Journal of Experimental Marine Biology and Ecology 533 (2020) 151473

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Exploring data from an individual stranding of a Cuvier's beaked whale in the Gulf of Taranto (Northern Ionian Sea, Central-eastern Mediterranean Sea)

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ARTICLE INFO

Keywords:
Strandings
Lagrangian drifting model
Ziphius cavirostris
Osteological collection
Museology

ABSTRACT

Cetacean stranding events are an important source of information and biological samples, providing data on species occurrence, distribution and population dynamics as well as on mortality rate induced by human activities or natural causes. This is even truer for species such as *Z. cavirostris*, whose elusive behavior has made its field observation difficult in many Mediterranean areas. Although the occurrence of single or serial strandings of this species and the rise in survey effort have increased the knowledge on its presence, there are still gaps in some Mediterranean areas, especially in the Gulf of Taranto (North-western Ionian Sea, Italy), where a critical habitat suitable for different cetacean species has been identified. Despite no sightings of *Z. cavirostris* having been recorded so far, ACCOBAMS designates the Gulf of Taranto as an Area of Special Concern for Beaked Whales (ASC-BW) on the basis of atypical mass strandings of 12 individuals which occurred in the Ionian Sea in 2011. Moreover, the conservation status of the *Z. cavirostris* has recently passed from Data Deficient to Vulnerable, according the IUCN criteria adopted for the Mediterranean Sea, thus showing the urgent need for basic information on its bio-ecological features, suitable habitat and threats at local or wider scale. This study is the first attempt at application in the Mediterranean Sea of a Lagrangian prediction model simulating the carcass drift trajectories to investigate the at-site sea origin of an individual of *Z. cavirostris* stranded in the Gulf of Taranto. Moreover, a necropsy was carried out to detect the possible cause of death and the results of bacteriological, biomolecular and toxicological analysis are provided. Finally, a collection of osteological data as well as the process of turning and displaying the skeleton in a museum are detailed, highlighting the importance of these exhibits from a scientific and educational point of view.

1. Introduction

Cetaceans can be affected by many diseases and stranded individuals often represent a potential zoonotic risk for human health (Bogomolni et al., 2008; Geraci and Ridgway, 1991; Hunt et al., 2008). Effective management for their immediate disposal is fundamental to reduce the possibility of infection (Cioppi et al., 2014; Tucker et al., 2018). State maritime authorities, health and research institutions and even voluntary associations have to collaborate in following national and international guidelines (i.e. ACCOBAMS-MOP7/2019/Doc 33, 2019), managing the transport and the treatment of the carcasses in a cost effective way, mostly in the case of such huge animals (C.Re.Di.Ma, 2014). On the other hand, stranding events are an important source of information and biological samples (Peltier and Ridoux, 2015). In fact, their recording could provide data on species occurrence, distribution and population dynamics as well as on mortality rate induced by

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https://doi.org/10.1016/j.jembe.2020.151473
Received 24 May 2020; Received in revised form 24 August 2020; Accepted 6 October 2020
Available online 19 October 2020

natural causes or human activities such as intensive underwater noise, marine traffic and fishery (Peltier et al., 2012, 2019, 2020). Such data have a higher ecological relevance in the case of rare and elusive cetacean species whose occurrence and abundance is mostly inferred from stranding events. This is the case of the Cuvier's beaked whale (*Ziphius cavirostris*, Cuvier, 1823), whose short duration of surfacing behavior and the inconspicuous blows as well as dives of more than 60 min (Baird et al., 2006, 2008; Heyning, 1989a; Tyack et al., 2006), have made its field observation difficult in many Mediterranean areas. The occurrence of single or serial standings and the rise in survey effort, that started from 1960 (Podestà et al., 2006, 2016), have increased the knowledge on its presence in the Mediterranean Sea, both in the western (e.g. Arcangeli et al., 2016; Azzellino et al., 2012; Cañadas and Vázquez, 2014; Gannier and Epinat, 2008; Moulins et al., 2007) and in the eastern basins (e.g. Akkaya Bas et al., 2016; Frantzis, 2009; Gomerči et al., 2006; Holcer et al., 2007; Kerem et al., 2012; Öztürk et al., 2011). In this light, *Z. cavirostris* is currently considered a regular living Mediterranean species whose conservation status has only recently passed from Data Deficient (Cañadas, 2012) to Vulnerable (Cañadas and Notarbartolo di Sciara, 2018), according to the IUCN criteria adopted for the Mediterranean Sea. In particular, Cañadas et al. (2018) investigated the distribution and abundance of *Z. cavirostris* throughout the Mediterranean Sea, mainly by modeling its habitat preference using multiplatform and multiyear sighting data and physiographic variables. The Alborán Sea, the Hellenic Trench, the Southern Adriatic Sea, the Ligurian Sea and the Tyrrhenian Sea were indicated as high-density areas for the species. The Northern Ionian Sea has been ranked as an area hosting preferential habitats because of its regional eco-physiographic features and sightings of *Z. cavirostris* which occurred in the Greek Ionian Sea. Therefore, although information on the species has increased, there is still a lack of knowledge in some areas of the Mediterranean Sea and especially in the Gulf of Taranto (North-western Ionian Sea), which has already been shown to be a critical habitat suitable for different cetacean species such as the striped and common bottlenose dolphins, Risso's dolphin and sperm whale (Azzolin et al., 2020; Bellomo et al., 2019; Carlucci et al., 2016, 2018a, 2018b, 2020; Maglietta et al., 2018, 2020; Renò et al., 2018, 2019; Santacesaria et al., 2019). Although the Gulf of Taranto is characterized by terraces descending toward submarine canyons reaching depth over 1000 m (Pescatore and Senatore, 1986; Capezzuto et al., 2010; Carlucci et al., 2018c), no sightings of *Z. cavirostris* have been recorded so far in the basin. Only two individual strandings were reported from 1945 to 1981 in this basin (Maio et al., 2014). In addition in the same area, 13 other individuals were reported by the Italian Stranding Network database (http://mammiferimarini.unipv.it/) in 10 strandings, which occurred from 1992 to 2012. In particular, in 2011 an atypical mass stranding occurred in the Ionian Sea. It consisted of 10 individuals of *Z. cavirostris* stranded on the western coast of Corfù (Greece) and 2 along the coast of Crotone (Italy), most probably linked to an Italian Navy exercise (Mare Aperto/Amphex 2011) conducted in the Gulf of Taranto (Podestà et al., 2016; Bernaldo de Quirós et al., 2019). This latter event led Parties within ACCOBAMS to designate the Gulf of Taranto as an Area of Special Concern for Beaked Whales (ASC-BW) where mitigation actions to prevent noise impacts on cetacean species should be applied before, during and after activities emitting intense underwater noise (ACCOBAMS, 2013).

Although making inferences from strandings is problematic, due to the possible influence of different factors which can introduce bias (i.e. current movements and coastal topography), such events may offer the possibility to collect information about the pattern of distribution of species because they very often spatially match with sightings records (Maldini et al., 2005; Peltier et al., 2014). A stranding site has to be considered as being potentially close to the area where an individual suffered the stress that led it to strand. In the light of this, the individual stranding of *Z. cavirostris* that occurred in the Gulf of Taranto has been investigated by means of a Lagrangian model to investigate the transport of its body by surface currents and its possible geographical origin. Moreover, a necropsy was carried out to detect the possible cause of death, to provide the results of bacteriological and biomolecular analysis as well as those of plasma-mass spectrometry applied in order to detect traces of heavy metals. Finally, an osteological analysis together with the process of recovery and display of the skeleton in a museum form are detailed, highlighting the importance of these exhibits from a scientific and educational point of view.

2. Materials and methods

2.1. Finding of the carcass

A stranded individual of *Z. cavirostris* was found on 11 December 2019 on the northern coast of San Pietro Island, in the Chéradi Islands Archipelago in the Gulf of Taranto, Italy (40°27′25,2"N, 17°9′36″E) (Fig. 2). Since the finding site is within an Italian Navy base, in accordance with the local Authorities involved in the Italian Stranding Network, the carcass was harnessed and towed via sea to a more suitable area within the harbor of Taranto. The necropsy was carried out applying the best practice on cetacean post mortem investigation and tissue sampling (ACCOBAMS-MOP7/2019/Doc 33, 2019), collecting photo-video documentation and basic morphometric data.

2.2. Numerical models of currents and the Lagrangian drift model

The at-site sea origin of the living individual is one of the most difficult questions in the case of a cetacean stranding. The answer to this question depends on two factors: the state of decomposition of the carcass which is influenced by the time spent by the body at sea after death, and the drift conditions (direction and strength of sea currents and winds) over the study area (Peltier and Ridoux, 2015). In this case, the study perimeter considered to investigate the origin of this *Z. cavirostris* ranged from the Southern Adriatic Sea to the Northern Ionian Sea (Fig. 1), because both areas are quite close to the stranding site and they are defined as high-density areas for occurrence of this species in the Central Mediterranean Sea (Cañadas et al., 2018). A Lagrangian model was used to simulate the transport of the Cuvier's beaked whale body by surface currents to the stranding site according the meso-scale water masses circulation (Civitarese et al., 2010). In particular, hydrodynamic (HD) and particle tracking (PT) modules of MIKE 3 FM, produced by the Danish Hydraulic Institute (DHI, 2016) (see Appendix A for more details on the hydrodynamic model implemented), were used to evaluate how hydrodynamic parameters affect possible drifts of the carcass within the analyzed area testing, by a forward-tracking simulation, the release of 1000 virtual independent floating particles from 12 hypothetical sources chosen from the closest possible to the occurrence areas of Z *cavirostris* in the Central Mediterranean Sea, within the study perimeter (Fig. 1). No settling was considered in any of the simulations and therefore no vertical settling velocity was introduced.

Considering that the time at sea of the carcass after death, was estimated to be in the range between 15 and 30 days, the release of the virtual floating particles was tested for each source to 15 (26th November 2019), 20 (21th November 2019), 25 (16th November 2019) and 30 days prior to the finding and the forward trajectories of these objects were calculated until 11 December 2019 (finding date). Each floating particle was subjected to transport by the modeled currents as well as a randomly fluctuating velocity (in both direction and magnitude) derived from the random walk model. The sources resulting in a cloud of virtual particles far from the finding area of the stranded individual were excluded from further analysis, whilst a sensitivity analysis was carried out for the sources whose model simulations provided a compatible result. This analysis consisted of the comparison of simulations performed starting from 4 vertices of an area of 37 × 20 km^2 centered at each hypothetical source tested as release points of virtual particles and it aimed to verify their reliability and the stability.

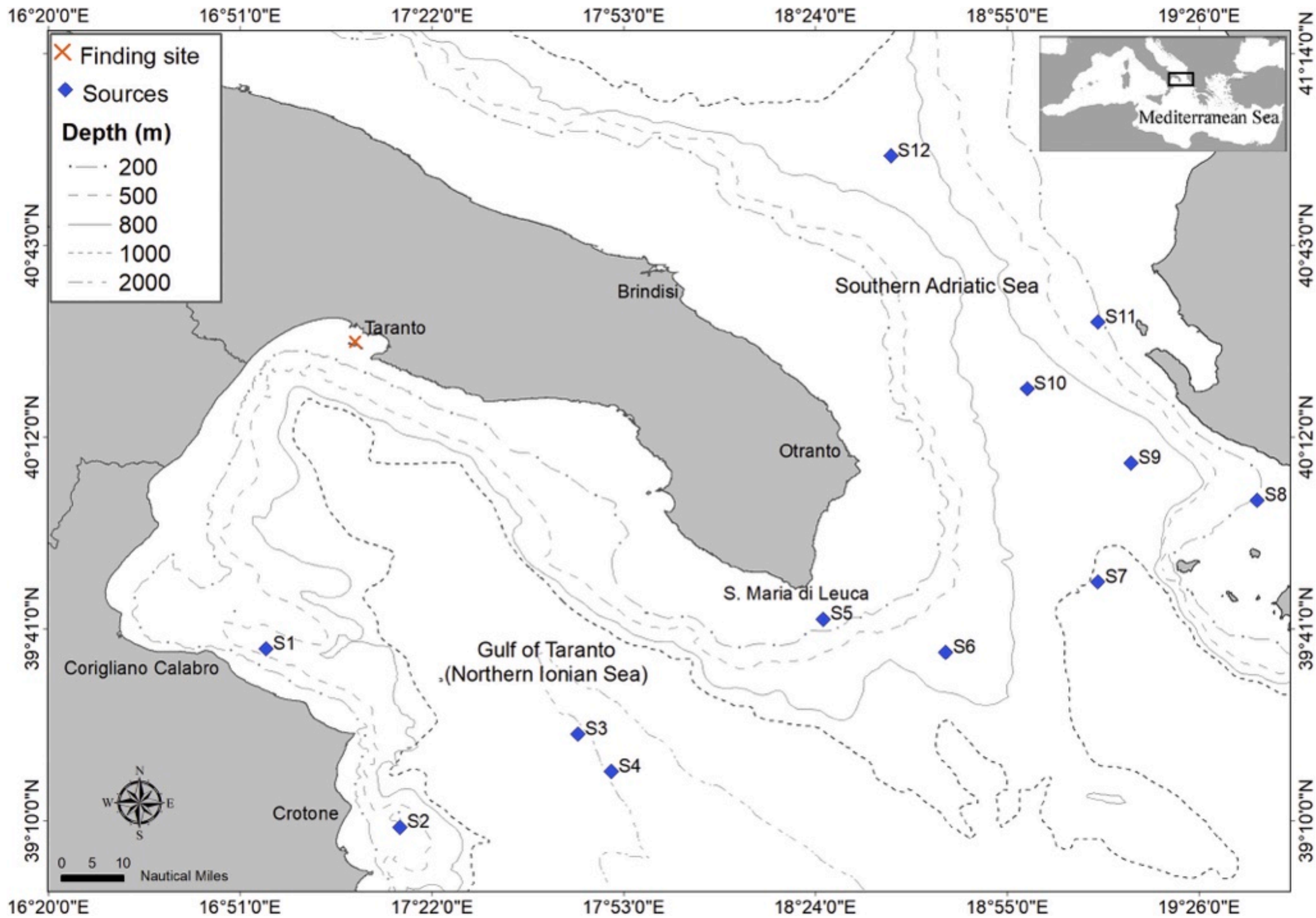


Fig. 1. Map of the study area with indication of finding site of *Z. cavirostris* and of the sources used in the simulation model.

2.3. Museum preparation process and osteological data collection

The preparation for museum display of a cetacean skeleton consists of a long and complex process starting from the disposal of the carcass, cleaning of the bones and assembly of the skeleton. On December 12th 2019 the disposal process of the carcass was carried out through a supervised offshore sinking to recover the skeleton for the subsequent processes. The carcass was towed to and sunk in the harbor of San Paolo Island, Archipelago of Chéradi Islands, in an isolated place that made it possible to remove and recover the skeleton. When the decomposition of the carcass was at an advanced stage and the bones were almost cleaned, the skeleton was recovered and moved to the "KETOS" Euro-Mediterranean Citizen Science center for the cleaning process of bones.

The bone cleaning phase is fundamental to remove the lipids and fatty acids from the bone matrix that tend to stain the bone brown and so whiten the skeleton. This was carried out according to an ad hoc protocol based on procedures reported in the literature (Rowley, 2015; Valle et al., 2016). The bones were boiled and then, soaked in a container with hydrogen peroxide 35% H_2O_2 (130Vol) to achieve complete whiteness of the bones. The bones bigger than the container were brushed, at least three times, with the hydrogen peroxide and the product was left on the bones for several hours. Although these procedures had a large risk of error by having organic matter first subject to 100 °C and then to a high concentration of H_2O_2 for extended periods of time, these processes effectively degrease and whiten the bones. In this case, the time spent for cleaning and whitening processes ranged from a minimum of 1 h to a maximum of 12 h, taking care to avoid damaging the bone tissue. It was necessary to repeat these phases up to three times to clean the skull completely. Finally, each bone was washed with a sodium hypochlorite solution (10% NaClO) and brushed hard to permanently remove the organic remains and to eliminate the odor from the bones. At the end of the cleaning phase, the dry bones were catalogued according to the literature (Andrews, 1908; Omura, 1972; Rommel et al., 2006), measured, weighed and photographed. Five bones were damaged during the recovery of the skeleton and the cleaning phase, and three were missing. All the damaged bones were repaired with epoxy resin to fix the broken pieces, whereas missing bones were recreated using 3D printing. For each missing bone, a 3D scan was realized by the photogrammetry of the homologous bone, using the open source software Meshroom (AliceVision - 3D Reconstruction Software). Finally, each bone was covered by an acrylic transparent varnish spray to preserve it from decay. Throughout the museum preparation process, the bones were on show to the public and a dedicated citizen science project was carried out with the aim of raising awareness of the conservation status of cetacean populations.

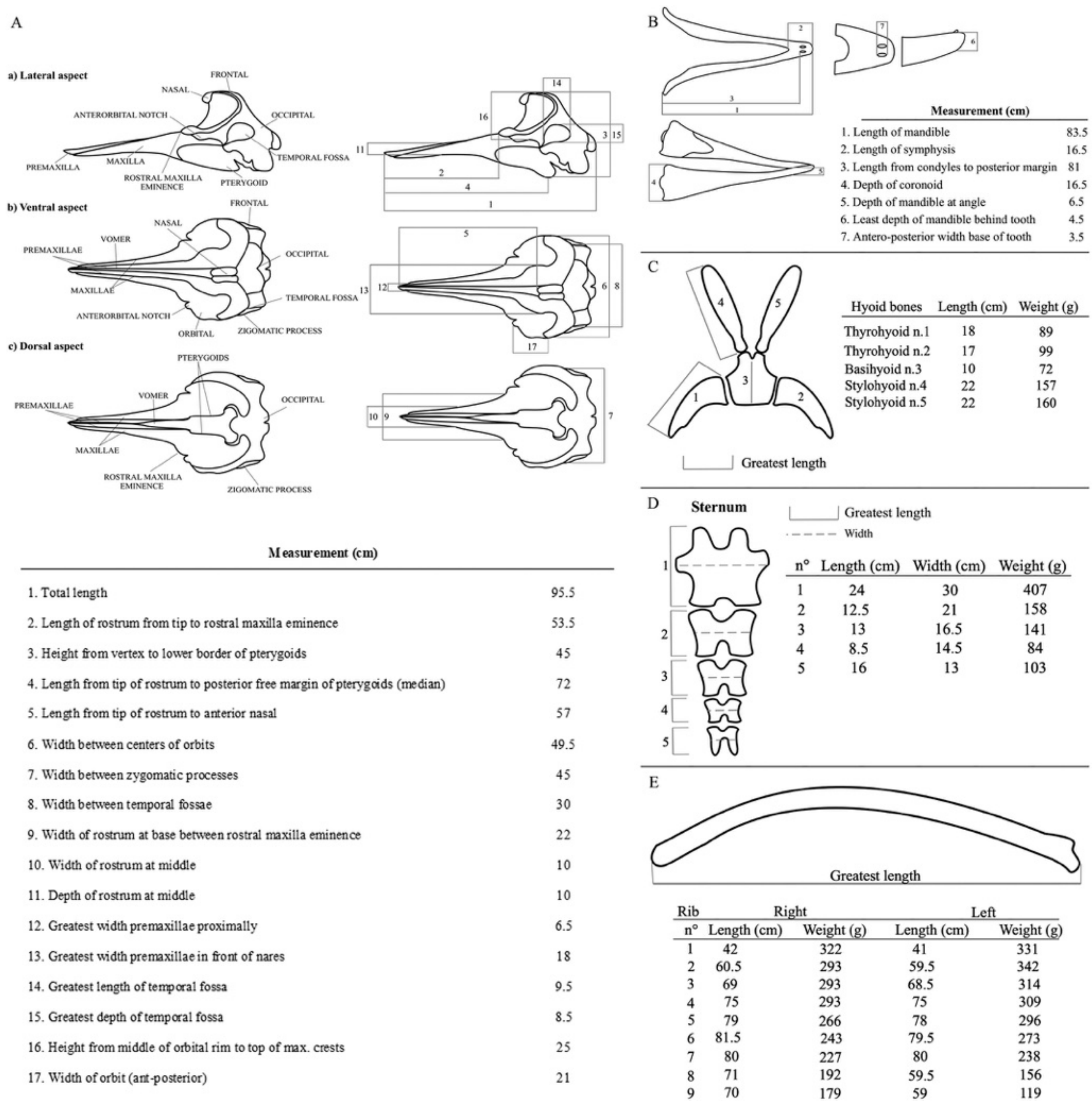
Osteological data are important indicators of several attributes of an individual such as the gender and the age (Cáceres-Saez et al., 2015; Guglielmini et al., 2002). *Z. cavirostris* shows a pronounced sexual dimorphism with mature males characterized by a deep excavation (prenarial basin) on the rostrum, a prominent mesorostral bone and the presence of two teeth that erupt from the gum (Heyning, 1989b; Moore, 1968; Omura, 1972; Rommel et al., 2006). The age of the individual can be estimated by the observation of sutures of the skull or by the quantity of the epiphyses fused to the centrum of each vertebra (Moore, 1968). The measurements of the skeleton can provide useful information on the species and lead to a comparative analysis between individuals from different regions.

In this study, all the bones of the stranded *Z. cavirostris* were examined, measured and weighted when possible. In particular, a suite of measurements was used to describe each part of the skeleton (Fig. 2), considering the data collected by Andrews (1908) as the main reference. Measurements were collected in a straight line parallel to the axis of each part of the bone. The width and height of centrum of vertebra was measured considering their front side.

3. Results

3.1. Necropsy of the carcass

The stranded individual of *Z. cavirostris* has been recorded with the ID number 13107 in the Italian Stranding Network database (http://mammiferimarini.unipv.it/). It was assessed as a female of 5.35 m in length and approximately 2000 kg. Basic morphometric data are reported in Fig. 3. The measurements of the girth at the cranial dorsal fin insertion and at the axillary pectoral flipper insertion were not collected due to the considerable size and the softening of tissues. The body was assessed to be in an advanced decomposition state (Decomposition Condition Category 4 (DCC), ACCOBAMS-MOP7/2019/Doc 33, 2019). During gross examination, despite the severe autolysis, massive parasitic cutaneous infestations associated with mineralized ulcerative findings were observed along with a reddish fluid in the main internal cavities. Furthermore, the aorta was severely affected by a diffuse, mural mineralization with bone metaplasia (also microscopically assessed) of the arterial wall, which had been deformed in its shape and



Measurement (cm) (A)

1. Total length	95.5
2. Length of rostrum from tip to rostral maxilla eminence	53.5
3. Height from vertex to lower border of pterygoids	45
4. Length from tip of rostrum to posterior free margin of pterygoids (median)	72
5. Length from tip of rostrum to anterior nasal	57
6. Width between centers of orbits	49.5
7. Width between zygomatic processes	45
8. Width between temporal fossae	30
9. Width of rostrum at base between rostral maxilla eminence	22
10. Width of rostrum at middle	10
11. Depth of rostrum at middle	10
12. Greatest width premaxillae proximally	6.5
13. Greatest width premaxillae in front of nares	18
14. Greatest length of temporal fossa	9.5
15. Greatest depth of temporal fossa	8.5
16. Height from middle of orbital rim to top of max. crests	25
17. Width of orbit (ant-posterior)	21

Measurement (cm) (B)

1. Length of mandible	83.5
2. Length of symphysis	16.5
3. Length from condyles to posterior margin	81
4. Depth of coronoid	16.5
5. Depth of mandible at angle	6.5
6. Least depth of mandible behind tooth	4.5
7. Antero-posterior width base of tooth	3.5

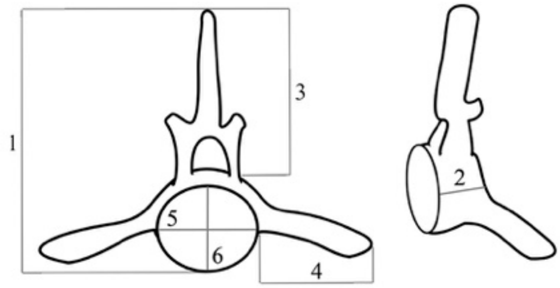
Hyoid bones	Length (cm)	Weight (g)
Thyrohyoid n.1	18	89
Thyrohyoid n.2	17	99
Basihyoid n.3	10	72
Stylohyoid n.4	22	157
Stylohyoid n.5	22	160

n°	Length (cm)	Width (cm)	Weight (g)
1	24	30	407
2	12.5	21	158
3	13	16.5	141
4	8.5	14.5	84
5	16	13	103

Rib	Right		Left	
n°	Length (cm)	Weight (g)	Length (cm)	Weight (g)
1	42	322	41	331
2	60.5	293	59.5	342
3	69	293	68.5	314
4	75	293	75	309
5	79	266	78	296
6	81.5	243	79.5	273
7	80	227	80	238
8	71	192	59.5	156
9	70	179	59	119

Fig. 2. Description and measurements collected for A) skull, B) lower jaw, C) hyoid, D) sternum, E) ribs, F) vertebrae, G) chevron and H) forelimb bones of individual of *Z. cavirostris* stranded in the Gulf of Taranto identified by the ID number 13107.

F

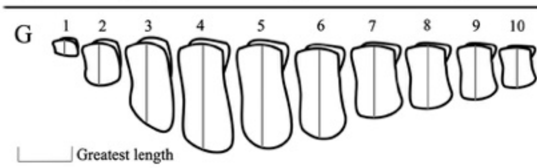


Measurement (cm)

1. Height (spine to edge of body)
2. Lenght of centrum (antero-posterior)
3. Height of spinous process
4. Length of transverse process
5. Width of centrum
6. Height of centrum

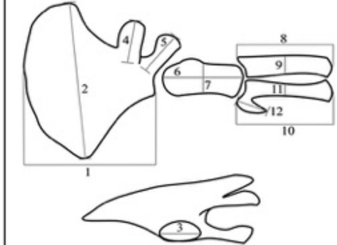
n°	Vertebrae	Weight (g)	Height (spine to edge of body) (cm)	Length of centrum (antero-posterior) (cm)	Height of spinous process (cm)	Length of transverse process (cm)	Width of centrum (cm)	Height of centrum (cm)
1-4	C.1-4	708	19.5	27	/	/	10	6.5
5	C.5	66	13.5	1	/	/	8	6.7
6	C.6	113	16	1.3	/	/	8.2	7
7	C.7	117	14.5	2	/	/	12	7.5
8	T.1	196	23	3	16	9	7	9
9	T.2	273	32.5	4	25	10	7	8
10	T.3	321	35	6	29	9	8	6
11	T.4	357	37	7	31	8.5	6	8
12	T.5	368	37	7	31	8.5	7.5	6.3
13	T.6	400	37.5	8	31	8.5	6	7.5
14	T.7	424	39	9.2	34	10.5	8	6.7
15	T.8	529	39	10.3	31	10	10.5	7.5
16	T.9	668	46	32	11	14	10	7.5
17	T.10	660	48	11.5	37.5	15	10	8.5
18	L. 1	728	44	12	30.5	14	10.5	10
19	L. 2	727	45.5	12	36	13	11	10.5
20	L. 3	749	47	12.3	36.5	15	11.2	11.5
21	L. 4	847	47	12.2	34.5	12.5	11	10
22	L. 5	916	48	13	36	14	11	10
23	L. 6	1046	49	13.5	37	12.5	13	11
24	L. 7	1108	49	14	38	12.5	12.5	11
25	L. 8	1146	51	14	38	11	12	12
26	L. 9	1290	51	15.3	38	12	11	12.5
27	Ca.1	1260	44.5	15.5	32.5	9	12	11.5
28	Ca.2	1378	44	16	31.5	9.5	12.5	12
29	Ca.3	1891	44	16	28.5	7.5	12.5	12
30	Ca.4	1932	39	16.5	25	4.5	15	15.5
31	Ca.5	1141	36	13.2	22	7	12	12
32	Ca.6	1088	31	13	18	6.5	12	12
33	Ca.7	1029	29	12.5	16	6.5	12	12
34	Ca.8	998	25	13	11.5	2	12	11
35	Ca.9	1071	24	13	6	/	15	14
36	Ca.10	885	20	10	4	/	10	9
37	Ca.11	529	16	9	3	/	9.5	8
38	Ca.12	299	8.5	5	/	/	9	8.5
39	Ca.13	162	7	4.5	/	/	8.5	7
40	Ca.14	88	6	4.5	/	/	7	6
41	Ca.15	69	7	4	/	/	6.5	7
42	Ca.16	46	5	4	/	/	5.5	5
43	Ca.17	24	3.8	2.5	/	/	4	2.5
44	Ca.18	12	4	3.3	/	/	2.5	4
45	Ca.19	5	2.8	2	/	/	3	2.8

G



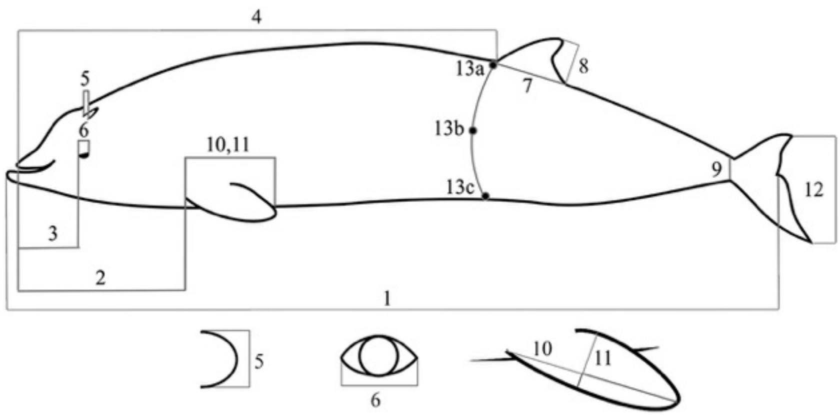
Chevron bones	Length (cm)	Weight (g)
n.1	2.5	8
n.2	8	30
n.3	16	75
n.4	20	158
n.5	19.2	134
n.6	16.2	146
n.7	13	120
n.8	12	105
n.9	10	63
n.10	7.5	40

H



Measurement forelimb bones (cm)	Right	Left
1. Length of scapula	27	26
2. Width of scapula (antero-posterior)	34.5	34.5
3. Width of glenoid fossa	7	7.5
4. Length of acromion	12	12
5. Length of coracoid	11.5	11.5
6. Lenght of humerus	17	16
7. Width of humerus	9	9.5
8. Length of radius	19.5	18.5
9. Width of radius	6	5
10. Lenght of ulna	20.5	20.5
11. Width of ulna	8	8
12. Lenght of olecranon	8.5	8

Fig. 2. (*continued*)



Measurement (cm)	
1. Total Length	535
2. Length from the mouth junction to the anterior insertion of the right pectoral flipper	135
3. Length from the anterior insertion of the eye to the mouth junction	75
4. Length from the mouth junction to the anterior insertion of the dorsal fin	330
5. Maximum length of the blowhole	14.5
6. Length of the ocular opening (eye)	5
7. Length of dorsal fin	40
8. Height of dorsal fin	30
9. Circumference at caudal peduncle	58
10. Length of pectoral flipper from the anterior insertion	60
11. Width of pectoral flipper	17
12. Width of fluke	135
13a. Measure of dorsal blubber thickness	3
13b. Measure of lateral blubber thickness	3.6
13c. Measure of ventral blubber thickness	3.8

Fig. 3. Morphometric data collected during the external analysis of the carcass of *Z. cavirostris* stranded in the Gulf of Taranto.

progress. Close inspection showed raised whitish hard plaques. No parasitic bodies were found in the vessel lumens and the absence of marine debris was noted along the entire digestive system. In detail, despite the poor preservation condition of the carcass, few samples of medulla oblongata, lung, liver, kidney, heart, muscular tissue, aorta and mesenteric arteries were collected for bacteriological and biomolecular analysis. Cultural examinations were carried out to investigate the presence of *Brucella* spp. (World Organisation for Animal Health, 2018), *Salmonella* spp. (UNI EN ISO 6579-1, 2017) and *Listeria* spp. (UNI EN ISO 11290-1, 2017). The Real Time PCR-restriction fragment length polymorphism (RT-PCR RFLP) technique was applied to detect Cetacean Morbillivirus (CeMV) (Verna et al., 2017), *Neospora caninum* and *Toxoplasma gondii* (De Craeye et al., 2011). Finally, hepatic, renal and muscular tissue samples were collected and analyzed using inductively coupled plasma-mass spectrometry in order to detect traces of heavy metals such as total arsenic, cadmium, mercury and lead (UNI EN 13805, 2014; UNI EN 15763:, 2010).

As a result of all these analysis, no bacterial infections by *Brucella* spp., *Salmonella* spp. or *Listeria* spp. were detected. On the contrary, biomolecular examinations confirmed the presence of Cetacean Morbillivirus in the myocardium and lung as well as *T. gondii* in the myocardium, lungs and kidney supporting a possible ongoing condition. Microscopic examination of arterial walls confirmed a chronic, mural fibrocalcific arteritis affecting the entire thickness of the examined aorta. Finally, traces of arsenic, cadmium and mercury were found in samples of liver, kidney and muscle tissue (Table 1).

3.2. Numerical models of currents and the Lagrangian drift model

The model of currents, implemented using data from the EU Copernicus Marine Service (https://marine.copernicus.eu/) (Appendix A), showed a strong eastern surface coastal current incoming from the South Adriatic Sea into the Gulf of Taranto in the Northern Ionian Sea during the investigated period. In particular, the surface current flowing into the Gulf of Taranto fed a cyclonic vortex (Fig. 4). Simulations performed at 15, 20, 25 and 30 days highlighted that only sources S3 and S9 proved plausible among those investigated, because the trajectories of the virtual particles reached the finding area of the stranded individual of *Z. cavirostris* (Fig. 5 and Fig. 6). Thereafter, the sensitivity analysis was only carried out for these two remaining sources at each temporal scale and showed both independence and stability of simulations ran from the vertices of the S3 source (see Fig.

Table 1
Concentrations of total arsenic, cadmium, mercury and lead recorded in the liver, kidney and muscle samples collected from the individual of *Z. cavirostris* stranded in the Gulf of Taranto.

Tissue	As (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
Liver	1.22	6.13	474.8	< LOQ
Kidney	0.52	20.4	33.1	< LOQ
Muscle	2.01	0.043	29.3	< LOQ

< LOQ indicates that the value is below the Limit of Quantification (LOQ = 0.012).

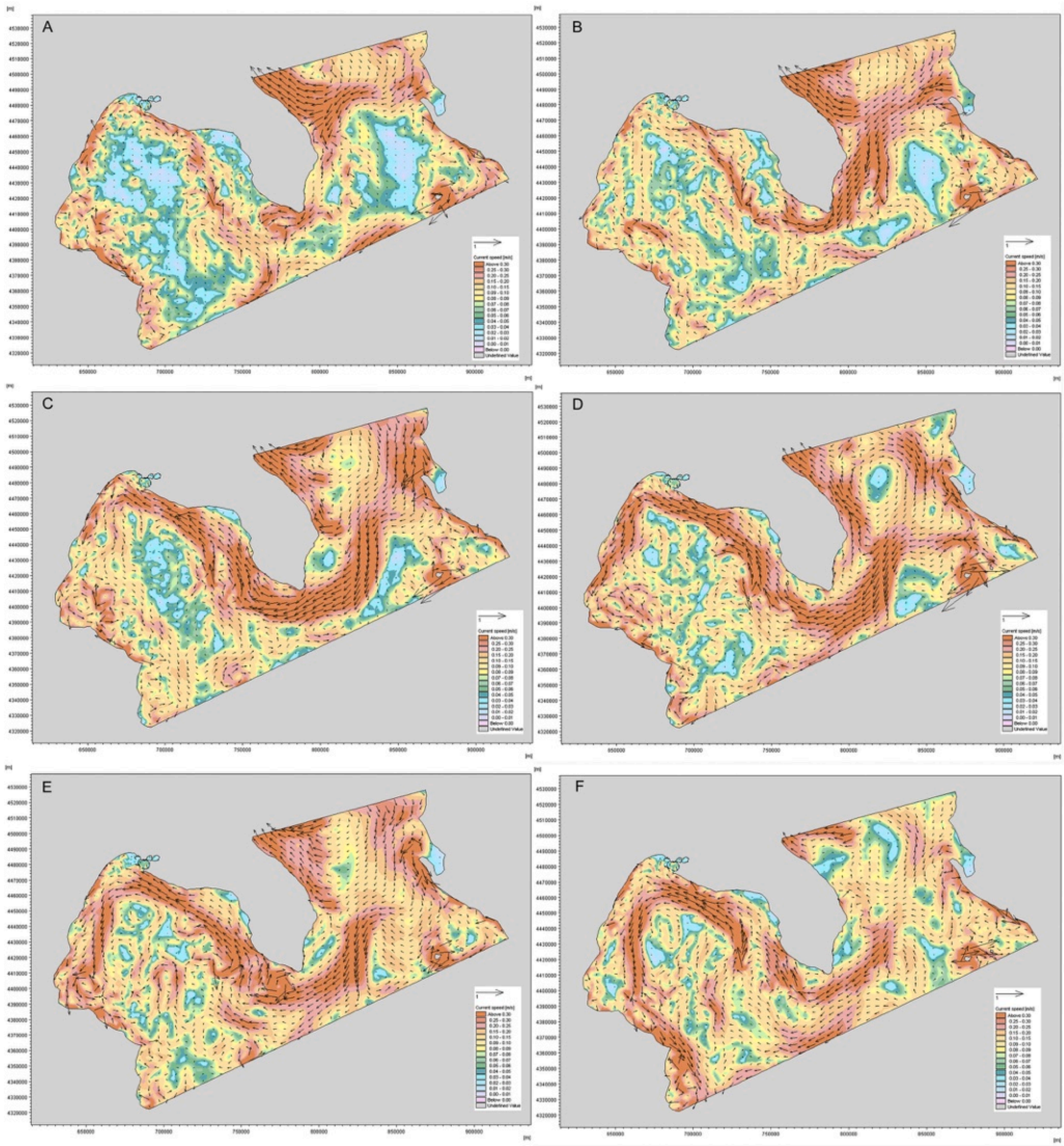


Fig. 4. Surface current field from MIKE 3 FM HD: a) 11 November 2019 at 12:00; (b) 16 November 2019; (c) 21 November 2019; d) 26 November 2019; e) 1 December 2019; f) 11 December 2019.

B1-B5 in Appendix B). The probability P(x) that the body was transported near the stranding site from source S3 with a release of particles at 15, 20, 25 and 30 days prior to the finding date were equal to 72%, 60%, 50% and 28%, respectively (Fig. 7).

3.3. Museum preparation process and osteological data collection

The museum preparation process lasted 3 months. The first month and a half involved the at-sea disposal of the carcass in its supervised offshore sinking area. In particular after one month, an inspection of the carcass was carried out by scuba divers to assess its state of decomposition and part of the skeleton (lower jaw, arms, ribs, cervical, thoracic and lumbar vertebrae) was recovered. After two weeks, the rest of the skeleton was also recovered and the whole re-assembly was completed. One months was required for the cleaning phase, in which three weeks were used to completely clean the skull of blood, lipids and fatty acids. Finally, two weeks where used to assemble the skeleton for its display in KETOS museum.

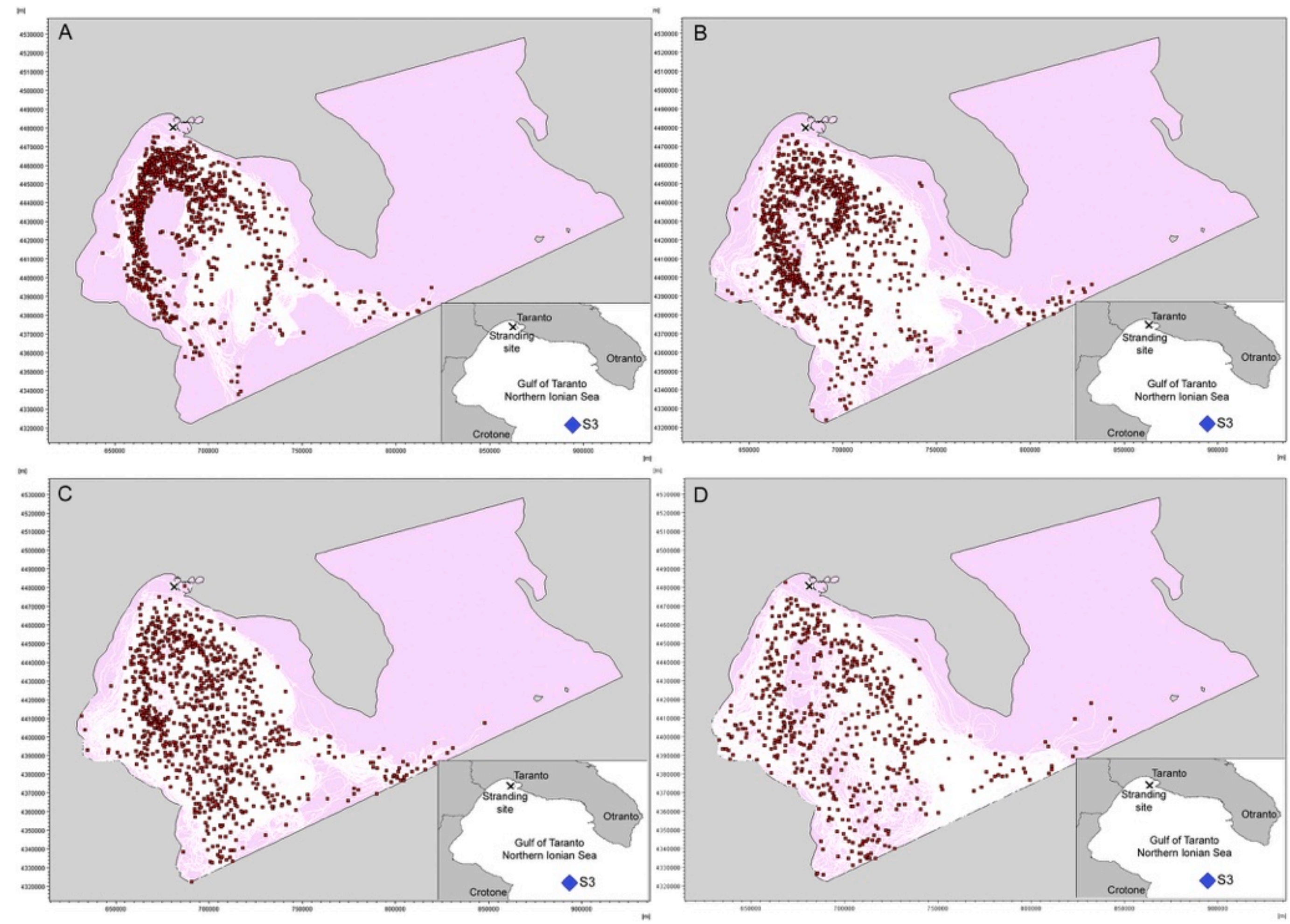


Fig. 5. The cloud of virtual particles (1000 in number) released from S3 a) 15 days, b), 20 days, c) 25 days and d) 30 days prior to the finding date of stranding on 11 December 2019.

The skeleton consisted of 138 bones collected and catalogued: skull, jaw, 5 hyoid bones, 18 ribs, 5 sternum bones, 2 scapulae, 2 humerus, 2 ulnae, 2 radii, 12 carpal bones, 10 metacarpal bones, 23 phalanges, 45 vertebra and 10 chevron bones. Only three phalanges of the left arm were missing after the whale had been removed from the disposal area.

The skull is 95.5 cm long and weighs 13 kg, and all the sutures are closed (Fig. 2A). The mesorostral bone is developed but it does not rise beyond the level of the pre-maxillaries. Moreover, no prenarial basin is developed. The mandible is 83.5 cm long and weighs 1940 g (Fig. 2B). Rami of the mandible are completely anchylosed together at symphysis. Each ramus is characterized by a hallow channel, which contained acoustic fat. Two small and cylindrical teeth not erupted from the gum are present on the jaw tip.

There are five hyoid bones (Fig. 2C). The two thyrohyoid (n°1–2) bones are not anchylosed with the basihyoid (n°3), whereas this latter, characterized by a trapezoidal form with two prominent conical notches on the anterior border is connected to two cylindrical stylohyoid (n°4–5).

The sternum consists of five segments connected by cartilage (Fig. 2D). The former is butterfly shaped and it is wider than its length. The latter segment is characterized by two elongated pieces separated throughout. There are nine paired ribs (Fig. 2E). From the 1st to the 7th, the ribs are double-headed and are articulated to the centrum of the vertebrae. The single head ribs are articulated with the transverse process of their respective vertebrae. The first ribs show a well-defined articular surface, which is compressed laterally and the broadest and shortest in the series.

The number of vertebrae in individuals of *Z. cavirostris* ranges from 42 to 49 and the species vertebral formula is C. 7, T. 9–11, L. 7–9, Ca. 19–22, according to Rommel et al. (2006). The vertebral formula in the case of this stranded individual is C. 7, T. 10, L. 9, Ca. 19 = 45 (all the measurements are shown in Fig. 2F). The first four cervical vertebrae are ankylosed by both their centra and neural arches, forming a solid bone as already seen for this species (Rommel et al., 2006). In addition, the fifth and the sixth vertebrae are only ankylosed by at their arches, whereas the centra was free. The 8th vertebra (1st thoracic) is the first with a spinous process (or neural spine) projected forward and acuminate, all the other spinous processes are inclined backward and obliquely truncated at the apices. The 35th vertebra (9th caudal) is the former without a transverse process, the 37th vertebra (11th caudal) is the first with arterial foramen and the 38th vertebra (12th caudal) is the first without a spinous process. The epiphyses of all vertebrae are fused to the centra and their sutures are obliterated. Furthermore, the posterior part of the 29th vertebra (3rd caudal) and anterior part of the 30th vertebra (4th caudal) are irregularly developed and ankylosed together at the center of the vertebral body (Fig. 8). The same abnormality was also found on the 35th vertebra (9th caudal) and 36th vertebra (10th caudal), over which an irregular pit is also present (Fig. 8). The neural canals are the dorsal, vertebral bony channels extending from the base of the skull to the tail, in which the spinal cord and associated blood vessels are contained. In deep diving cetaceans, such as *Z. cavirostris*, the ventral retia mirabilia of the caudal region are contained in the bony channel formed by the chevron bones which are located on the ventral aspect of the spinal column (Rommel et al., 2006;

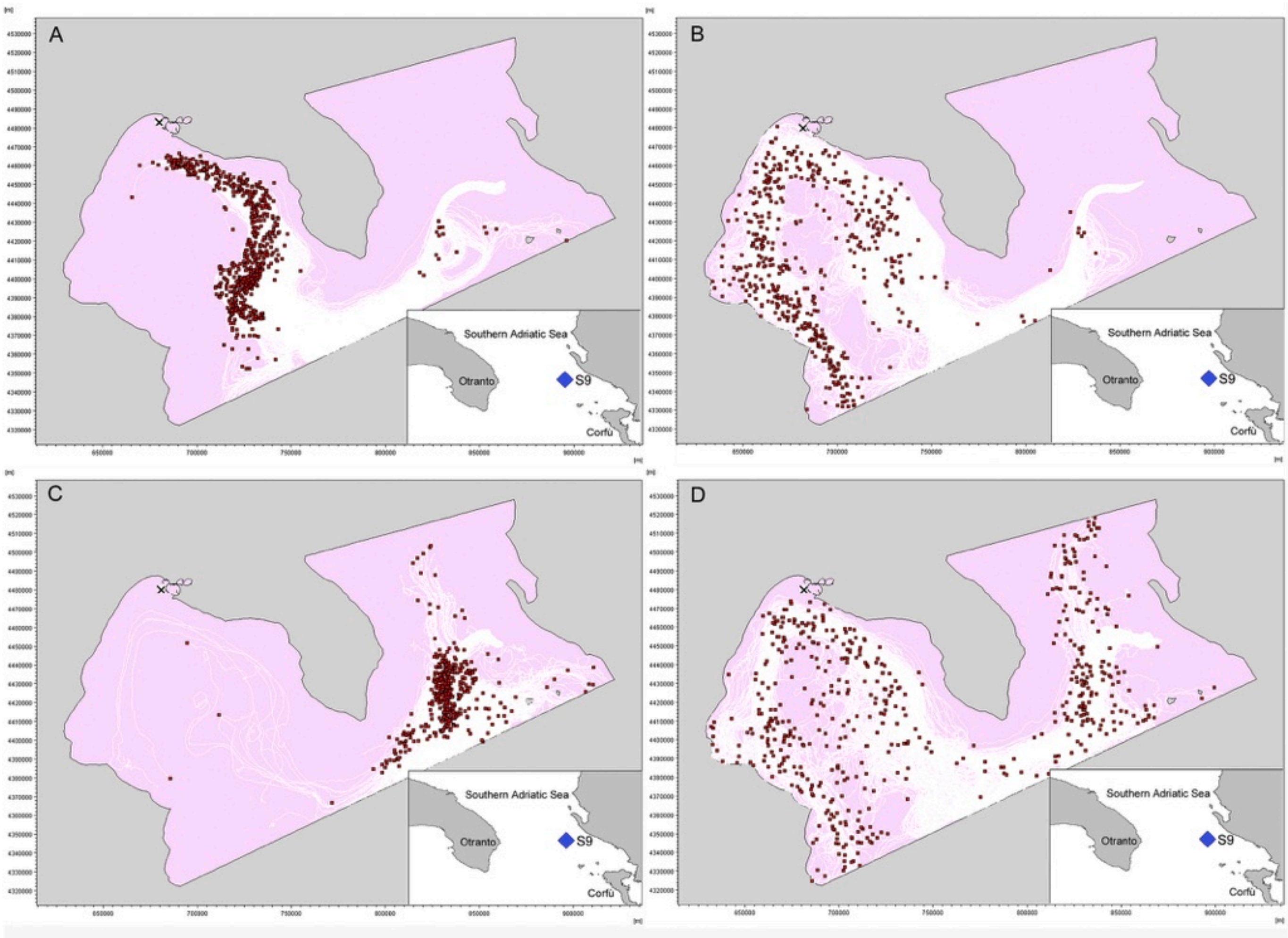


Fig. 6. The cloud of virtual particles (1000 in number) released from S9 a) 15 days, b) 20 days, c) 25 days and d) 30 days prior to the finding date of stranding on 11 December 2019.

Cozzi et al., 2016). From the 28th vertebra (2nd caudal) the inferior median carina is divided posteriorly to form articular facets for the attachment of the chevron bones.

There are 10 chevron bones (Fig. 2G). The 1st chevron bone is the smallest and consists of a pair of wing-like laminae, acuminate and free distally. The laminae forming the 2nd chevron bone are joined at their truncated distal ends but they have no spine. From the 3rd chevron bone, the spine becomes longer up to a maximum length of 20 cm.

Finally, measurements of the forelimb bones are reported in Fig. 2H. The scapula is fan-shaped and it weighs 346 g. The acromion is broad, laterally compressed, concave on its inner side and protrudes strongly upward. Its superior margin overlaps the coracoid border. The coracoid is shorter and narrower than the acromion, with an expanded tip. The humerus, that weighs 306 g, is short and thick with a proximal extremity divided into a head and external tuberosity, separated by a well-marked bicipital groove. The radius is straight and slightly concave internally. It is obliquely truncated to the proximal extremity and it weighs 149 g. The ulna is sickle-shaped due to the presence of a prominent olecranon and it weighs 81 g. The carpus is composed of six bones distributed in two rows. One small pyramidal-shaped ossification was found in the carpus, but no reference on this bone can be found in the literature. There are five metacarpal bones and the formula for the phalanges is: I_0, II_4, III_4, IV_3, V_2.

4. Discussion

Determining the cause of death in a stranded cetacean is a highly complex procedure in most instances. Extensive pathological analyses are required but the logistics and decomposition of the carcass at the time of the necropsy may preclude pathological examinations. However, despite its advanced decomposition state, an inherent serious pathological condition was revealed in the Cuvier's beaked whale during its post mortem and laboratory examinations. The aorta was severely affected by a diffuse, mural fibrocalcific arteritis with bone metaplasia of the arterial wall deforming its shape and progress. Neither parasitic bodies in the vessel lumens nor bacterial infection were detected. Biomolecular examinations confirmed the presence of Cetacean Morbillivirus (CeMV) in the myocardium and lung, adding further evidence that Ziphiidae are susceptible to morbillivirus infection like other cetacean species (Di Guardo et al., 2013; Mazzariol et al., 2012; Van Bressem et al., 2014). The presence of *T. gondii* in the myocardium, lungs and kidney support the hypothesis of a disseminated protozoan infection, as reported in other CeMV infected cetaceans (Obusan et al., 2019). Traces of total mercury, cadmium and arsenic were also found in the liver, kidney and muscular tissue indicating a possible metal contamination lightly affecting the stranded individual. However due to the decomposition state of the carcass,

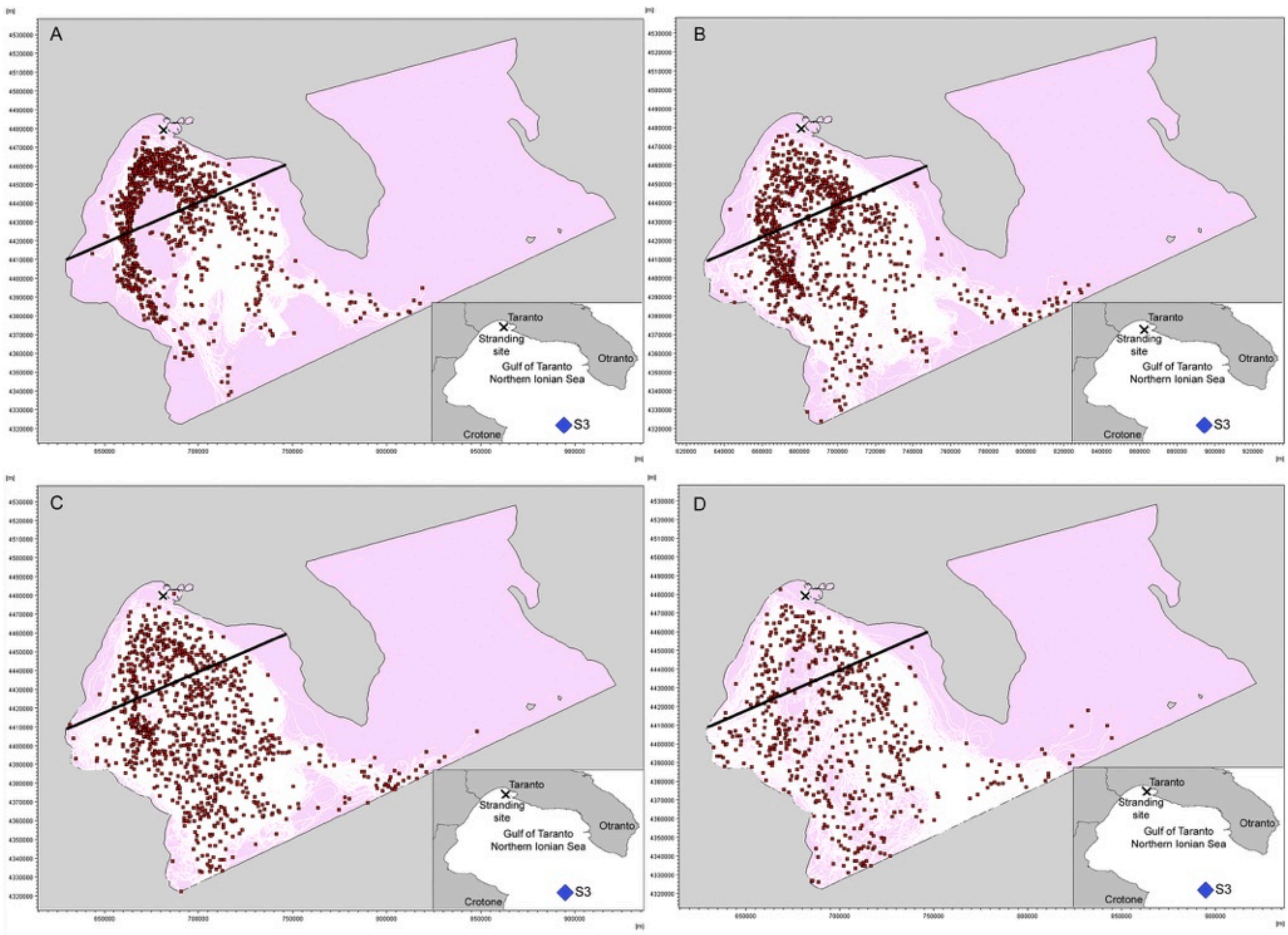


Fig. 7. The cloud of virtual particles (1000 in number) released from S3 a) 15 days, b) 20 days, c) 25 days and d) 30 days prior to the finding date, with indication of area, delimited by a straight dark line, defined for the calculation of probability P(x).

caution is required in any further considerations on this latter result. Much of the pathological evidence associated to *Z. cavirostris* is linked to the description of gas and fat embolic syndrome associated with sonar military exercises or traumatic conditions (Fernández et al., 2005; Bernaldo de Quirós et al., 2019). However, the inflammatory changes associated with mineralization and bone metaplasia are also frequently encountered findings that make a whale sick and weaken it. In fact, as indicated by Diaz-Delgado et al. (2016), a fibrosing arteritis with many degenerative associated changes also including evidence of mineralized tissue in the mesenteric arteries and several aortic tracts was reported to be the leading cause of a Cuvier's beaked whale stranding in the Canary Islands (Eastern Atlantic). In particular, this study reported that arteritis and changes in the vascular dynamics were associated with vascular *Crassicauda* spp. infection, as similarly observed in 2015 and 2017 in two different individuals of *Z. cavirostris* stranded in the Tyrrhenian Sea (Western-central Mediterranean Sea), in which arteritis was coupled with a CeMV infection (Centelleghe et al., 2017; C.Re.Di.Ma, 2017; Mazzariol personal communication).

The approach adopted to investigate the at-site sea origin of the living whale before its stranding consisted of the implementation of a Lagrangian drift prediction model simulating the carcass drift trajectories. Modeling considered the drift duration, as retrospectively estimated by the decomposition state of the animal, the hydrodynamic model based on the surface currents as well as the direct wind drag (tangential shear stress) and form drag (pressure differences across the item), or the 'sail' effect (a force at an oblique angle to the wind direction). Since the forward-tracking in time simulation may not be a sensitive method compared to the back-tracking one (Durgadoo et al., 2019), the reliability of the sources with trajectories compatible with the stranding site was assessed through a sensitivity analysis. Remarkably, the sensitivity analysis excluded the Southern Adriatic Sea as an origin area for the stranded individual and alternatively indicated the southernmost portion of the Gulf of Taranto in the Northern Ionian Sea as the most plausible death location and origin of the Cuvier's beaked whale. In addition, consistently with deductions from the necropsy analysis, it was also possible to confirm with a probability ranging from 60 to 72% that the death of the individual plausibly occurred between 15 and 20 days before the carcass finding date.

To the best of our knowledge, this is the first attempt at application of an oceanographic modeling approach to identify the origin source at sea of a cetacean stranded in the Mediterranean Sea. This innovative methodology is mostly derived from the strategies adopted for simulating oil spills (e.g. De Carolis et al., 2013; De Padova et al., 2017) or marine debris drifts (e.g. Durgadoo et al., 2019) and is comparable with similar studies applied on the stranding dynamic of sea turtles (Santos et al., 2018) and cetacean species carried out in the North Atlantic on *Delphinus delphis* (Peltier et al., 2012, 2016) and *Phocoena phocoena* (Peltier et al., 2013).

This finding highlights that the Gulf of Taranto where depths exceed 1000 m could be considered an important habitat for *Z. cavirostris* as has already been demonstrated for *G. griseus* and *P. macrocephalus* (Bellomo et al., 2019; Carlucci et al., 2020; Maglietta et al., 2018). This

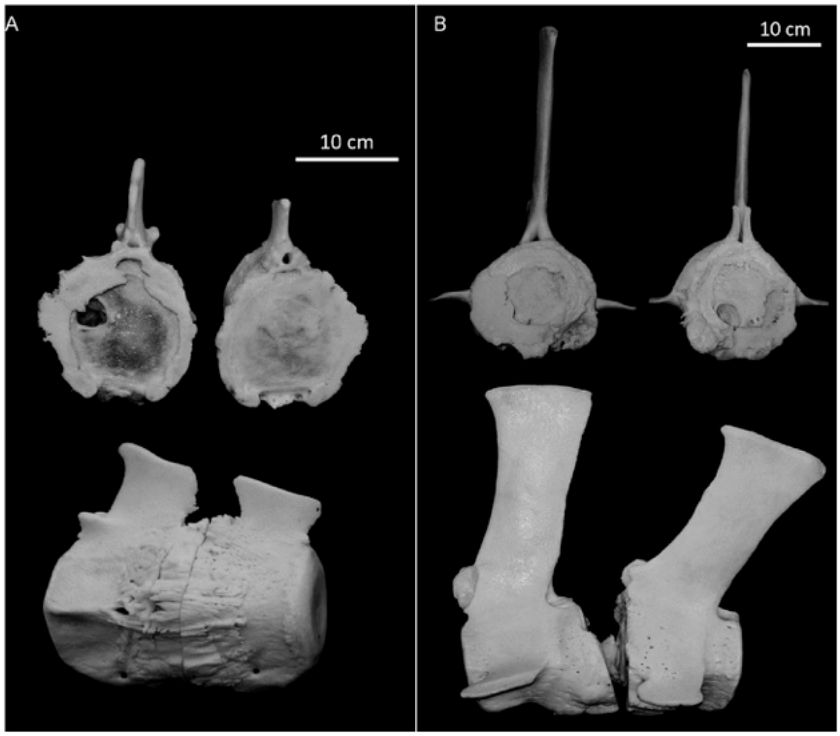


Fig. 8. Two pairs of irregular developed and ankylosed vertebrae as observed in the individual of *Z. cavirostris* stranded in 2019. In a) 29thand 30th vertebra, in b) 35th and 36th vertebra. The posterior part of the first vertebra was fused with the anterior part of the consecutive vertebra.

reinforces the indication provided by ACCOBAMS (2013) of the need for mitigation action to prevent noise impacts on cetacean species (Gordon et al., 2003 and references therein), mostly in the case of marine oil and gas exploration in the area (http://www.va.minambiente.it; Monaco et al., 2016).

The sinking disposal of the carcass in a restricted-access location proved to be the most natural cost effective method providing no impact on human health, fisheries resources, or marine ecosystems. Despite the slow-down of biological activity related to cooler temperatures during winter, the soft tissue decomposition only took one and a half months, making it a good time-saving technique compared with other methods such as the burial of the carcass (Valle et al., 2016). In addition, this method allowed a mostly cleaned skeleton to be recovered at the end of the decomposition process.

The museum preparation process described in this study represents an example of effective collaboration between research institutes and local authorities. The stranding of a cetacean, such as the individual of *Z. cavirostris* stranded in the Gulf of Taranto, is usually deemed as a problem for the disposal. In this case, the postmortem procedures and investigations carried out with a multidisciplinary approach turned this event into an opportunity to gain data on this species, to organize an osteological collection available for comparison of individuals of different sex, age and from different areas (Gomerči et al., 2006). Moreover, the opportunity offered to the general public to observe the skeleton is important, making it possible for them to learn about the anatomy of cetaceans as well as their evolution and conservation status on the regional and global scales.

The osteological data collected from the skeleton confirmed that the stranded whale was an adult female. In fact, the closure of the skull sutures, the complete ankylosis of epiphyses and the vertebral bodies as well as the development of mesorostral bone indicate an adult individual. The presence of two conical teeth covered by the gum and the lack of the prenarial basin overlap with analogous descriptions of female individuals (Omura, 1972) (Fig. 9). Concerning the abnormality observed on the 9th and 10th caudal vertebra, similar degenerative findings are frequent in cetaceans, especially in the lumbar and caudal

Fig. 9. Comparison between two skulls of *Z. cavirostris* stranded in 2019 and 1945 in the Gulf of Taranto. The skull of the adult male (on the right) shows the prenarial basin which is lacking in the adult female (on the left).

region of the vertebral column and are often associated with advanced age (Alexander et al., 1989; Cozzi et al., 2009; Prescher, 1998; Sweeny et al., 2005). However, despite the possible associated rigidity and impaired swimming, this condition has never been associated with a stranding event.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2020.151473.

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