

Insight in some aspects of the reproductive biology, morphometry and age of *Ctenopteryx sicula* (Cephalopoda: Ctenopterygidae) in the Adriatic Sea

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*The first record of the deep-sea species *Ctenopteryx sicula* is reported from the Adriatic Sea. A total of six specimens of this rare species were caught, with bottom trawl and pelagic trawl nets, in the scope of the Deep Sea Research of the bathyal fauna in the South Adriatic Pit in 2008 and 2010. Specimens were caught at depths down to 1200 m, therefore making it the deepest and northernmost finding of this species in the Mediterranean Sea. The dorsal mantle lengths of analyzed specimens ranged between 21 and 60 mm. Only in females, a visceral photophore on the ventral side of the inc sac was detected. Some new information on morphometric body and beak features was given in the paper, including the description of beak pigmentation. A number of increments in statoliths revealed that individuals were between three and five months old. A positive linear correlation between the length of statoliths and number of increments/age in days was detected. Macroscopic and histological samples of gonads proved that one specimen was sexually mature (August), four specimens were in stages of sexual maturation (May and August) and two were immature (March). The results indicated that the maturation process of the species in the South Adriatic Sea starts in spring and the full sexual maturity is reached during summer.*

Key words: *Ctenopteryx sicula*, first record, Adriatic Sea, reproductive biology, morphometry, age

INTRODUCTION

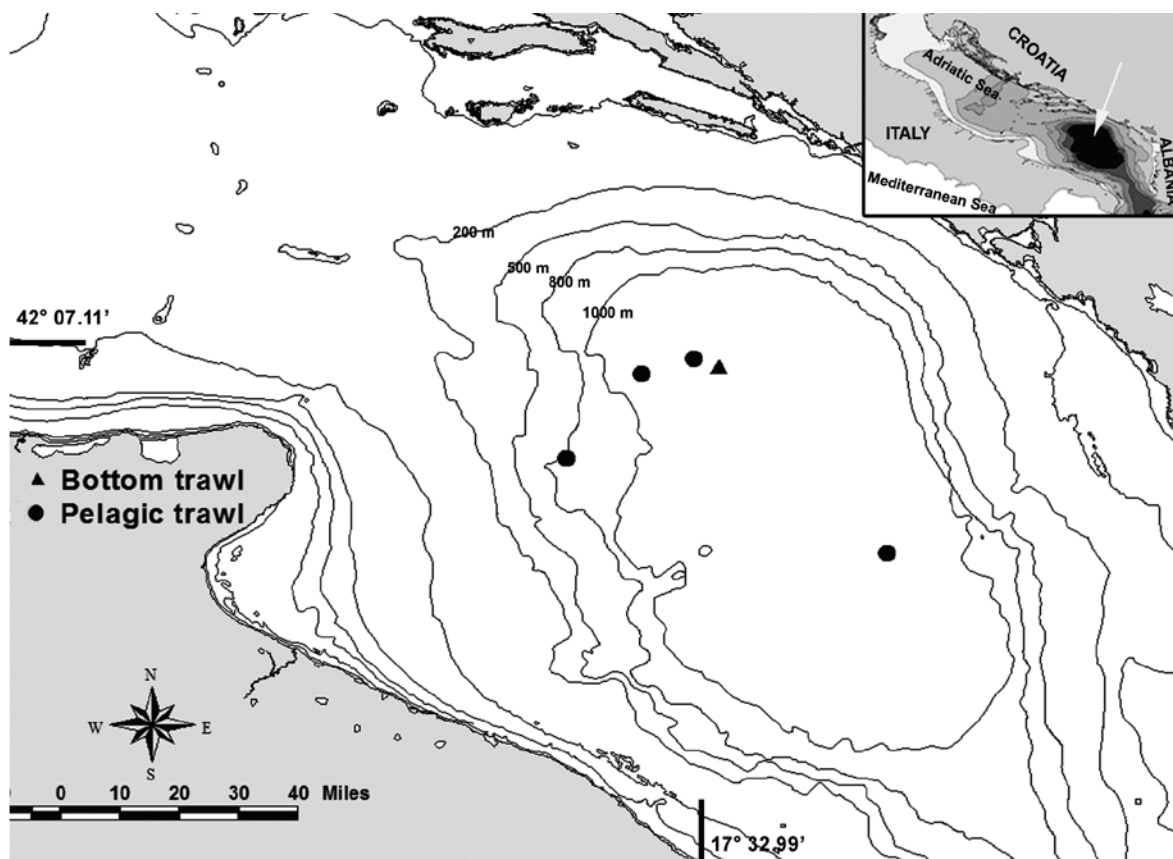
Ctenopteryx sicula (Verany, 1851) is a pelagic cephalopod species inhabiting tropical to subtropical waters, characteristic for its strong diel vertical migrations and adults descending to mesopelagic and bathypelagic depths (JEREB & ROPER, 2010).

In the Mediterranean, findings of this species were previously reported in the Eastern Mediterranean: North-eastern Ionian Sea (LEFKADITOU *et al.*, 2003a), North-western Ionian (D'ONGHIA *et al.*, 1998), southern Aegean (LEFKADITOU *et al.*, 2003b), in the Western Mediterranean: DEGNER (1925), ADAM (1967), RUBY & KNUDSEN (1972), MANGOLD (1973), MANGOLD & BOLETZKY (1987), QUETGLAS *et al.* (2013) and in the North-western Mediterranean: off Tuscany and Latium coasts (BIAGI *et al.*, 2002), the Balearic Sea (QUETGLAS *et al.*, 2000), the Catalan and Northern Tyrrhenian Sea (SÁNCHEZ *et al.*, 1998) and off the Iberian

Peninsula Mediterranean coast (GONZÁLEZ & SÁNCHEZ, 2002).

The teuthofauna of the Adriatic Sea has been investigated lately through several studies and the knowledge on this group of organisms increased significantly. Aside from the studies on single species of cephalopods, several studies have been done on community level, based on data from bottom trawl surveys (KRSTULOVIC ŠIFNER *et al.*, 2005, 2011). These investigations helped to improve the list of Adriatic cephalopods obtained through previous studies at eastern and western side of the Adriatic coast (GAMULIN-BRIDA & ILIJANIĆ, 1972; BELLO, 1990).

Although investigations on cephalopods have been intensified in the last decade, the information on deep-sea teuthofauna is still scarce. This study proves that *C. sicula* inhabits deep waters of the South Adriatic and it also gives some new information on distribution, reproductive biology, morphometry and age of this uncommon pelagic species.



ig.1. Position of the stations where *Ctenopteryx sicula* was caught in the South Adriatic Pit

MATERIALS AND METHODS

Biological samples were collected during four surveys in deep waters of the South Adriatic Pit: one in 2008 and three in 2010 with the research vessel "Andrea" in the scope of the FAO AdriaMed project. In surveys done in August 2008 and May and August 2010 synthetic bottom trawl net, GOC 73, with large vertical opening (2-3 m) and 20 mm stretched mesh size at the cod-end, was used (BERTRAND *et al.*, 2002). Besides the bottom trawl net, during surveys in March, May and August 2010, commercial pelagic trawl net with 16 mm stretched mesh size at the cod-end was used for the sampling. Only one specimen of *C. sicula* was registered during survey in 2008, while a total of five specimens were registered during surveys in 2010. The first specimen was caught with bottom trawl net and five others with pelagic trawl. Geographic positions of positive stations are presented in Fig. 1 while basic data about the positive stations and specimens with assigned identification numbers (ID) are indicated in Table 1.

Morphological features of *C. sicula* specimens were recognized using keys of MANGOLD & BOLETZKY (1987) and NESIS (1987), most char-

acteristic being wide mantle flattened from above and an unusual „ribbed“ fin composed of a series of soft „rays“, especially long at the posterior end of the mantle, joined in most of their length by a thin membrane (Fig. 2). The specimens were preserved in 4% formalin and deposited in the Cephalopod collection of the University Department of Marine Studies in Split, Croatia and in the Laboratory for Marine Biology in Fano, Italy.

A total of 13 body morphometric characters (Table 2) and 7 beak morphometric characters (Table 3) were measured with a precision of 0.01 mm by digital caliper following CLARKE (1986). Beak pigmentation was determined mainly according to the darkening degree of lateral walls and wings in both jaws.

For histological analysis of the reproductive tissue, sections of testis and ovary from two individuals in most advanced maturity stages, a male (ID1) and a female (ID5), were processed for routine histological preparation. The tissue was dehydrated in a graded ethanol series (from 70% to 100%), cleared in xylene and embedded in paraffin. Histological sections were cut at 5 μ m, stained with haematoxylin-eosin and then mounted permanently for microscopic analysis.

Table 1. Data on collection of six individuals of *Chtenopteryx sicula* caught in the South Adriatic Pit in 2008 and 2010

	ID1	ID2	ID3	ID4	ID5	ID6
Year	2008	2010	2010	2010	2010	2010
Month	August	March	March	May	August	August
Fishing Gear	Bottom trawl	Pelagic trawl	Pelagic trawl	Pelagic trawl	Pelagic trawl	Pelagic trawl
Depth (m)	1200-1200	1100-1170	850-895	1150-1119	1180-1190	1170-1190
Position	42°05.800' N	42°04.720' N	41°51.620' N	42°15.770' N	42°07.106' N	41°36.693' N
(beginning)	17°38.000' E	17°02.480' E	17°07.720' E	17°35.290' E	17°02.988' E	18°12.065' E
Position	42°11.480' N	41°59.580' N	41°45.480' N	42°15.540' N	42°02.333' N	41°44.545' N
(ending)	17°34.630' E	17°01.070' E	17°14.760' E	17°28.050' E	17°08.578' E	18°00.282' E
DML (mm)	50	21	22	35	54	60
	8.99					
BW (g)		0.9	1	38	13.5	21.2
Sex	Male	Female	Female	Male	Female	Female
Maturity	2b	1	1	2a	2b	3a

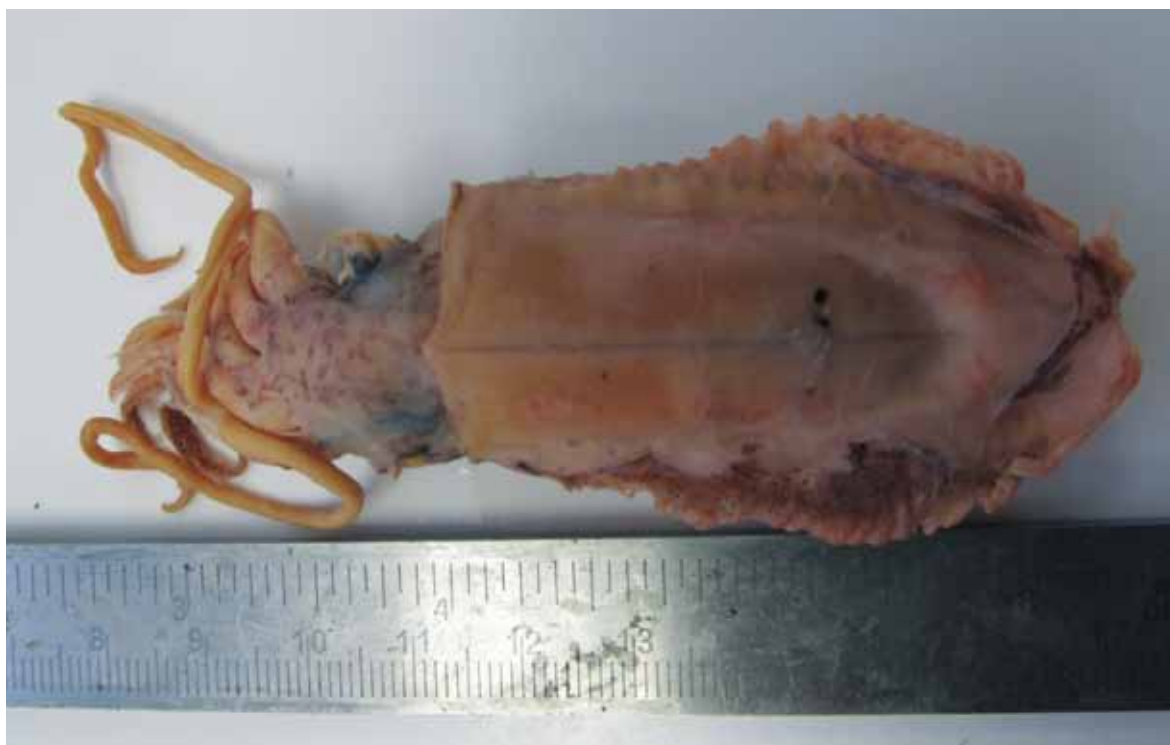


Fig.2. Preserved female specimen of *Ctenopteryx sicula* photographed in the laboratory (dorsal view, ML = 54 mm)

An attempt was made to determine the individual age of collected specimens. Age in days was assessed by using method based on statolith microstructure. Statoliths were mounted with their concave side up onto microscopic slides using Crystal Bond™ thermoplastic cement melted on the hot plate warmed at around 120 °C. Once the cement hardened, statoliths were

ground and polished using lapping film sheets of 30, 12, 5 and 0.05 μm . Statolith growth increments were examined and counted from the natal ring to the edge of the dorsal dome under the transmitting light microscope Olympus BX51 (magnification at 400 x).

Hatching date was estimated using back-calculation method by subtracting age in days

Table 2. Body morphometric characters (mm) of *Ctenopteryx sicula* from the Adriatic Sea

Body morphometric characters		ID1	ID2	ID3	ID4	ID5
TL	total length	207	-	-	134	137
DML	dorsal mantle length	51	21	22	35	54
HL	head length	24	8.86	7.43	10	15
FL	fin length	45	22.17	18.89	37	55.5
AL4	arm IV length	35.46	13.10	13.25	23.08	30.51
AL3	arm III length	33.05	10.13	9.35	19.33	29.90
AL2	arm II length	30.88	9.11	12.59	19.03	29.28
AL1	arm I length	28.43	9.26	9.75	16.85	25.84
FW	fin width (max near bottom)	14	7.80	6.13	8.32	9.06
MW	mantle width	24	11.22	9.35	18	21
ED	eye diameter	16	4.29	4.91	-	-
FuL	funnel length	17.28	6.98	9.33	-	18
FuW	funnel width	12.04	4.28	5.71	-	15

Table 3. Upper and lower beak morphometric characters (mm) of *Ctenopteryx sicula* from the Adriatic Sea

Upper beak characters		ID1	ID2	ID3	ID4	ID5
HL	upper hood length	3,64	1,69	1,82	2,52	4,24
RL	upper rostral length	1,14	0,61	0,59	0,93	1,33
WL	upper wing length	1,26	0,56	0,64	0,9	0,92
CL	upper crest length	5,21	-	-	3,75	4,73
JW	upper jaw angle width	1,12	0,33	0,39	0,7	1,07
LW_a	width of the lateral wall	1,87	-	-	1,65	1,76
Lower beak characters		ID1	ID2	ID3	ID4	ID5
HL	lower hood length	1,55	0,54	0,78	1,15	1,72
RL	lower rostral length	0,72	0,44	0,5	0,82	1,14
WL	lower wing length	2,18	1,09	1,18	1,63	2,91
CL	lower crest length	2,61	1,27	1,36	2,01	3,99
JW	lower jaw angle width	1,21	0,35	0,47	0,79	1,32
BL	length of the base line	3,75	-	-	3,27	3,94

from date of capture, adopting the hypothesis “one statolith increment per day” (GONZÁLEZ *et al.*, 1996; ARKHIPKIN *et al.*, 1999, 2000).

RESULTS

Morphometry

Collected body morphometric characters were presented for each specimen without further statistical analyses (Table 1). Obtained measures indicated that the fourth arm in the species was the longest while the first, second and third arm lengths varied with no evident pattern. Percentage of the mantle width in the mantle length was in the usual range for the species, between 39 and 53%.

The darkening process of beak was accentuated with animal growth and maturity. The beak pigmentation of all immature specimens (ID2, ID3, ID4) was very similar. Rostral tip of the upper beak showed dark brown coloration, hood only had initial light brown pigmentation, while lateral walls had no pigmentation at all. Pigmented parts of the lower beak were rostrum and the anterior part of the hood with slight pigmentation of the shoulder. The upper and lower beak of maturing male individual (ID1) had an intense dark brown pigmentation of the rostrum and brown pigmentation of the hood. Furthermore, pigmentation of the lateral walls in both beaks comprised 2/3 of their length, while the wings of the lower beak were surrounded by

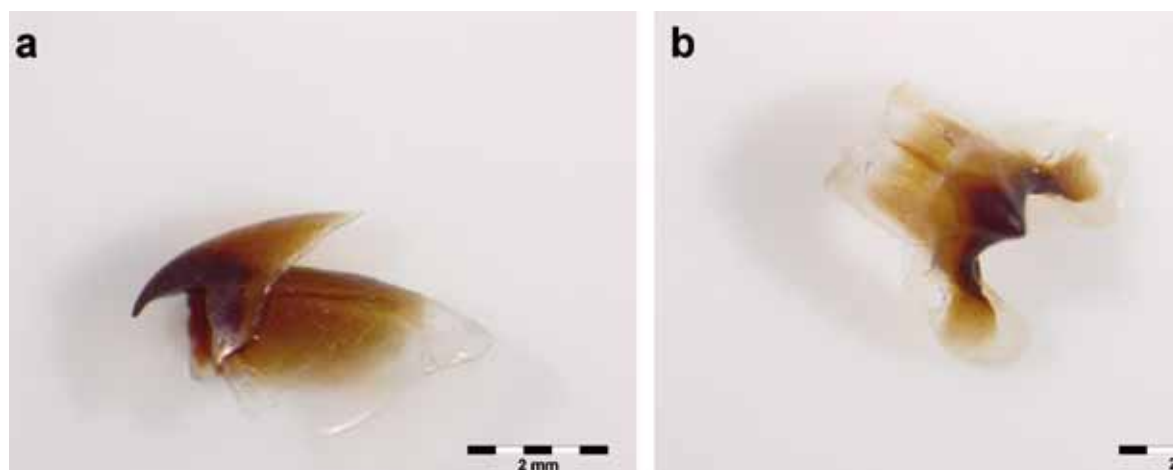


Fig.3. Beaks of *Ctenopteryx sicula*: A) upper beak; B) lower beak

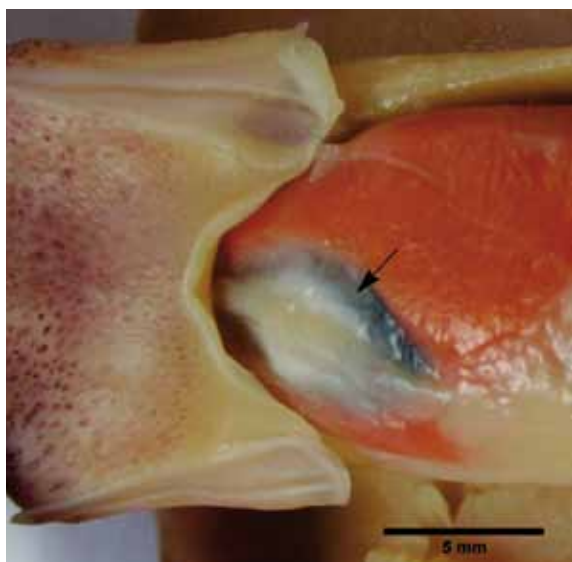


Fig. 4. Intestinal photophore from the female of *Ctenopteryx sicula* (ventral view, ML = 54 mm)

translucent border (Fig. 3). In maturing female (ID5), pigmentation of the upper beak also increased towards the hood end, while superior part of the lateral wall showed soft brown coloration. In the lower beak, initial pigmentation of the wings progressed to the hood with evident increase in the lateral wall pigmentation.

Reproductive biology

In all examined females visceral photophore was visible while in males this structure was not detected. In immature females photophore was small but clearly visible on ventral side of the ink sac under the intestine, while in the female in advanced stage of maturation as well as in the mature female it was very large with characteristic yellowish ball lying on the concave surface of the ink sac and covered with light-reflecting layer (Fig. 4).

The gonads of *C. sicula* were positioned in the posterior portion of the visceropericardial

coelom. The testis of the male (ID1) used for the histological analysis in this study weighed 0.11 g and measured 15.58 mm and 6.41 mm in length and width, respectively. In the testis, spermatozoa were found in the lumina of seminiferous tubules indicating maturity stage of the animal (Fig. 5a). The germinal epithelium consisted of spermatocytes placed near the inner seminiferous tubule wall, spermatids and spermatozoa filling the lumen of the seminiferous tubule. In the examined specimen 4 fully formed spermatophores were found with lengths of 4.25, 3.45, 3.38 and 4.74 mm (mean 3.96 ± 0.66 SD). Based on micro and macroscopic examination of gonads and reproductive system, it was concluded that this specimen was in an advanced stage of maturation.

The female specimen (ID5) was also examined histologically for the state of gonads and it was observed that the ovary was in previtellogenic stage with primary oocytes and oocytes surrounded by follicular cells (Fig. 5b). Primary oocytes had nucleus with several nucleoli, while surface of growing oocytes had follicular cells that just begun to multiply.

Age estimates

The number of counted statolith increments was between 89 and 146 (Fig. 6, Table 4). According to the number of increments, analyzed individuals were between 3 and 5 months old. Small, immature individuals were in the third and fourth month while larger, maturing individuals were in the fifth month of their lives. There was a positive linear correlation between the length of statoliths and number of increments/age in days ($R^2 = 0.865$).

Table 4. Length of statoliths (SL) and number of increments (age in days) of *Ctenopteryx sicula* from the Adriatic Sea

	ID1	ID2	ID3	ID4	ID5
SL (mm)	2.11	1.38	1.35	1.86	2.04
Age (days)	131	104	89	124	146

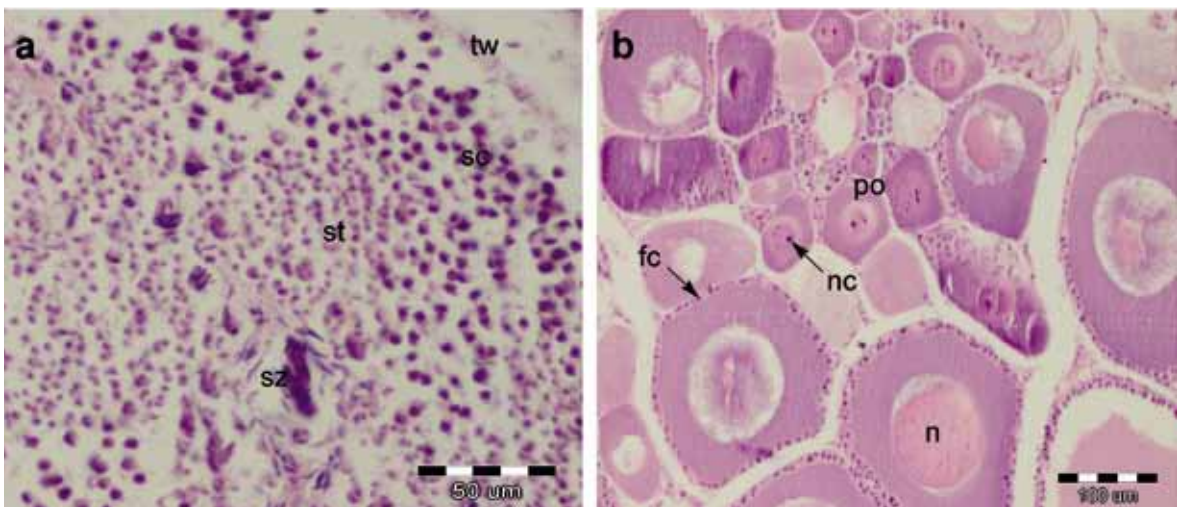


Fig.5. *Chtenopteryx sicula*: A) histological section of mature testis showing seminiferous tubule with different type of germ cells: seminiferous tubule wall (tw), spermatocytes (sc), spermatids (st) and spermatozoa (sz); B) histological section of previtellogenic ovary showing: primary oocytes (po) with several nucleoli (nc) and oocytes with large nucleus (n) surrounded by follicular cells (fc)

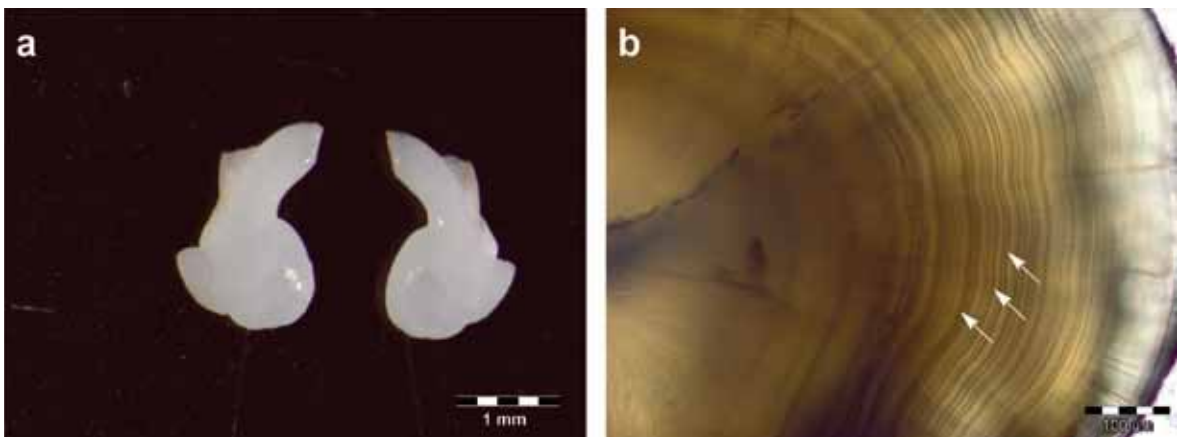


Fig.6. *Chtenopteryx sicula*: A) statoliths; B) detail of the statolith with growth increments (arrows)

DISCUSSION

Chtenopteryx sicula can be found in a wide depth range, but it primarily inhabits deeper, meso- and bathypelagic waters and most of the records are from depths over 500 m (QUETGLAS *et al.* 2013). The previous maximum depth recorded for this species in the Mediterranean was 1099 m (VILLANUEVA, 1992) and depth at which the species was caught by bottom trawl net in the Adriatic Sea in 2008 was 1200 m (depth registered at the beginning and at the end of the trawling). Although there might be a possibility that the specimen was caught during hauling at

depths above 1200 m, the present findings may be considered not only the northernmost but also the deepest in the Mediterranean. One of specimens had mantle length 60 cm and it is one of the largest recorded specimens of *C. sicula* from the Mediterranean (D'ONGHIA *et al.*, 1998; QUETGLAS *et al.*, 2013). The main reason, why this species was never previously reported from the Adriatic Sea, was the lack of systematic deep-sea investigations in the area. Until recently, data on deep-sea teuthofauna in the Adriatic Sea have been very scarce and most of information on bathyal and bathypelagic cephalopods came from the studies on stomach contents of deep

diving mammals and large fish caught in the southern Adriatic Sea, as cephalopods are very important prey for many large fish and mammals (BELLO, 1985; KOVAČIĆ *et al.*, 2009). In these studies some deep sea cephalopods, already known from the Mediterranean, were identified based on the beaks found in the stomachs of the analyzed specimens. As these predator species are highly migratory, there was no clear evidence if these cephalopods were caught as prey in the Adriatic Sea or in some other parts of the Mediterranean. Nevertheless, the species *C. sicula* had never been reported in the Adriatic Sea, neither as a caught specimen nor through the beaks determined from the stomachs of the large predators so these findings are the first record and proof that this species inhabits deep waters of the Adriatic Sea. One of the reasons why *C. sicula* was missing from the Adriatic cephalopod species list when compared to the Mediterranean, was the fact that most of investigations of the teuthofauna in the Adriatic had been done using bottom trawl nets, fishing gear primarily targeting demersal species, which reduced significantly the probability of catching this or any other pelagic species.

Although considered as a tropical to subtropical cosmopolitan species (NESIS, 1987; JEREB & ROPER, 2010), due to the poor understanding of the species-level systematics in this genus, the presence of *C. sicula* outside the Mediterranean Sea is uncertain (YOUNG & VECCHIONE, 2006). Previous descriptions of the species from the Mediterranean are not very clear and indicate some differences which are basis for the ongoing dispute whether there may be two species of genus *Chtenopteryx* in the Mediterranean, currently going by the name *C. sicula*. JOUBIN (1900) described three individuals of about 50 mm ML found in the stomach of a dolphin caught off

Corsica and PFEFFER (1912) described the species based on juvenile specimens in poor condition of 13 mm ML and smaller. The most detailed description of the species was given by NAEF (1921-23) from the sample captured off Naples. This description of the species was based on the specimen without the visceral photophore. In this study examined female specimens of *C. sicula* had visceral photophore. YOUNG & VECCHIONE (2006) also examined a specimen of *C. sicula* from the Mediterranean Sea that had the visceral photophore but they didn't indicate the sex of the examined specimen. Our analyses based on 6 individuals revealed the presence of the visceral photophore only in females so this characteristic could be sex determined as is the presence of dorsally directed photophore in posterior mantle cavity in males.

The results based on the analyses of maturity stages and age of the examined specimens indicate that the maturation process starts when specimens are in the fifth month of their life and it probably takes another two or three months to achieve the full sexual maturity. When samples are compared by season it seems that the maturation process of *C. sicula* in the South Adriatic Sea starts in spring and full sexual maturity is reached during summer months.

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Upoznavanje značajki reproduktivne biologije, morfometrije i starosti vrste *Ctenopteryx sicula* (Cephalopoda: Ctenopterygidae) u Jadranu

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SAŽETAK

U radu su opisani prvi nalazi dubokomorske vrste *Ctenopteryx sicula* u Jadranskom moru. Pridnenom i pelagičnom mrežom ulovljeno je ukupno šest primjeraka ove rijetke vrste u sklopu istraživanja dubokomorskih zajednica u južnojadranskoj kotlini 2008 i 2010. godine. Primjerci su ulovljeni na dubinama do 1200 m te predstavljaju najdublji i ujedno najsjeverniji nalaz ove vrste u Mediteranu. Dorzalna dužina plašta analiziranih jedinki kretala se od 21 do 60 mm. Samo u ženki nađeni su visceralni fotofori na ventralnoj strani vrećice sa crnilom. U radu su date nove informacije vezane uz obilježja kljuna, uključujući detaljan opis pigmentacije. Broj zona prirasta u statolitima ukazuje na starost jedinki između tri i pet mjeseci. Utvrđena je pozitivna korelacija između dužine statolita i broja zoni prirasta/starosti u danima. Makroskopski i histološki uzorci gonada potvrdili su da je jedna jedinka bila spolno zrela (kolovoz), četiri jedinke su bile u stadiju spolnog sazrijevanja (svibanj i kolovoz), a dvije su bile spolno nezrele (ožujak). Rezultati ukazuju na to da proces spolnog sazrijevanja ove vrste u južnom Jadranu počinje u proljeće, dok se puna spolna zrelost doseže tijekom ljeta.

Ključne riječi: *Ctenopteryx sicula*, prvi nalaz, Jadransko more, reprodukcija, morfometrika, starost

