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Age estimation and growth of striped dolphins *Stenella coeruleoalba* stranded along the coasts of south-western Italy

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Abstract
The knowledge of demographic traits such as longevity, growth rates and age at sexual maturity is crucial for understanding the structure of a population in its natural environment and implementing appropriate strategies for its management and conservation. Based on counts of growth layer groups in sections of decalcified teeth using the paraffin technique, we estimated the age and growth of 25 individuals of striped dolphin (*Stenella coeruleoalba*) found dead stranded along the coast of Campania and Calabria (south Italy, central-western Mediterranean) from 2013 to 2018. Seven individuals, with TL of 100–110 cm, were calves under 1 year old. The oldest male and female individuals were 19 and 14 years old, respectively. Growth curve estimated using the Gompertz growth model (GGM) showed that in *S. coeruleoalba* male growth trajectories are partly in accordance with those reported in other studies on the same species from different Mediterranean areas. The high frequency (28%) of calves strongly suggests that females of this species use the marine area all around the south-western Italian coasts to give birth to their offspring. Furthermore, a comparison with the estimated age of striped dolphins from other Mediterranean marine areas shows that the longevity of the individuals examined in this study is much lower. Our study provides information toward understanding the demographic traits of *S. coeruleoalba* from Mediterranean Sea. The results reported here can be useful for future research aimed at understanding population structure, mortality patterns and the effects of anthropogenic activity on the survival of this species in this marine area.

Keywords: Stenella coeruleoalba*, dolphin, south-western Italy, age estimation and growth, tooth sections*

Introduction

The striped dolphin, *Stenella coeruleoalba* (Meyen 1833) (Cetartiodactyla, Delphinidae), has a wide distribution, from tropical to warm-temperate waters of the Atlantic, Pacific and Indian oceans, as well as many adjacent seas, including the Mediterranean (Braulik 2019). Morphological and genetic evidence suggests that the Mediterranean and eastern North Atlantic striped dolphin populations are isolated from each other, with reduced or absent gene flow across the Strait of Gibraltar (Calzada & Aguilar 1995; Garcia-Martinez et al. 1995; Aguilar & Gaspari 2012). Within the Mediterranean, some clinal variations in body length among populations have been observed, suggesting restrictions in gene flow between areas (Calzada & Aguilar 1995) or even different demographic characteristics of distinct populations. In general, reduced gene flow may be a result of geographic isolation, genetic and chromosome differentiation (Mezzasalma et al. 2013, 2017a, 2017b). Evidence of genetic differentiation between the Adriatic and Tyrrhenian Seas has been also found

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(Gaspari et al. 2007), but currently there is no information available on different age structures in Mediterranean populations of *S. coeruleoalba*.

Based on the International Union for Conservation of Nature (IUCN) Red List criteria, *S. coeruleoalba* is listed as "Least concern" worldwide (Braulik 2019) but "Vulnerable" in the Mediterranean, owing to several threats to which this species is susceptible and the limited conservation actions currently taking place in this geographical area (Aguilar & Gaspari 2012). In the past, the Mediterranean population of *S. coeruleoalba* was strongly affected by accidental catches in pelagic nets for swordfish fishing (Magnaghi & Podestà 1987; Podestà & Magnaghi 1989; Notarbartolo Di Sciara 1990). From the end of the last century, despite a ban in the Mediterranean by all European Union countries, including Italy (EC Reg. 1239/98), *S. coeruleoalba* continued to suffer from illegal capture although to a lesser extent than in the past (Tudela et al. 2005; Fortuna et al. 2007). Furthermore, over the last 30 years, several morbillivirus epizootics were responsible for many die-offs of Mediterranean striped *S. coeruleoalba* (Aguilar & Raga 1993; Raga et al. 2008; Rubio-Guerri et al. 2013; Casalone et al. 2014; Profeta et al. 2015).

Despite its importance from a conservation point of view, information on the age structure of the Mediterranean population of this cetacean is scarce and limited to only a few studies (Calzada et al. 1994, 1997; Marsili et al. 1997, 2001). Data on age determination of Italian striped dolphin are also reported in Guglielmini et al. (2002).

One of the most widely used methods to estimate the individual age of odontocetes is based on the interpretation of the growth incremental layers which may be recognized in tooth sections (Scheffer & Myrick 1980; Evans et al. 2002; Read et al. 2018). For many species of odontocetes it has been possible to define growth layer groups (GLGs), where each group represents the amount of mineralized tissue formed per year (e.g. Myrick Jr et al. 1984; Hohn et al. 1989; Myrick Jr & Cornell 1990). Counts of GLGs in the tooth dentine is the common practice for assessing the age of odontocetes because dentine is the most developed mineralized tissue of the tooth (Perrin & Myrick 1980; Luque et al. 2009; Murphy et al. 2014). However, some researchers have obtained more accurate estimates for older dolphins when cementum is used (Cockcroft & Ross 1990). The distinctness of dentinal GLGs may vary according to the species and is influenced by the growth pattern of the dentine (Klevezal & Myrick 1984; Evans et al. 2007). In addition, the identification of GLGs may be complicated by the presence of accessory layers within the dentine, i.e. adjacent layers discernible in a GLG (*sensu* Scheffer & Myrick 1980), and other tooth-tissue alterations, such as pulp stones in the pulp cavity and dentinal resorption (Luque et al. 2009, 2013; Della Bianca et al. 2012; Read et al. 2018). The reading efficiency of GLGs and, consequently, the reliability of the age estimate can be also influenced by the tooth preparation technique (Hohn & Fernandez 1999; Evans et al. 2002). Luque et al. (2009) showed that the paraffin technique is a viable method and represents a cost-effective alternative to other techniques when preparing teeth of small odontocete species for age determination.

In this study, we aimed to estimate the individual age and growth of striped dolphins (*Stenella coeruleoalba*) stranded along the coast of Campania (Maio et al. 2019) and Calabria (south Italy, western Mediterranean) from 2013 to 2018 using the counts of GLGs on stained sections of decalcified teeth using the paraffin technique.

Materials and methods

Sampling

We analyzed the teeth of 25 individuals (17 males, 8 females) stranded along the coasts of Campania and Calabria (Southern Italy) from 2013 to 2018 (Figure 1; Supplementary data, Table S1). Sex was determined

Figure 1. Stranding areas (open ovals) of the striped dolphins (*Stenella coeruleoalba*) examined in this study.

by visual inspection of the gonads. For each animal, total body length (TL) was measured from the tip of the rostrum to the notch of the tail (American Society of Mammalogists 1961). During the necropsy, a portion with at least 3 teeth was collected from the middle of the lower jaw of each dolphin and fixed in 75% ethanol, exchanging the ethanol solution once a day for 5 days. Teeth were successively removed from the jaw bone using a scalpel blade, cleaned of soft tissue, and then stored in 75% ethanol until laboratory analysis.

Histological technique for age determination

Tooth histological sections were prepared according to Luque et al. (2009), with slight modifications specified below. After gently rinsing in tap water, teeth were decalcified. Since the decalcification can affect the quality of the tooth histological sections and therefore their interpretation, for each animal, two decalcification solutions were used: RDO (Apex Engineering Products Corporation, Illinois, USA), a commercial rapid decalcifying agent of which hydrochloric acid is the principal active ingredient, and 5% nitric acid. The decalcification end point was defined as when teeth appeared translucent and pliable (Murphy et al. 2014; Read et al. 2018). The duration required for optimal decalcification varied between samples in relation to the decalcifier (type and concentration), tooth size and age, with longer times required for larger and older individuals. Using RDO, decalcification time ranged from 8 to 12 h for smaller individuals (TL < 150 cm) and up to 24 h for larger individuals. Using 5% nitric acid, decalcification time ranged from 12 to 20 h for smaller individuals (TL < 150 cm) and up to 48 h for larger individuals. After decalcification, the teeth were washed in running tap water for 6 h, dehydrated through a series of graded ethanol baths, cleared with Bioclear (Bio Optica, Milano, Italy) and then infiltrated with molten paraffin (60°C). Longitudinal serial paraffin sections (15 μm thick) were obtained using a standard rotative microtome (Reichert-Jung/Leica 2045, Germany), stained with Mayer's Hematoxylin (30 min) and mounted with Bio Mount HM resin (Bio-Optica, Milano, Italy).

Tooth sections were examined using both a Leica EZ4 stereo microscope and a Motic BA340 compound light microscope, equipped with a digital camera. The former microscope was used to check for the best-stained sections closest to the midline of the tooth and to obtain a complete histological view (low magnification) of the sections; the latter allowed us to observe tooth sections at higher magnification which were successively photographed with the digital camera and stitched together into a single image. The acquired images of the tooth sections were optimized with respect to contrast and intensity using Adobe Photoshop 6.0 in order to enhance the distinctiveness of the GLGs. The count of GLGs was performed independently by three researchers (FMG, NM, CHL) and without prior knowledge of the TL or sex of the specimens. In the case of discrepancies in the GLG count, the sections were read again until a final consensus was reached.

Growth models

The growth of *S. coeruleoalba* was described using the GGM fitted to the age–length data according to other studies on odontocetes (Calzada et al. 1997; Venuto et al. 2020).

The GGM equation is as follows:

$$l_t = L_\infty e^{e^{-k(t-I)}}$$

where l_t is the total body length at age t; L_∞ is the asymptotic length at which growth is zero; e is the base of the natural logarithm; k is the growth coefficient that defines the shape of the curve; and I is the age at the inflection point.

L_∞, k, and their asymptotic confidence intervals (CIs) were estimated using a non-linear regression procedure by means of the Growth II software (Henderson & Seaby 2006).

Results

Dentinal GLGs were visible on all tooth sections, and their count was the same in teeth for which the two decalcification methods produced a similar result (Figure 2). Accessory lines, which are subannual incremental layers (*sensu* Read et al., 2018), were often observed, especially within the first 3–4 GLGs following the neonatal line (NNL).

Estimated ages ranged from 0 to 19 years old (Figure 3). Seven individuals (5 males, 2 females), with TL 100–110 cm, were calves under 1 year of age (Figure 4); 7 individuals (3 males, 4 females), with TL 134–156 cm, were 1–7 years old; 11 individuals (9 males, 2 females), with TL 162–210 cm, were 9–19 years old. The oldest male and female were 19 (TL = 210 cm) and 14 (TL = 194 cm) years old, respectively.

Since the female sample was too small, the growth curve was estimated using GGM only for males (Figure 5). The estimated asymptotic total body length in males (L_∞ ± CI: 195.7 ± 0.4 mm, k ± CI,

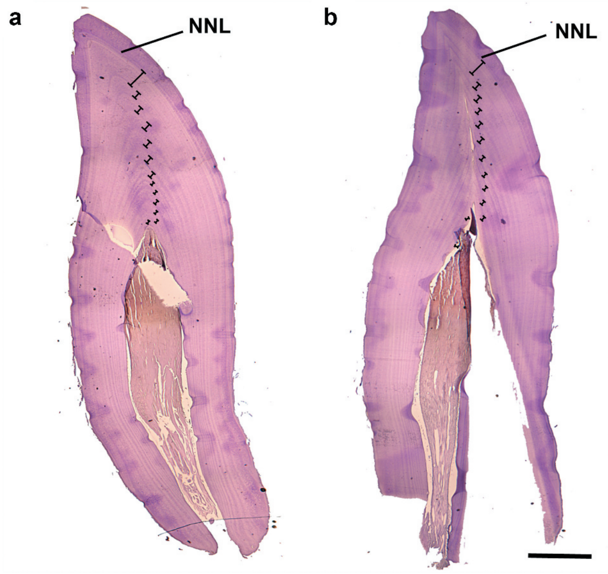

Figure 2. Representative tooth sections of *Stenella coeruleoalba* (ID Sc166277) obtained using different decalcifiers. The sections were obtained from two different teeth of the same individual. (a) Tooth decalcified using RDO; (b) tooth decalcified using 5% acid nitric. The neonatal line (NNL) and GLGs (⊢⊣) in dentine are indicated. In (b) one less GLG (n = 12) compared to (a) (n = 13) is visible, probably due to the greater erosion of the pulp cavity. Scale bar = 1600 μm for both figures.

0.39 ± .1.32) was lower than the maximum total body length observed for this sex (TL: 210 cm).

Figure 3. Tooth section of *Stenella coeruleoalba* calf under 1 year old (ID Sc9793). NNL: neonatal line. Scale bar = 1100 μm.

Discussion

This study shows that the longevity of striped dolphins stranded along the coasts of south-western Italy (central-western Mediterranean) between 2013 and 2018 is much lower than that recorded in previous studies on striped dolphins stranded along Mediterranean coasts, and highlights the variability in the age structure of *S. coeruleoalba* populations in this marine area. In fact, in this study the estimated ages of the oldest male and female were 19 and 14 years, respectively, whereas other studies yielded estimates of 28 and 32 years for males and females, respectively, stranded along the Spanish coasts (Calzada & Aguilar 1995; Calzada et al. 1997), and 29 years (for the two sexes combined) for animals stranded along a wide coastal area all around the Italian peninsula (Marsili et al. 2001).

There are several possible explanations for the age structure of our sample, such as greater susceptibility to diseases in adults due to the effects of pollution or allopatric geographical distribution of adults and juveniles, as suggested in other studies (Calzada et al. 1994). In particular, it might be hypothesized that the age structure of our sample was affected by die-offs, mainly due to morbillivirus, that have frequently occurred in the Mediterranean striped dolphin population in the last few decades (Pertoldi et al. 2000; Valsecchi et al. 2004; Casalone et al. 2014). Furthermore, it has been suggested that animals dying from morbillivirus may venture more frequently toward the shore than those dying of old age (Valsecchi et al. 2004). Our study sample also had a very high percentage (about 90%) of animals infected with morbillivirus (data not shown). However, further targeted studies are needed on a larger sample to clarify the link between morbillivirus and age structure of Mediterranean striped dolphins.

Another interesting finding of this work is the high frequency (n = 7; 28% of our sample) of calves under 1 year old. We hypothesize that they very likely died in marine areas next to the stranding localities, for the following reasons. Most of the calf carcasses (n = 4) were in good condition (decomposition stage code 2,

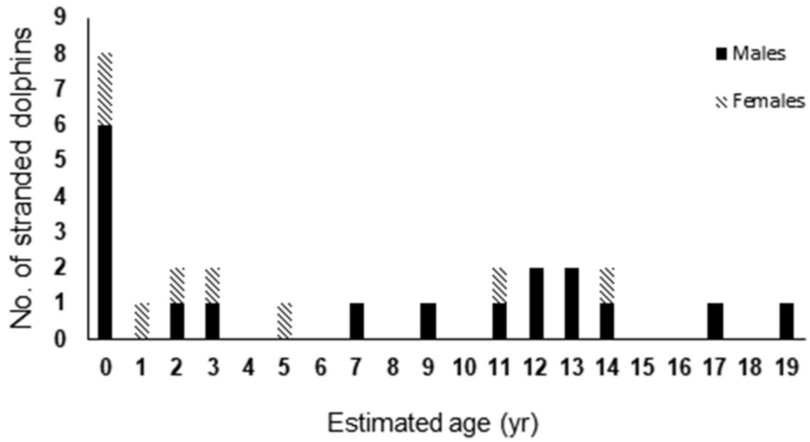

Figure 4. Age distribution of *Stenella coeruleoalba* analyzed here (n = 25) using tooth sections.

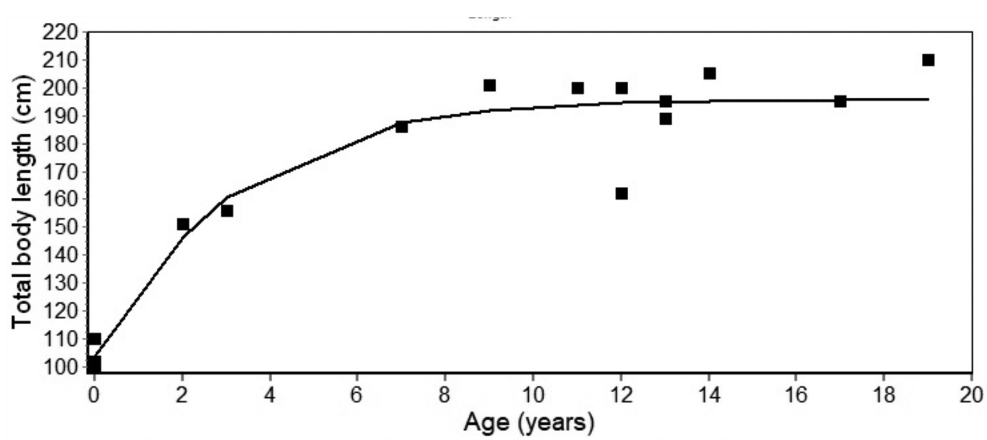

Figure 5. Gompertz growth curves for males of *Stenella coeruleoalba*. Growth parameters are given in the text.

according to Geraci & Lounsbury 2005), while the remaining calves (n = 3) were in the initial decomposition state (decomposition stage code 2–3). In addition, the weather conditions and the average wind speed were stable and moderate, respectively, during the days of the stranding and those immediately preceding our research (see https://www.ilmeteo.it/portale/archivio-meteo). Finally, the trend of the speed and direction of surface currents, reported monthly for different stranding areas, was parallel to the coastlines (Istituto Idrografico della Marina 1982). Therefore, our results for the calves strongly suggest that the marine area along the Tyrrenian coasts of Campania and Calabria and Jonian coasts of Calabria (southern Italy) is used by the species to give birth to the offspring and as a nursery. This conclusion is in accordance with previous studies relating to the Salerno coast, where at least 15 calves under 1 year old were recorded between 1986 and 2019 (Maio et al. 2012, Banca Dati Spiaggiamenti: http://mammiferimarini.unipv.it). Furthermore, we collected calves both during May–early October (n = 4) and January–March (n = 3) (see Supplementary Table S1). Therefore, it is likely that in the study area *S. coeruleoalba* has two reproduction periods, roughly corresponding to the summer–early autumn and the late winter period, respectively, as previously suggested by Marini et al. (1992), Mussi et al. (1997) and Mussi and Miragliuolo (2003).

The GGM showed that in *S. coeruleoalba* male growth trajectories are partly in accordance with those reported in other studies on the same species from different Mediterranean areas (Di Meglio et al. 1996; Calzada et al. 1997; Marsili et al. 1997, 2004). Indeed, we also observed initially high growth rates in males, until reaching an asymptote that likely corresponds to their attainment of sexual maturity. In our study, the asymptote begins at 12 years of age and a TL of 195 cm. In the study by Calzada et al. (1997) the asymptote was approached at 18 years of age and a TL of 200 cm in males. In the papers of Marsili et al. (1997, 2001, 2004), where the data for the two sexes were combined, the age of sexual maturity was estimated at 9 years, at a TL of about 190 cm. Similarly, Di Meglio et al. (1996) reported that males of Mediterranean striped dolphins reach their asymptotic length, and then sexual maturity, at 8–9 years. Therefore, our findings are consistent with the observation that the males of *S. coeruleoalba* from central-western Mediterranean can reach sexual maturity at a much lower age than that reported by Calzada et al. (1997). However, confirmation of the age at sexual maturity of striped dolphin males predicted on the basis of the growth curve can only be obtained via inspection of the gonads, which unfortunately were not available for this study.

To conclude, although this study was limited by the number of animals examined, it provides interesting information toward understanding the demographic traits of *S. coeruleoalba* from the Mediterranean Sea. The results reported here can useful for future research aimed at understanding mortality patterns and the effects of anthropogenic activity on the survival of this species in this marine area, as performed also for other cetacean species (Maio et al., 2016) and other marine vertebrates (see Guarino et al. 2020).

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary material

Supplemental data for this article can be accessed here.

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