Frontiers in Marine Science

TYPE Original Research
PUBLISHED 01 April 2025
DOI 10.3389/fmars.2025.1567441

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OPEN ACCESS

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RECEIVED 27 January 2025
ACCEPTED 17 March 2025
PUBLISHED 01 April 2025

CITATION
Agus B, Battaglia P, Bellodi A, Cannas R, Coluccia E and Cuccu D (2025) Age and growth of *Todaropsis eblanae* (Ommastrephidae) through comparison of statoliths, beaks and eye lenses. *Front. Mar. Sci.* 12:1567441. doi: 10.3389/fmars.2025.1567441

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Age and growth of *Todaropsis eblanae* (Ommastrephidae) through comparison of statoliths, beaks and eye lenses

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The age composition of *Todaropsis eblanae* from the Sardinian waters (western Mediterranean Sea) was studied for the first time through the analysis of growth increments and the comparison of three structures: statoliths, beaks and eye lenses. The analysis was performed on 270 wild specimens of both sexes at different sizes (45-200 mm of mantle length; 6.98-443 g of total weight) and maturity stages (immature, maturing and mature) caught from July to September by trawl net. Significant differences in growth and length-weight relationship were observed between sexes, due to females reach a larger size than males. All the three structures had dimensions positively correlated with the size of the animals and showed clearly readable growth increments. Low values of IAPE, CV and PA confirmed the accuracy and good reproducibility of age readings. Eye lenses showed a very high number of growth increments (106-640), and a daily deposition was excluded. In contrast, beaks and statoliths showed NI values (70-316 and 73-310, respectively) always consistent with the size and maturity of the specimens, then a daily deposition has been suggested and their value compared. Moreover, the Mann-Whitney W-test confirmed a highly significant relationship between the number of growth increments in beaks and statoliths, suggesting that the beak can be considered a valid alternative to statoliths for age estimation in *T. eblanae*. The absolute growth rates confirm that females grow faster than males. Both sexes showed a higher initial growth rate, which gradually decreases, with the highest values at the age of 101-151 days, before reaching sexual maturity. According to a semelparous cycle, the estimated ages for the largest mature female (310-316 days) and male (288-292 days) suggest a lifespan of less than one year. Overall, the age and growth results reported, although referred to a specific area and a short sampling, could represent useful knowledge for a correct evaluation and management of this important commercial species in the future.

KEYWORDS

***Todaropsis eblanae*, age, growth, statoliths, beaks, eye lens, Mediterranean**

10.3389/fmars.2025.1567441

1 Introduction

The lesser flying squid *Todaropsis eblanae* (Ball, 1841) is a neritic demersal species occurring in the eastern Atlantic Ocean from 61° north to 36° south latitude, in the Mediterranean Sea, the western Indian Ocean, the western Pacific Ocean, the South China Sea and Australian waters. Recent findings extend its distribution north to the Arctic (Roper et al., 2010). The distribution and abundance of *T. eblanae* (Hastie et al., 2009; Oesterwind et al., 2014; Lauria et al., 2016) and some reproductive aspects related mainly to the Atlantic and Indian populations (Hernández-García, 2002; Sabirov et al., 2012; Robin et al., 2002; Petroni, 2012) have been investigated. A genetic study on this species indicated the existence of at least 3 genetically isolated populations in the Eastern Atlantic (Dillane et al., 2005). Within the ICES European areas, commercial catches of *T. eblanae* together with other Ommastrephid squids such as *Illex coindetii*, *Ommastrephes caroli* and *Todarodes sagittatus* account for at least 49.8%-65.9% of total squid catches from 2015 to 2020 (FAO, 2020; Lishchenko et al., 2021). In the central western Mediterranean and in particular in Italian waters, *T. eblanae* represents a conspicuous fraction of bottom trawl commercial catches, forming the commercial category "totani" together with *Illex coindetii* (Belcari, 2017; Roper et al., 2010). The need to properly identify landings of Ommastrephid species has been emphasized because many stocks have not yet been assessed (Belcari et al., 2015 and Arkhipkin et al., 2015). Knowledge of biological aspects such as reproduction, lifespan, age and growth are essential elements for stock assessment and sustainable exploitation of cephalopods species (Arkhipkin et al., 2021). Generalizing, cephalopods are known for having short life cycles (max 2-3 years) and rapid and continuous growth that ends rather abruptly with the maturation of the gonads. The time allotted for growth can be modified mainly by temperature and available food (Forsythe and Van Heukelem, 1987).

To date, the analysis of the hard structures (i.e. statolith, beak, gladius, stylet, cuttlefish bone and eye lens) is the most widely used method to assess the age and growth of cephalopods. These structures can indeed 'remember' ontogenetic events by forming periodic growth increments. Daily deposition in beak has been validated for *Octopus vulgaris* (Perales-Raya et al., 2014) and then, this structure has been used for studying aging in several species (Xavier et al., 2022). Until now, the deposition of growth increments in squid beaks has been validated only on some Ommastrephid species such as *Dosidicus gigas*, *Ommastrephes bartramii*, *Illex argentinus* and *Todarodes pacificus* (Sakai et al., 2007; Liu et al., 2015; Fang et al., 2016).

The most common structures used to study age in cephalopods are the statoliths, despite their preparation being laborious and time-consuming; however, their reliability and daily periodicity of microincrements have been demonstrated in several cephalopods (Arkhipkin et al., 2018; Agus et al., 2024) and also in Ommastrephid squids (Hurley et al., 1985; Nakamura and Sakurai, 1991). Other hard structures, such as stylets, gladius, and eye lenses, have also been investigated to assess their potential use in age estimation (Arkhipkin and Bizikov, 1991; Hermosilla et al., 2010; Rodríguez-Domínguez et al., 2013; Agus et al., 2018). Growth patterns in the eye lens have been studied in *Sepia officinalis*, *Enteroctopus megalocyatus*, *Octopus maya*, *Loligo vulgaris*, *Loligo forbesii* and *Ommastrephes caroli* (Clarke, 1993; Baqueiro-Cárdenas et al., 2011; Rodríguez-Domínguez et al., 2013; Agus et al., 2018, 2021) but results were not always satisfactory (Rodríguez-Domínguez et al., 2013). Focusing on *T. eblanae*, preliminary data on the age and growth have so far been investigated by analyzing the statoliths of specimens from the north-east Atlantic (Robin et al., 2002), African waters (Arkhipkin and Laptikhovsky, 2000) as well as in Mediterranean individuals from Ligurian (Cavanna et al., 2008) and Ionian waters (Fotiadis et al., 2015). In view of the fragmentary knowledge of the biology of this species in the Mediterranean Sea, age and growth are addressed through the analysis and comparison of three hard structures (statolith, beak and eye lens) in the two sexes at different sexual maturity providing a complete picture of the life history of *T. eblanae*.

2 Materials and methods

2.1 Sample analysis

Overall, 270 individuals of *T. eblanae* were collected at depths between 30 to 570 m from the waters around Sardinia (Central-Western Mediterranean Sea, FAO GFCM Geographical subarea GSA 11) from July to September 2023 during Mediterranean International Trawl Survey activities (MEDITS, Spedicato et al., 2020) (Supplementary Figure 1). Dead specimens were immediately frozen on board and then transported to the laboratory for analysis. Dorsal mantle length (DML) was measured to the nearest 1 mm and total weight (TW) was recorded to the nearest 0.01g. For each squid, sex and maturity stage were assigned following the macroscopic scale in Follesa and Carbonara (2019). Length–weight relationships were calculated separately for each sex, according to the function $TW = a \times DML^b$, where TW is the total weight (g), DML is the Dorsal mantle length (mm), a is the intercept of the regression and b is the regression coefficient or slope. The growth was assessed by comparing the slope b to the isometric value of 3, by Student's t-test, between males and females. Moreover, The Kolmogorov-Smirnov (KS) two-sample test was used to investigate differences between the length-frequency and total weight distributions by sexes (Zar, 1999). All statistical tests were performed using the software Statgraphics Centurion XVI.

2.2 Hard structures: extraction and processing

Statoliths were surgically extracted from the cephalic cartilage and their maximum length (SL) was taken, with an accuracy of 0.01mm from the end of the dorsal dome to the top of the rostrum (Supplementary Figure 2). According to Ceriola and Milone (2007), the concave side of statolith was mounted on a microscope slide using Crystal Bond TM thermoplastic resin dissolved at 110–140°C. Then, both sides of the statoliths were grinded and polished using lapping film sheets with 30, 12, 5 and 0.5 µm grades. The total number of growth increments (NI) was counted from the natal ring towards the edge of the lateral dome (Ceriola and Milone, 2007). Eye lenses, once extracted, were cleaned with distilled water, measured using a digital gauge (accurate to a tenth of a millimeter) (Supplementary Figure 3) and fixed in 5% formalin, according to Baqueiro-Cárdenas et al. (2011). Lenses were kept under running water for 24 h and then put under a laminar flow hood for a few hours, to allow the complete drying of the structure (Agus et al., 2018). Then, they were cut into two parts, incorporated into epoxy resin and ground using a lapping machine with waterproof sandpaper at different grit (320, 600, 800 and 1000) until a section of 1 mm thickness was attained. The sections were mounted on a microscope slide and stained using Gill's Ematoxilin and Eosin and examined using an optical microscope equipped with a CANON EOS 1100 D camera to take digital images (magnification: 100× and 400×). Growth increments (NI) were counted starting from the center (nucleus) until the outer end of the lens. To avoid double counting, each eye lens increment was marked and numbered using the Tps_Dig2 software. Beaks were removed, cleaned with distilled water and stored in 75% ethanol and according to Clarke (1986) the following measurements were taken: upper crest length (uCL) and the upper rostral length (uRL) (Supplementary Figure 4). Following Liu et al. (2015, 2017) only the upper beak was considered for age analysis. Upper beaks were sectioned following the sagittal axis along the posterior edge of the hood and crest to the rostral tip, keeping the cutting line slightly to one side, to avoid any possible damage to the Rostrum Sagittal Section (RSS). One of the two sections was embedded in epoxy resin, left to harden for 24 h and glued to a microscope slide. Then, it was ground parallel to the cutting plane using a lapping machine with waterproof sandpaper 320, 600, 800 and 1000 grit, to approach the rostrum sagittal section. Beak increments (NI) were observed and photographed using the same optical microscope already described (magnifications:100x). NI in the RSS were counted slightly further from the Internal Rostral Axis (IRA). The morphometric measurements of all the three hard structures were related to the size of the animals (DML), through a regression analysis.

2.3 Data analysis and age estimation

The increment counting (NI) in the three structures were performed twice by three experienced readers independently, assuming a daily deposition of the bands (Arkhipkin et al., 2018).

The precision and accuracy of the overall readings were assessed by the Index of Average Percent Error (IAPE) (Beamish and Fournier, 1981), calculated as follows:

$$IAPE = N^{-1} \sum [R^{-1} \sum (|X_{ij} - X_j)X_j^{-1}]100$$

where N equals the number of samples aged, R represents the number of readings, X_{ij} is the ith age determination of the jth fish and X_j equals the average age calculated for the jth fish. In addition, the Coefficient of Variation (%CV) (Chang, 1982) and the Percentage of Agreement (%A) were also calculated.

The relationship between NI in the three different structures and the size of the animals (DML) were tested with 4 different curve models: linear, exponential, power and logarithmic and the Akaike Information Criterion (AIC) was calculated to choose which model best describes the growth of the sampled specimens (Akaike, 1974).

The absolute growth rate (AGR; mm d−1) was calculated separately for beaks and statoliths for each 50-day interval by sex using the following equation (Gonzalez et al., 1996):

$$AGR = R2 - R1/T2 - T1$$

TABLE 1 Composition of sample per sex and number of increments (NI) counted in statoliths, beaks and eye lenses of 270 *T. eblanae* specimens, at different sexual maturity stages.

Sex	Maturity stage	DML (mm)	TW (g)	NI Statoliths	NI Beaks	NI Eye lens
Undetermined N= 40		45.00–60.00 [52.95 ± 5.38]	6.98–20.05 [13.14 ± 4.33]	73–100 [88 ± 9]	70–120 [86 ± 13]	106–500 [354 ± 101]
Males N=110	**Immature** N=28	61.00–84.00 [72.18 ± 7.80]	17.56–51.40 [29.97 ± 10.14]	102–140 [125 ± 12]	89–142 [123 ± 15]	200–503 [381 ± 127]
	Developing + Maturing N= 46	80.00–135.00 [110.06 ± 16.35]	34.65–170.00 [96.12 ± 38.04]	134–184 [157 ± 16]	130–185 [156 ± 15]	250–562 [442 ± 79]
	Mature N= 36	105.00–142.00 [129.69 ± 8.22]	81.17–179.83 [151.24 ± 22.67]	150–288 [277 ± 9]	142–292 [276 ± 10]	330–600 [453 ± 76]
Females N= 120	**Immature** N= 35	62.00–110.00 [81.78 ± 14.99]	21.30–81.64 [44.01 ± 19.36]	110–148 [133 ± 13]	106–150 [134 ± 10]	259–560 [386 ± 70]
	Developing + Maturing N= 43	75.00–160.00 [116.30 ± 23.26]	37.55–252.30 [115.50 ± 60.07]	131–200 [163 ± 21]	129–195 [161 ± 20]	220–547 [423 ± 70]
	Mature N= 42	136.00–200.00 [180.05 ± 12.99]	167.13–443 [354.75 ± 66.12]	184–310 [240 ± 24]	173–316 [234 ± 25]	250–640 [469 ± 80]

Mean and standard deviation values are reported in square brackets. DML, dorsal mantle length; TW, total weight.

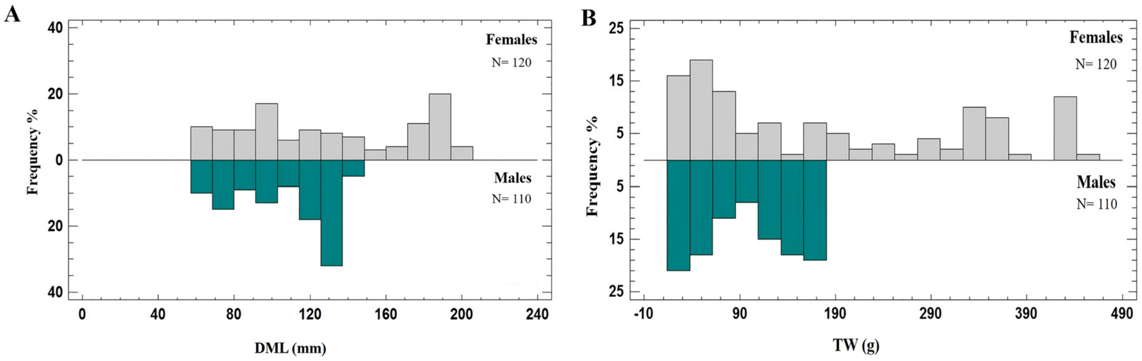

FIGURE 1
The frequency distributions of length and weight of *Todaropsis eblanae* for females (grey) and males (green): **(A)** dorsal mantle length (DML); **(B)** total weight (TW).

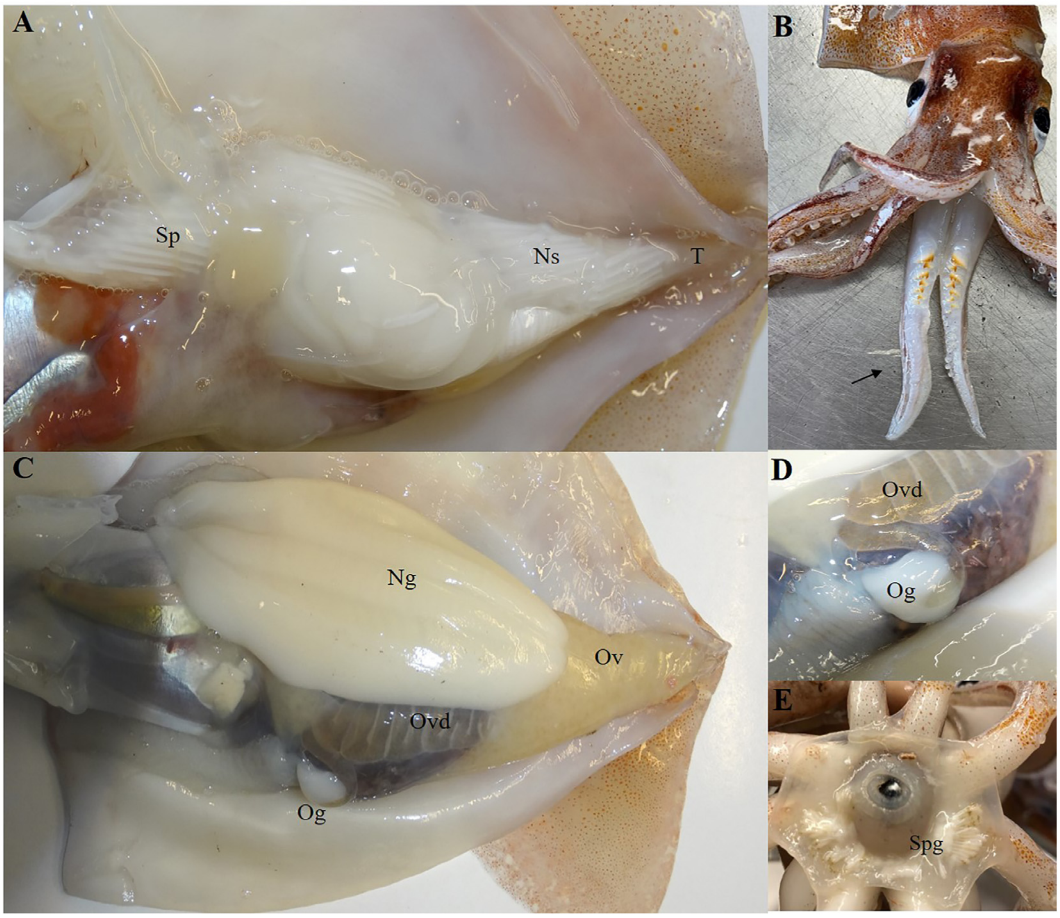

FIGURE 2
Macroscopic evidence of sexual maturity in male **(A, B)** and in female (CDE) of *Todaropis eblanae*. **(A)** male sexual apparatus; **(B)** ventral arms hectocotilized; **(C)** female sexual apparatus; **(D)** oviduct with ripe eggs and oviducal gland; E Buccal mass with spermatangia implanted. T, Testis; Ns, Needham's Sac; Sp, spermatophores; Ov, Ovary; Ovd, Oviduct; Og, Oviduct gland; Ng, Nidamental gland; Spg, spermatangia.

where R1 and R2 are the average ML (mm) at the beginning (T1) and end (T2) of the time interval, respectively. NI counted in both statoliths and beaks were correlated using a linear regression. Before being compared with the Mann-Whitney W-test to check whether significant differences existed between the increments of the two structures, the Shapiro-Wilk normality test was performed. All statistical analysis were made using R (4.4.2 version).

3 Results

3.1 Sample analysis

The sample was composed of 110 males (DML range = 61-142 mm; TW range = 17.56-179.83 g), 120 females (DML range = 62-200 mm; TW range = 21.30-443.00 g) at different maturity stages (immature, developing, maturing and mature), whereas the sex of forty specimens was not easily distinguishable macroscopically and they were classified as undetermined (DML range = 45-60 mm; TW range = 6.98- 20.05 g) (Table 1). The frequency distribution of length and weight, by sex are shown in Figure 1.

The length-weight relationship in females ($TW= 0.0003 \times DML^{2.6773}$; $r^2 = 0.98$), differed significantly from males ($TW= 0.0002 \times DML^{2.7883}$; $r^2 = 0.97$) with t-test $P< 0.005$. The Kolmogorov-Smirnov two sample test ($P<0.05$) further confirmed that females reach a statistically significant larger size and weight than males. Macroscopically all mature males had left and right ventral arms hectocotilized and a developed reproductive system with packed spermatophores in the Needham's Sac (Figures 2A, B). Mature females had large Nedamental's glands and ripe oocytes in the ovary and oviducts ready to be laid (Figures 2C, D). Mating signs were observed in females for the presence of implanted spermatangia in the buccal mass (Figure 2E), both in mature (36%) and maturing (12%) individuals.

3.2 Hard structures

The total length of the statolith (SL) ranged from 0.53 mm (45 mm DML, 8.29 TW; undetermined) to 1.35 mm in the biggest mature female (200 mm DML and 443 g TW). In the same specimens we also recorded the smallest (2.6 mm) and the largest (9.83 mm) eye lens diameter. As regard to the beak measurements, the smallest upper beak (uRL=2.11 mm, uCL=7.40 mm) was found in an undetermined specimen of 45 mm DML and the largest (uRL=8.70 mm, uCL=26.0 mm) belonged to the above mentioned biggest female. The linear relationship between the morphometric measurements of the three hard structures (statolith, beak, eye lens) and the size (DML) of the animals showed a significant positive correlation in all samples examined (Figure 3).

3.3 Data analysis and age estimation

All 270 statoliths, upper beaks and extracted eye lenses, were available for ageing analysis and were legible, clearly showing regular and well-defined growth increments (Figures 4A–C). IAPE, CV and PA values testify to a high level of accuracy and reproducibility of the

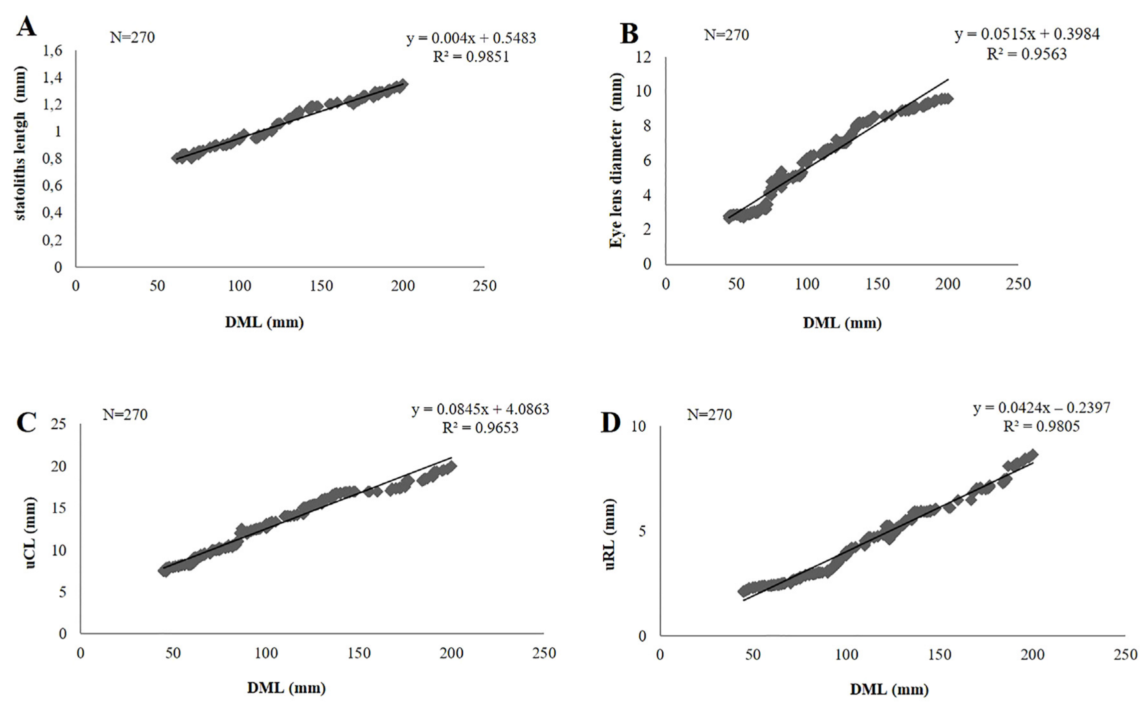

FIGURE 3
Linear relationship between the measurements of statoliths **(A)**, eye lens **(B)** and beaks **(C, D)** *vs.* dorsal mantle length (DML) in *Todaropsis eblanae.*

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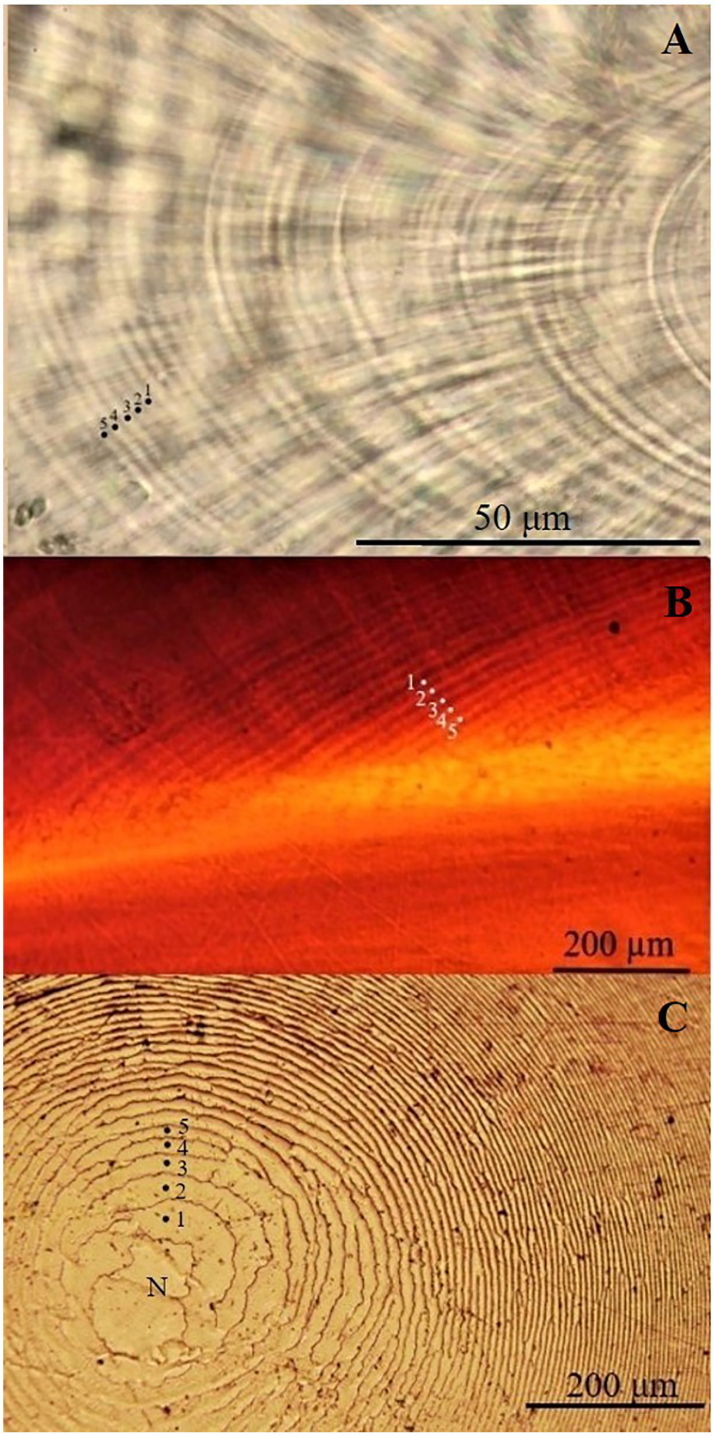

FIGURE 4
Growth increments marked with numbers (1-5) in statolith **(A)**, upper beak **(B)** and eye lens **(C)** belonged to a maturing male of 126 mm DML and 123.14 g TW of *Todaropsis eblanae*.

readings in all structures (Table 2). The count of NI in statoliths showed similar values to those in beaks. The lowest values of 73 NI in the statoliths and 70 NI in the beaks were recorded in the smallest specimen (45 mm DML, 8.29 g TW; undetermined) while the highest values were found in the largest mature female (200 mm DML, 443.0 g TW) with 310 NI and 316 NI in the two structures, respectively. As for males, the highest numbers of NI in statoliths (288) and beaks (292) were observed in a mature specimen of 142 mm DML and 177.0 g TW (Table 1). NI counted in the eye lenses, in both sexes and for all maturity stages, were always considerably higher than those counted in the statoliths and beaks, even in the indeterminate specimens (average of 354 NI) reaching up to 600 NI in the eye

TABLE 2 Summary of the ageing precision and accuracy results for each hard structure analysed.

Hard structures	N	Ageing Precision and Accuracy		
		CV (%)	PA (%)	IAPE (%)
Statoliths	270	0.7	86.7	0.41
Beaks	270	1.2	80.4	1.32
Eye lens	270	1.8	65.0	1.44

lenses of the largest animals (Table 1). For both sexes and for the entire sample, the AIC test (Supplementary Tables 1, 2) showed that the linear model best describes the relationships between NI of statoliths and of beaks, with DML (Figure 5). A good relationship (R^2 = 0.9966) was found plotting the increments counted in the statoliths against those observed in the beaks for each specimen (Figure 6). The Shapiro-Wilk test showed a not normal distribution (W=0.93, *p*-value = 0.0) and the application of the Mann-Whitney test (*p*-value =0,929878) confirms that there is not a statistically significant difference between the two variables at the 95,0% confidence. Daily growth rates, calculated separately for the two sexes and the two structures (beaks and statoliths), were highest at a young age and then gradually decreased. (Table 3). Females grow faster than males. Regarding the statoliths, the highest rates were recorded in age group II (101-151 days) with values respectively of 1.74 mm/d in females and of 1.67 mm/d in males. Considering the beaks, females showed higher values of 1.71 mm/day and 1.01 mm/day respectively in the age classes II (101-151 day) and III (152-202 days) while males in the I class<100 (1.25 mm/day). Regarding sexual maturity, indeterminate and immature specimens were only present in the first two age classes:<100 and 101-151 days of age, for both structures. Maturing males were in class II from 101 to 151 days of age, while maturing females were between 152 and 202 days of age (class III) for both statoliths and beaks. Mature specimens appear in age class II for males and class III for females, indicating that males reached maturity earlier than females. Considering beaks and statoliths respectively, males begin maturity at 142 and 150 days, while females at 173 and 184 days.

4 Discussion

The sample of *Todaropsis eblanae* analyzed in this study showed that females are significantly larger than males in mantle length and weight, as already reported in literature (Roper et al., 2010). The maximum sizes recorded in Sardinian waters for females (200 mm DML;443 g WT) and males (142 mm DML; 180 g WT) are in agreement with other Mediterranean observations (Mangold-Wirz, 1963; Cavanna et al., 2008), but are smaller than in north Atlantic populations, where females reach a maximum size 290 mm DML against 220 mm DML in males (Robin et al., 2002). The significant differences between sexes found in the length-weight relationship were also observed in most of the studied regions in both the Atlantic and Mediterranean as well as a negative allometry (regression coefficient b<3) (Belcari et al., 2015) The present study represents the first age estimation in *T. eblanae* through the use of multiple hard structures (i.e., upper beaks, eye lenses, statoliths), implementing the so far available fragmentary information on the age of the species, obtained exclusively through the analysis of statoliths in both the Atlantic (Arkhipkin and Laptikhovsky, 2000; Robin et al., 2002) and the Mediterranean Sea (Cavanna et al., 2008; Fotiadis et al., 2015).

All the hard structures showed measurements positively correlated with the size of the animals and were easily readable with well-defined growth increments suggesting the feasibility of their use for age determination. In these structures, the overall CV, PA and IAPE values related to the counting of growth increments, demonstrate a high level of accuracy and reproducibility of readings (Campana, 2001). Although statoliths are the most common structures used for ageing in cephalopods and their reliability and daily periodicity have been validated or hypothesized in several

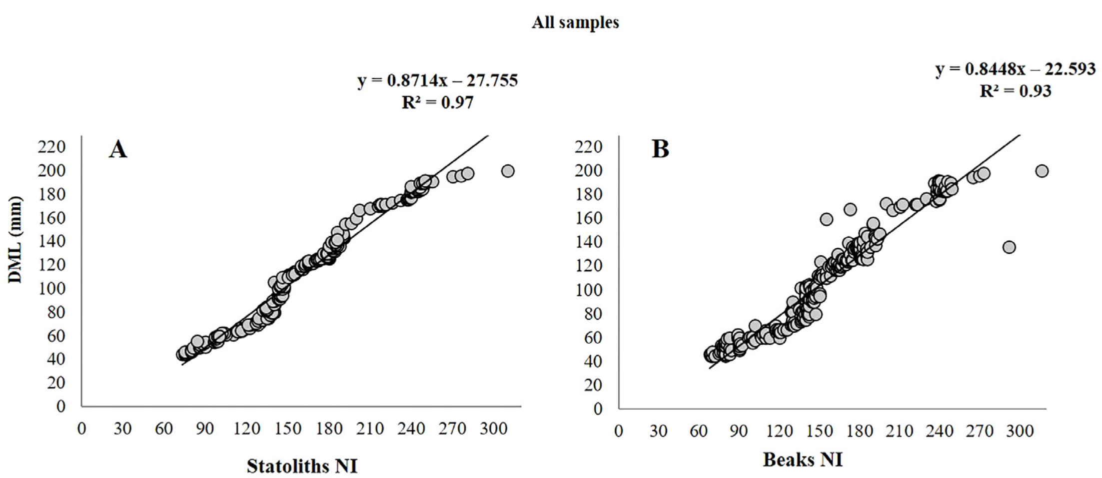

FIGURE 5
Plots of the number of increments (NI) counted in the statoliths **(A)** and in the beaks **(B)** *vs*. dorsal mantle length (DML) of 270 *Todaropsis eblanae*.

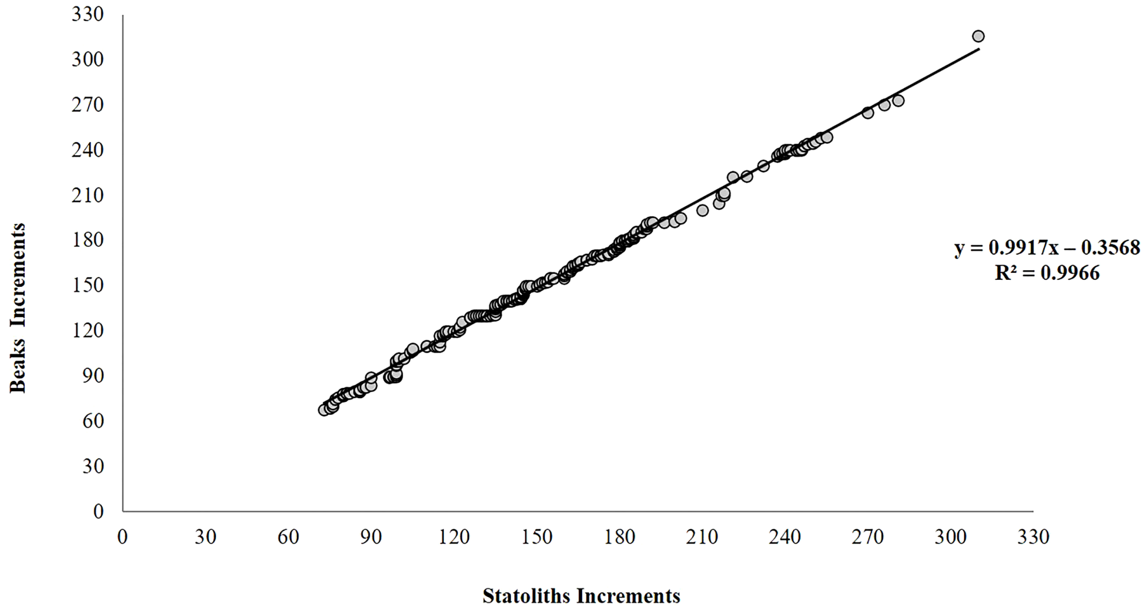

FIGURE 6
Relationship between the number of increments counted in beaks and statoliths belonging to the same specimens (N= 270) of *Todaropsis eblanae*.

species (Arkhipkin et al., 2018; Agus et al., 2024), in terms of processing effort, the smaller size of statoliths compared to beaks and eye lenses resulted in more laborious and time-consuming laboratory work. As regards the eye lens, to date, a daily deposition according to the size of the animals was suggested only in *L. vulgaris*, *L. forbesii*, and *O. caroli* (Agus et al., 2018, 2021). A sub-daily deposition has been proposed instead for the lenses of *S. officinalis*, *E. megalocyatus* and *O. maya* (Clarke, 1993; Baqueiro-Cárdenas et al., 2011; Rodríguez-Domínguez et al., 2013). In the present analysis, the considerable number of increments (up to 600) counted in the eye lenses of *T. eblanae*, significantly higher than those obtained with statoliths and beaks, clashes with a daily deposition, suggesting a different increment periodicity. Considering these results and according to Rodríguez-Domínguez et al. (2013), although this structure is considered attractive as a potential tool for aging, its use is not successful in all cephalopod species, and this variation suggests the need for further studies for its validation. On the contrary, the NI obtained from statoliths and

TABLE 3 Absolute growth rate (AGR) for mantle length for age class of increments in statoliths and beaks of *Todaropsis eblanae* by sex.

Sex	Class	Age Class (days)	Statoliths		Beaks	
			DML x̄ ± SD	AGR	DML x̄ ± SD	AGR
Males N= 110	I	<100	–	-	61.50 ± 1.0	**1.25**
	II	101-151	83.72 ± 14.71	**1.67**	86.35 ± 14.77	1.00
	III	152-202	120.00 ± 6.08	0.73	117.00 ± 5.13	0.70
	IV	203-253	127.70 ± 7.10	0.16	128.00 ± 7.23	0.30
	V	254-304	131.40 ± 7.84	0.06	131.00 ± 8.20	0.17
	VI	>305	–	-	-	-
Females N= 120	I	<100	–	-		
	II	101-151	86.48 ± 15.00	**1.74**	85.35 ± 14.30	**1.71**
	III	152-202	134.67 ± 14.65	0.96	135.90± 6.07	1.01
	IV	203-253	181.20 ± 7.05	0.90	181.70 ± 7.16	0.90
	V	254-304	195.00± 2.94	0.28	194.17 ± 2.79	0.25
	VI	>305	200	0.12	200	0.12

AGR absolute growth rate (mm d-1), x̄ average, SD standard deviation; The highest values of AGR are given in bold.

beaks were similar and correlated to the size of the specimens of both sexes, as well as consistent/congruent with sexual maturity, in agreement with a daily deposition. The number of increments obtained from beaks and their positive comparison with statoliths (confirmed by Mann-Whitney test) prove for the first time that this hard structure is a promising and simple tool for age estimation also in *T. eblanae*, as already observed for other Ommastrephid squids: *O. bartrami* (Fang et al., 2016), *D. gigas*, *I. argentinus*, *Stenotheuthis oualaniensis* (Liu et al., 2015), *O. caroli* (Agus et al., 2021). Assuming daily increments in both statoliths and beaks and considering that the samples were taken mostly in summer, it can be assumed that the smallest, non-sexually determinable specimens of *T. eblanae* (around 13 g and 88 days old) were able to hatch in spring and that the large mature specimens, following a semelparous cycle, were close to death at less than a year old. Furthermore, our results showed a higher initial growth rate for both sexes (higher in females), which gradually decreases, with the highest values at the age of 101-151 days, before reaching sexual maturity. The same trend was observed also in Atlantic (Arkhipkin and Laptikhovsky, 2000). The attainment of maturity, which in males occurs at a smaller size (105 mm DML; 82 g WT) and age (142-150 days) than in females (136 mm DML; 167 g WT; 173-184 days), is in agreement with previous observations made in the Atlantic (Hernández-García, 2002; Gonzales et al., 1994; Rasero, 1996; Zumholz and Piatkowski, 2005) and in the Mediterranean Sea (Mangold-Wirz, 1963; Cavanna et al., 2008; Fotiadis et al., 2015). This precociousness in male maturation prompts males to mate with females that are not yet ready, as confirmed by the presence of implanted spermatangia in 12% of the buccal mass of maturing females. This characteristic (behavior) already observed for *T. eblanae* off NorthWest Africa (Hernández-García, 2002) is common in other cephalopods, especially octopods (e. g. Mangold, 1983; Cuccu et al., 2013; Agus et al., 2021, 2022).

5 Conclusions

This study, provides for the first time in *T. eblanae* the analysis of age at different stages of maturity using beaks, statoliths and eye lenses, and give a complete picture of the biological history of this species in Sardinian waters. Considering that advances in age estimation of cephalopods could enable the identification of seasonal cohorts and age-based assessment (Pierce et al., 2019), our results update to the still fragmentary knowledge on *T. eblanae* in the Mediterranean Sea that needs to be validated in other regions and environmental conditions in the future.

Overall, the results suggest a daily deposition for beaks and statoliths. The strong relationship between the increments observed in the beaks and statoliths confirms their potential use for determining age in *T. eblanae* of different sex, size and maturity stage. However, although the beak and the statolith provide similar results, the use of beak for age determination is preferred to the statolith because of the simplicity of its analysis. As for the eye lens, the high number of increments, considerably higher than that of statoliths and beaks, does not support daily deposition suggesting the need for further studies to assess which alternative deposition rate characterizes this structure.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with local legislation and institutional requirements, because the animals analysed were found dead.

Author contributions

BA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. PB: Data curation, Investigation, Methodology, Writing – review & editing. AB: Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing. RC: Data curation, Formal Analysis, Writing – review & editing. EC: Data curation, Formal Analysis, Writing – review & editing. DC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Data for the purposes of the analyses were obtained from the International Mediterranean Trawl survey MEDITS within the Data Collection Framework (EU Reg. 199/2008).

Acknowledgments

We would like to thank the crew of the ship 'Gisella' employed for the sampling.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1567441/full#supplementary-material

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