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First stranding event of a common minke whale calf, *Balaenoptera acutorostrata* Lacépède, 1804, reported in Spanish Mediterranean waters

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At least six mysticete species have been reported to date in the Mediterranean Sea (Notarbartolo di Sciara and Birkun 2010). The fin whale, *Balaenoptera physalus*, is the only mysticete species with a stable population in the Mediterranean Sea, which differs genetically from Atlantic populations (Giménez et al. 2013 and references therein). Other mysticete species have been previously recorded as either sighted or stranded in this area (Notarbartolo di Sciara and Birkun 2010). The humpback whale, Megaptera novaeangliae, and the common minke whale, B. acutorostrata, are considered rare visitors to the Mediterranean, with less than a one sighting or stranded record per species, per year (IUCN 2012). The sei whale, B. borealis, the North Atlantic right whale, Eubalaena glacialis, and the gray whale, Eschrichtius robustus, are less common and considered to be vagrant species in the Mediterranean Sea (Notarbartolo di Sciara and Birkun 2010). A few individuals of these species have been reported in the western Mediterranean, and they presumably came from North Atlantic populations which entered the Mediterranean waters via the Strait of Gibraltar (IUCN 2012).

The common minke whale, *B. acutorostrata*, is the smallest species of the family Balaenopteridae, occurs regularly in coastal and offshore waters worldwide, and feeds mainly on krill, copepods and small schools of fish (Pierce et al. 2004). Two subspecies are recognised, the North Atlantic minke whale, *B. a. acutorostrata*, and the North Pacific minke whale, *B. a. scammoni*. Another unnamed subspecies was found in the Southern Hemisphere, considered a dwarf form that comes genetically closer to the North Atlantic minke whale (Rice 1998; Pastene 2006).

Common minke whales from North Atlantic populations are born with an average body length of between 2.4 m and 2.8 m. After the lactation period, which lasts between four and six months, calves are weaned when their body size reaches between 4.3 m and 5.5 m. Sexual maturity is attained when total body length of males and females reaches 8 m and 8.6 m, respectively (Van Waerebeek et al. 1999; Harms et al. 2008; Perrin and Brownell 2009; Christiansen et al. 2014).

We report a stranding event of a common minke whale calf, *B. acutorostrata*, in the Spanish Mediterranean for the first time. We used different morphological traits to determine the age of the individual, i.e., total body length, closure of the cranial sutures and ossification degree of the pectoral flipper. This is new information that extends previous knowledge on the common minke whale in this area and raises questions about the use of western Mediterranean waters by this species.

Methods

On 28 April 2014, a dead small whale was found stranded at Santa Pola, Alicante, (eastern Spain, 38°12'N–0°34'W) (Fig. 1). The specimen was transported to an open municipal site to be examined where the carcass condition was scored according to the criteria proposed by Geraci and Lounsbury (2005) and Pugliares et al. (2007). A necropsy was done by personnel from the University of Valencia and the Oceanografic of Valencia, following the protocol of the marine mammal and marine turtle stranding network of the Valencian Community (Gozalbes et al. 2010).

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Fig. 1. Stranding and sighting events of common minke whale *Balaenoptera acutorostrata* in the Spanish Mediterranean taken from Notarbartolo di Sciara and Birkun (2010) and unpublished sources (Rosa Canales, Cetáceos y Navegación and Programa de Gestión Sostenible del Medio Marino Andaluz de la Consejería de Medio Ambiente y Ordenación del Territorio, personal communication, June 25, 2014). A triangle indicates the present report on the Santa Pola coast (Valencian community). Small stars represent stranding events of common minke whales of around 4–4.5 m in length (except for the one in 2014, which was 3 m length), whereas large stars represent sighting events of adult common minke whales.

External and internal gross pathology inspections were made in situ. Tissues from the skin, blubber, muscle, liver, heart, brain, kidney and ovaries were collected for further genetic, pollutant and limited histological analyses. All the collected samples were either frozen or preserved in 10% formalin. The stomach, liver, intestine, kidneys and lungs were examined for parasites. Stomach contents were collected and preserved in 70% ethanol to be analysed in the laboratory. The skull was also preserved to determine the degree of bone ossification. Diagnostic imaging of the left pectoral flipper was done by radiographic evaluation. Radiographs were taken with a Philips Practix 400 unit (Philips Medical Systems) and a Kodak Direct view Classic CR System (Carestream Health), using 35×43 cm Kodak cassettes (Kodak PQ Storage Phosphor Screen, Carestream Health) in a dorsal-ventral projection. Focal distance was 1 m and exposure values were 60 Kv and 5.2 mAs. The flipper was placed directly on the film cassette to avoid magnification. The digital image was processed afterwards with the Kodak Acquisition Software (Onyx-RAD Diagnostic Viewer). The degree of epiphyseal ossification (i.e., epiphyses and their fusion to the diaphysis) was graded according to Ogden et al. (1981), as follows: Stage 0 = no secondary ossification

centre present; stage 1 = secondary ossification present, but occupies < 50% of the width of the adjacent bone; stage 2 = secondary ossification that occupies > 50% of the adjacent bone; stage 3 = distance between the bone epiphysis and metaphysis begins to reduce; stage 4 = physis begins to close; stage 5 = physis is completely closed; stage 6 = the physeal region is replaced with mature bone tissue.

Small parts of a gelatinous mass, found in the stomach, were used for molecular analyses, as it was not possible to identify them morphologically. Genomic DNA was extracted using a standard DNA extraction method. Partial 28S rDNA and partial mitochondrial cytochrome c-oxidase subunit I (COI) sequences were amplified through polymerase chain reaction (PCR). Contiguous sequences were assembled and sequence identities were checked using the Basic Local Alignment Search Tool (BLAST) available from GenBank (Benson et al. 2005).

Results

Using the decomposition code system in stranded animals, the carcass of the animal found was categorised as code 4: advanced decomposition (Geraci and Lounsbury 2005). Skin cracked and sloughing, characteristic strong odour, soft muscles, viscera often identifiable, and a gasfilled gut, were just some of the characteristics found in the carcass that corresponded to this code level (Fig. 2a). The condition that the carcass was in allowed us limited examinations and samples were collected only from skin, blubber, muscle, liver, heart, brain, kidney and ovaries. According to air temperature records (ca. 26°C), it was estimated that the animal died a few days before it was found. Total body length (from snout to fluke notch) was 300 cm; as from the snout to the tip of the dorsal fin was 206 cm and from the snout to the anterior insertion of flipper was 94 cm. Measurements of the left pectoral flipper, dorsal flipper and fluke, expressed as maximum length \times maximum width, were 44 cm \times 17 cm, 16 cm \times 15 cm, and 22 cm × 81 cm, respectively. Measurements of the blubber and skin thickness were 2 cm and 0.1 cm respectively; these were taken from the median point of the girth-line immediately behind the tip of the pectoral flipper.

The specimen was identified as a female common minke whale, *Balaenoptera acutorostrata*, based on body size, sharply pointed rostrum and presence of a white band across each flipper, all of which are diagnostic traits of individuals from northern populations of this species



Fig. 2. Images of the stranded common minke whale calf, *Balaenoptera acutorostrata*, on the Santa Pola coast (Valencian Community). (a) Complete animal, (b) left pectoral flipper; black arrow indicates the white band across the flipper (c) X-ray of the left pectoral flipper showing the carpal and elbow area; white and black arrows indicate incompletely and non-ossified epiphyses, respectively, (d) cranium, dorsal view and (e) cranium, posterior view showing incomplete ossified cranial sutures. Scale bars indicate 1 m (a) and 10 cm (b, d, e).

(Cawardine 1995). Total body length was 3 m, which is indicative of a nursing calf (Van Waerebeek et al. 1999; Christiansen et al. 2014). In our specimen, and based on the observations made on the degree of joint suture given by Walsh and Berta (2011) for baleen whales, the basioccipital-exoccipital and the supraoccipitalexoccipital cranial sutures were partially closed (Fig. 2e). An X-ray done on the left pectoral flipper revealed an image where the distal epiphyseal secondary nucleus of ossification of the radius and ulna in the carpal region was not yet present; thus stage 0 was considered according to the scale for epiphyseal ossification by Ogden et al. (1981). In contrast, late stage 1 or early stage 2 was considered for the proximal epiphyses secondary nucleus of ulna ossification. The primary ossification nucleus of five carpals was starting to mineralise (Fig. 2c).

The external examination did not reveal an emaciated body condition, and no evident lesions or injuries related to disease or anthropogenic interaction. The umbilicus was observed as being completely closed and apparently well epithelised, and no evidence of the meconium was found. The internal examination revealed no macroscopic lesions, abnormalities or parasites in the gastrointestinal tract, kidneys or lungs. It was not possible to identify the gelatinous mass found in the stomach based on morphology. Similarly, the 788-bp sequence of 28S rDNA and the 445-bp sequence of the partial COI obtained from the gelatinous mass did not display enough nucleotide similarities when compared with other published sequences from the GenBank database. DNA sequences are available upon request.

Discussion

Three main observations support the idea that the individual found stranded on the Spanish Mediterranean coast is a common minke whale, B. acutorostrata of less than a vear old: i.e., total body length, cranial sutures not completely closed and the incipient ossification degree of the left pectoral flipper. From these, total body length specifically suggests that the animal was still a nursing calf. Common minke whales are born with a total body length of between 2.4 m and 2.8 m, and are weaned when they are between 4.5 m and 5.5 m in length (Van Waerebeek et al. 1999; Harms et al. 2008; Perrin and Brownell 2009; Christiansen et al. 2014). Therefore, animals whose body length is between 2.8 m and 4.3 m can be defined as nursing calves. The 3-metre total body length of the common minke whale found in the Spanish Mediterranean matches the age of a calf that was likely still nursing. The high level of decomposition of the animal when it was found precluded us from examining the milk content in the stomach. However, previous reports of common minke whale calves with a body length less than 3.5 m have shown that when dissected, animals still had milk in various digestive stages along the gastrointestinal tract; in both reported cases, the animals had recently died and carcasses were still fresh during the necropsy (Harms et al. 2008; Shoham-Frider et al. 2014). In this context, the unidentified gelatinous mass found in the stomach might have occurred as an accidental ingestion.

Previous studies have shown that mammals are born showing some degree of incomplete closure of cranial sutures in the posterior skull portion, and that skull bones start to fuse during early postnatal growth (Rager et al. 2014). In balaenopterids, the sequence at which these sutures ossified indicates the relative age of young animals (Walsh and Berta 2011). The degree of incomplete closure of the cranial sutures surrounding the foramem magnum (basioccipital-exoccipital and supraoccipitalexoccipital) observed in the found specimen was consistent with the description given by Walsh and Berta (2011) on calves aged under 1 year, in which the mentioned cranial sutures are partially closed; complete joints are tightly closed in common minke whales, when the total body length of individuals reaches approximately 4.2 m (Walsh and Berta 2011).

Radiographies are a widely employed methodology followed to investigate age class in stranded cetaceans (see Calzada and Aguilar 1996, and Del Castillo et al. 2014 for dolphins; and Stockin et al. 2008 for baleen whales). The X-ray done on the left pectoral flipper of the stranded common minke whale indicated an early bone development, which is compatible with a very young animal. For cetaceans, the proximal epiphyses of the ulna may ossify before the age of 1, whereas the distal epiphyses of this area may start and continue to ossify after the first year of life (Del Castillo et al. 2014). This proximo-distal gradient of ossification of the epiphyses was also observed in the X-ray done on the pectoral flipper of the common minke whale nursing calf (Fig. 2c). Similar images with an early mineralisation stage of the secondary ossification nucleus in the epiphyseal region of the humerus, radius and ulna have been observed in other species of cetacean calves (see Ogden et al. 1981; Calzada and Aguilar 1996; Stockin et al. 2008; Del Castillo et al. 2014).

Common minke whales are frequently sighted off the Atlantic coast of the Iberian Peninsula and the subtropical archipelagos in the Northeast Atlantic (Canary Islands), where some have been considered sexually immature animals according to body length (Van Waerebeek et al. 1999). Through satellite tracking a subadult common minke whale was tagged in Skagen (north Denmark) in June 2003. The animal migrated southward and reached Cape Verde, the Azores and the Canary islands. After three months, the device stopped transmitting a signal in the Mediterranean Sea, near Majorca (Teilmann et al. 2005). These observations suggest that common minke whales from the Atlantic Ocean might occasionally enter the Mediterranean via the Strait of Gibraltar, and indeed there are records of sighted and stranded individuals of this species in coastal and offshore waters of Spain, Morocco, Tunisia, France and Italy (Van Waerebeek et al. 1999; Notarbartolo di Sciara and Birkun 2010). More than a decade ago, Van Waerebeek et al. (1999) suggested that common minke whales might gave birth in the Mediterranean. This idea was based on the fact that common minke whale calves (between 3 m and 3.6 m in length) have been reported in the western Mediterranean on the coasts of France and Italy in the months of February (1878), April (1998) and May (1991) (Van Waerebeek et al. 1999; Notarbartolo di Sciara and Birkun 2010). Fewer common minke whales have been reported in the eastern Mediterranean in Georgia, Greece, Israel and Turkey (Verriopoulou et al. 2001; Notarbartolo di Sciara and Birkun 2010; Öztürk et al. 2011; Shoham-Frider et al. 2014).

Only two previously published reports of common minke whales are documented in Spanish Mediterranean

waters: one stranded individual in an advanced state of decomposition found on the coast of Malaga (Andalusia, South Spain) that measured 4.5 m and was found in October 1996 (Van Waerebeek et al. 1999), and one sighting event of a single adult in the province of Almeria (Andalusia) in July 2006 (Notarbartolo di Sciara and Birkun 2010). Information from two other unpublished reports of the species is available: one juvenile, with a 4-metre total body length, stranded on the coast of Almeria (Andalusia) in April 2004 (Programa de Gestión Sostenible del Medio Marino Andaluz de la Consejería de Medio Ambiente y Ordenación del Territorio, personal communication, June 25, 2014), and one sighting of an adult near Cape Tiñoso (37°31'N-1°10'W, Murcia, Southeast Spain) in April 2011 (Rosa Canales, personal communication, June 20, 2014) (Fig. 1). The above records of common minke whales in the Spanish Mediterranean might raise a flag for putative higher occurrence of the species in the area. The finding of a 3-metre long common minke whale in the Valencian Community is the first record of a nursing calf in Spanish Mediterranean waters.

According to the above evidence, the finding of a putative young calf in the study area is compatible with the idea that the animal either entered the Mediterranean with her mother or that the mother delivered in the Mediterranean. Further monitoring of sightings and strandings of the species are needed to evaluate species' distribution, migratory patterns and reproduction habits in the western Mediterranean.

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