BW = -1.749 + 2.884 log CL	30	0.985	**
log BW = -0.086 + 2.888 log WL	30	0.957	**
log BW = -0.387 + 2.840 log LWa	30	0.931	**
log BW = 0.493 + 2.867 log JW	30	0.984	**
Lower beak			
log ML = 1.45 + 1.147 log HL	31	0.930	**
log ML = 1.49 + 1.023 log RL	31	0.956	ns
log ML = 1.08 + 1.302 log CL	31	0.949	**
log ML = 1.19 + 1.157 log WL	31	0.948	**
log ML = 1.02 + 1.250 log BL	30	0.953	**
log ML = 1.59 + 1.157 log JW	31	0.960	**
log BW = 0.101 + 2.711 log HL	31	0.927	**
log BW = 0.196 + 2.424 log RL	31	0.955	**
log BW = -0.768 + 3.115 log CL	31	0.958	**
log BW = -0.490 + 2.756 log WL	31	0.953	**
log BW = -0.916 + 2.960 log BL	30	0.953	*
log BW = 0.430 + 2.736 log JW	31	0.957	**

When taking into consideration that this species reaches maximum mantle length of 300 mm, that males mature at about 200 mm ML (Kubodera, Piatkowski, Okutani, & Clarke, 1998), and also that with aging the growth rate of individuals slows down, it can be concluded that *A. lichtensteinii* is a slow-growing species. Arkhipkin and Nigmatullin (1997), by analysing the microstructure of the statoliths, revealed that species *Onychoteuthis banksi*, of the family Onychoteuthidae, was one of the slowest growing species among cephalopods. As stated by Dubinina (1977) the species of the genus *Onykia*, from the same family, had even lower growth rate than *Onychoteuthis*. Jackson (1993), by analysing *Onykia ingens* statolith microstructure, observed two very distinctive zones, opaque and translucent, that author attributed to the transition from an epipelagic to a demersal habitat in this species, and evidence for the hypothesis was drawn from analogous features observed within teleost otolith microstructure and from available information on *Onykia*. This feature was not observed in the statoliths microstructure of *A. lichtensteinii* analysed in this study, which therefore indicates that this species spends its whole life in the pelagic environment, probably just changing its depth distribution in the water column.

It has been found that paralarvae and juveniles of the deep-sea squids migrate in the shallower parts of pelagic with more prey available for their feeding, while adults at the spawning season descend to deep-sea areas (Jackson *et al*., 1998; Arkhipkin & Laptikhovsky, 2010). Based on the results of the study it can be assumed that only paralarvae and juvenile stages of *A. lichtensteinii*, driven by currents and water masses from the Mediterranean, appear in areas of the South Adriatic Pit. This early stages are probably driven from the Levantine basin through Otranto Straits to the Adriatic Sea by currents carrying water masses of the surface and intermediate layer Levantine waters from the Mediterranean (Robinson, Leslie, Theocharis, & Lascaratos, 2001; Zore-Armanda, 2000). Specimens found in the Aegean Sea were also immature with mantle lengths ranging between 55 and 106 mm and it was also the case in most of the species findings in the Mediterranean (Lefkaditou *et al*., 2000).

This study presents the first analysis of *A. lichtensteinii* beak morphometry in the Adriatic Sea. Analyses of beaks from the stomachs of predators are potentially a very useful tool for assessment of the biomass of deep-water squids. Clarke (1986) stated that it is only necessary to identify and measure either upper or lower beak, since they will both give the same information, and also proposed the relationship between lower rostral length and body weight as a good measure for assessment of the biomass of cephalopods based on beaks, and subsequently number of authors based their analysis on measures of the lower beak. In this study relationships between different upper and lower beak measures and body weight/mantle length were calculated. Using relationships of both beaks is in accordance with conclusions of recent studies which proved that a ratio between upper and lower beaks is not even in most of the cases, so using both upper and lower beaks have to be considered to improve the assessment of the contribution of different cephalopods to predator diets and estimation of the cephalopod mass (Xavier, Phillips, & Cherel, 2011).

Relationships between the lower beak rostral length (LRL) and mantle length, and between LRL and body weight of *A. lichtensteinii* found in the stomach of Risso's dolphin, *Grampus griseus*, from the central Mediterranean (Würtz, Poggi, & Clarke, 1992), are similar to those obtained in this study. Clarke (1986) calculated regression of the wet weight in grams against LRL for the family Onychoteuthidae but *Ancistroteuthis* specimens were not available for the calculation so the relationship was not so similar to the one found in this study. This is not surprising as beaks of *A. lichtensteinii* are very different from other onychoteuthids in their general proportions (Clarke, 1986). Previous finding of the species collected in the south Adriatic Sea and neighbouring north Ionian Sea and Gulf of Taranto, were related to the findings of beaks in the stomachs of large predators, e.g. in stomachs of the swordfish, *Xiphias gladius*, a species feeding dominantly on pelagic cephalopods, where *A. lichtensteinii* was one of the most abundant cephalopod preys species (Bello, 1991). Kovačić, Đuras Gomerčić, Gomerčić, Lucić, and Gomerčić (2010) didn't find remains of the species in the stomach contents of the Cuvier's beaked whales in the Adriatic Sea, although *Ziphius cavirostris* is feeding almost exclusively on pelagic squids (Santos *et al.*, 2001). *A. lichtensteinii* and some other deep-sea pelagic species could be much more abundant in the water column than previously assumed and therefore also potentially interesting for fisheries (Bello, 1991; Quetglas *et al.*, 2013). Future studies should be focused on assessment of the abundance and also on clarification of behavioural and distributional patterns of *A. lichtensteini* in the South Adriatic Sea which, based on the present knowledge, represents one of the most important macropelagic species in the food web energy transfer of the mesopelagic and bathypelagic habitat, both as a prey and as a predator.

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