J. Black Sea/Mediterranean Environment
Vol. 18, No. 1: 67-75 (2012)

SHORT COMMUNICATION

Unusual mass mortality of cetaceans on the coast of the Turkish Western Black Sea in summer 2009

Arda M. Tonay[1,2]*, Ayhan Dede[1,2], Ayaka Amaha Öztürk[1,2], Didem Ercan[3], Antonio Fernández[4]

[1]Faculty of Fisheries, Istanbul University, 34470, Laleli-Istanbul, TURKEY
[2]Turkish Marine Research Foundation (TUDAV), 34820, Beykoz, Istanbul, TURKEY
[3]Faculty of Fisheries, Muğla University, 48000, Muğla, TURKEY
[4]Institute for Animal Health. University of Las Palmas de Gran Canaria, Canary Islands, SPAIN

***Corresponding author:** atonay@istanbul.edu.tr

Abstract

Between mid-July and early August, an unusual mass mortality of cetaceans was detected on the Turkish Western Black Sea coast. 114 cetaceans (53 harbour porpoises, 9 common dolphins, 7 bottlenose dolphins, 45 unidentified) were reported dead and nine common dolphins stranded alive, 6 of which were returned to the sea. Only two freshly dead common dolphins were subjected to a complete necropsy, sampling and tissue analysis. Tissue samples from brain, lung, spleen, liver, kidney, muscle and testis were fixed in formalin and embedded in paraffin. Histological analysis was conducted and immunohistochemical technique to detect morbillivirus antigen was applied. RNA and cDNA samples were examined for dolphin morbillivirus (DMV) detect by reversetranscription–PCR (RT-PCR) and conventional PCR. Main histopathological lesions were multifocal non-purulent meningitis, severe diffuse alveolar oedema in lung samples, multifocal periportal macrovacuolar hepatic steatosis, interstitial and tubular degeneration with protein casts and calcifications in renal medulla. No evidence for DMV infection was analytically found in both dolphins, and the cause of this unusual mortality is still uncertain.

Keywords: *Delphinus delphis*, *Phocoena phocoena*, *Tursiops truncatus*, stranding, dolphin morbillivirus (DMV)

Introduction

The Black Sea is almost an enclosed sea and therefore its ecology is more affected by environmental problems than open seas (Zaitsev 2008). There are three cetacean species living in the Black Sea; the harbour porpoise (*Phocoena phocoena*), bottlenose dolphin (*Tursiops truncatus*) and common dolphin

(*Delphinus delphis*) (Ozturk 1996). Cetaceans in the Black Sea have been faced with several threats such as accidental catch in fishing gears (bycatch), habitat degradation and mass mortality events (Birkun 2008). In general, cetacean mass mortality events develop slowly over days, weeks or months, and three main factors are known to cause these events; infectious diseases, naturally occurring toxins and environmental changes (Cowan 2009; Hall and Harwood 2009). These threats may overlap, and any of them can be increased by anthropogenic factors (Hall and Harwood 2009). Among infectious diseases, morbillivirus is one of the most lethal and widespread causes of mortality in marine mammals (Van Brassem 2009).

Apart from mass mortality events of harbour porpoises as a result of ice entrapment by hydrometeorological conditions such as in 1993 (Birkun 2008), increased mortality or mass mortality were observed in the Azov Sea in 1999, 2002 and 2008 (Gol'din 2009). Mass mortality events in the Black Sea were reported for common dolphins in 1990 (unknown reason) and 1994 in Ukraine, Romania, Russia and Bulgaria due to morbillivirus, and for all three species but mostly harbour porpoises in Ukraine, Russia and Bulgaria in 1989 and 1990 due to severe pulmonary nematodosis and complicated by bacterial super-infection (Birkun *et al*. 1999; Birkun 2002; Birkun 2008). In 2003, the first marine mammal mass mortality was reported on the Turkish Western Black Sea coast, which affected mostly harbour porpoises (Tonay *et al.* 2008). In summer 2009, a new mass mortality occurred, this time affecting mostly common dolphins. The aim of this study is to report that unusual event and describe a series of analyses done with two fresh necropsied common dolphins in order to understand the cause of the death.

Materials and Methods

Between mid-July and early August, a series of cetacean strandings were reported along the 200 km-long Turkish Western Black Sea coast between Yalıköy and Şile (Figure 1). During this mass mortality event, at least 114 cetaceans (53 harbour porpoises, 9 common dolphins, 7 bottlenose dolphins, 45 unidentified) were reported dead (2.7 individuals per km), and nine common dolphins stranded alive (Table 1). Six of them were returned alive to the sea. In the field study (along the Thracian coast on July 30th and along the Anatolian coast on August 4th) and through the stranding network, a total of 33 cetacean specimens (15 common dolphins, 8 harbour porpoises, 7 bottlenose dolphins, 3 unidentified) were confirmed, some of which were also examined. In addition to those, a total of 87 individuals (45 harbour porpoise, 42 unidentified), stranded in the second half of July, were also reported by the locals in the region during the field study.

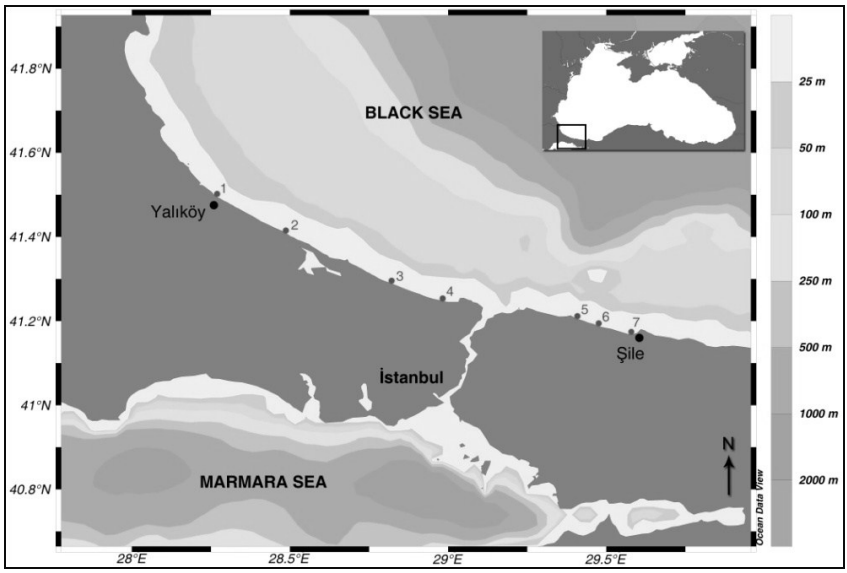

Figure 1. Study area and locations where live common dolphins stranded.

Table 1. Information on live strandings of common dolphins.

Case No	Number of dolphins	Date	Consequence
1	1	1 August	Returned to sea
2	1	30 July	Died
3	3	3 August	Returned to sea
4	1	6 August	Died
5	1	27 July	Returned to sea
6	1	24 July	Returned to sea
7	1	15 July	Died

Tissue samples (brain, lung, spleen, liver, kidney, muscle and testis) of two common dolphins were fixed in buffered formalin and processed routinely to be included in paraffin blocks. Sections from these samples were taken and stained routinely using the Hematoxylin and Eosin techniques as well as PAS technique. An immunohistochemical technique in order to detect morbillivirus antigens was carried out on paraffin tissue sections following the technique described previously (Fernández *et al.* 2008).

For the RNA isolation and cDNA synthesis 10–30 mg frozen tissue samples (kidney, liver, spleen, lymph nodules and brain) were used. The total RNA was isolated with RNA isolation kit (ROCHE High Pure RNA Tissue Kit) and 1 mg of total RNA was converted to cDNA by a kit (ROCHE First Strand cDNA Synthesis Kit for RT-PCR) according to the manufacturer protocol. The RNA

and cDNA samples were examined for DMV nucleic acid by reverse transcription–PCR (RT-PCR) and conventional PCR. RT-PCR reaction kit (ROCHE Titan One Tube RT-PCR Kit) was used according to the manufacturer protocol. The nested primer sets of universal morbillivirus primers used were:
UPN1: 5′-ACAAACCNAGRATTGCTGAAATGAT-3′
UPN2: 5′-CTGAAYTTGTTCTGAAYTGAGTTCT-3′
N1a: 5′-ACTATYAARTTYGGNATNGAACNATGT-3′
N2a: 5′-CTGCACTRAAYTTGTTYTGRAYNGAGT-3′ (Raga *et al*. 2008).

The PCR reaction mixtures included 100 ng template cDNA positive control, 100 pmol of each Primer and 2xPCR master mix (Fermentas). Amplification was performed using the Biometra thermal cycler and the following parameters: 94°C for 2 min, followed by 30 amplification cycles including denaturation at 94°C for 45 s, primer annealing at 50°C for 45 s, and extension at 72°C for 1 min, and a final extension step of 72°C for 5 min (Tom Barrett, 2009, Personal communication). PCR products were visualized on a UV transilluminator and size estimated against GeneRuler 50bp DNA Ladder (Fermentas SM0371) after Etbr staining and gel electrophoresis (1.5% TAE agarose gels, 90Volt and 30 min).

Results and Discussion

Many cetaceans died in the summer of 2009 in the Black Sea as a whole; between late May and July in the Northern Black Sea (Birkun 2009) and between July and early September in the southern part (this study). A total of 23 cetaceans (mainly common dolphins) stranded alive in Ukraine (13), Georgia (5) and Bulgaria (5) (Birkun 2009).

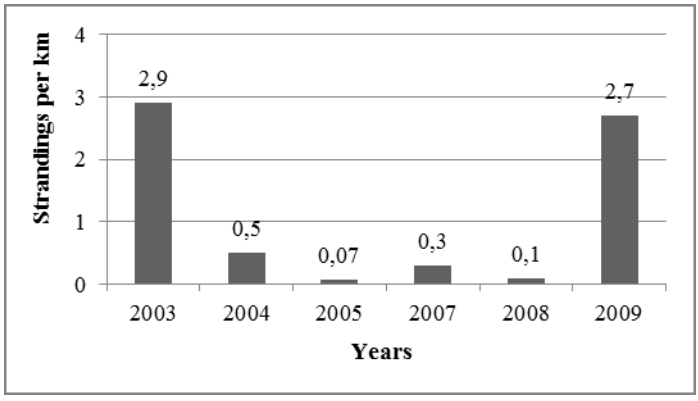

Figure 3. Strandings per km during 2003-2005 (Tonay *et al.* 2008), 2007-2009 summer in the study area. (There was no monitoring effort in 2006).
The stranding rate of this event was much higher than the past stranding rates on the Turkish Western Black Sea coast, except in 2003 (Tonay *et al*. 2008) (Fig.

3). The present event, thus, can be considered as an unusual one. However, a high rate of harbour porpoise stranding, especially as a result of bycatch in bottom gillnets used in turbot fishery, is usually observed in late spring and summer (Tonay and Ozturk 2003; Tonay *et al*. 2008). Therefore, it could be assumed that the majority of harbour porpoises found during this event were related to bycatch.

As many stranded individuals were at the advanced stage of decomposition due to hot weather, only two fresh specimens of stranded common dolphins were examined (Cases 2 and 4). Both animals were observed swimming abnormally before beaching. They died in a short time. The dolphins were in good physical conditions. No injury was observed. And during the necropsy, the main findings were related with empty stomachs, enlarged livers and spleens with abnormally brownish-yellowish appearance in the liver and areas of fibrosis in the spleen capsule (Figure 2).

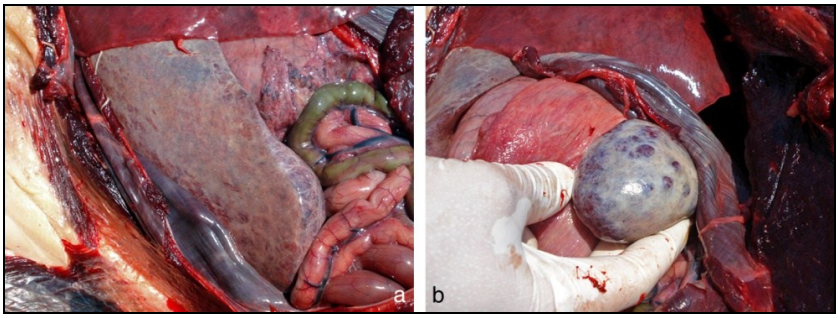

Figure 2. Necropsy of one of the stranded common dolphins (n.2). a) Abdominal cavity showing part of the liver and intestine b) Spleen.

Main histopathological lesions were: Multifocal non-purulent meningitis (Figure 4a); severe diffuse alveolar oedema in all analyzed lung samples; multifocal periportal macrovacuolar hepatic steatosis (Figure 4b); interstitial and tubular degeneration with protein casts and calcifications in renal medulla (Figure 4c, d). No lesions were detected in the rest of the organs.

No morbillivirus antigen was detected in the brains, lungs or spleens of the two common dolphins analysed immunohistochemically. PCR products were also negative for DMV. Although both dolphins were negative for morbillivirus, this possibility cannot be ruled out in this unusual mortality due to the low number of animals analysed. Still, other possible causes such as biotoxins and/or brucellosis need to be investigated.

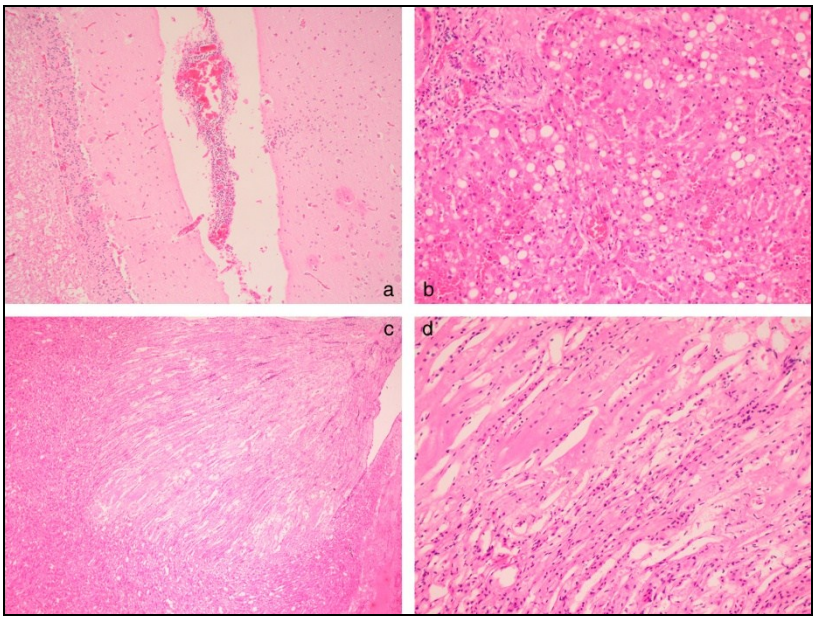

Figure 4. Histological images of the tissues of abnormal organs. **a)** Nervous tissue: Perivascular cuffing with many mononuclear cells (non-purulent meningitis) **b)** Many hepatocytes showing intracytoplasmic lipidic vacuoles of different size. Macrovacuolar hepatic steatosis and periportal lymphocytic pericolangitis **c)** and **d)** Interstitial and tubular hyaline degeneration in the renal medulla.

Mass mortality events due to morbillivirus has previously been reported in common dolphins in the Black Sea in 1994 (Birkun 2008), as well as in close sea areas, like in the Mediterranean Sea in 1990-1992 and 2007 affecting striped dolphins (Van Bressem 2009). In addition, serological examination of bycaught harbour porpoises in Georgia, Bulgaria and Ukraine (from 1997 to 1999) revealed positive morbillivirus-neutralizing antibody titres (Müller *et al.* 2002). Morbillivirus-specific antibodies were also detected in the blood serum of the bottlenose dolphins in Taman Bay on the east cost of the Kerch Strait in 2003-2007 (Alekseev *et al.* 2009). These data allow us to think that the Black Sea can be an endemic area for morbillivirus in cetaceans, and therefore, new outbreaks are expected to take place in the future as implied by Birkun (2008).

However, scientists and governments should also keep in mind other important key anthropogenic and climatic issues, since die-offs in the past concurred with drastic decline in the abundance of prey of cetaceans, anchovy and sprat, which were severely affected by overfishing combined with the consequences of water pollution (*e.g.,* eutrophication and water hypoxia) and population explosion of alien ctenophore *Mnemiopsis leidyi*. This may suggest a cause and effect relationship between prey scarcity and common dolphin and harbour porpoise mass mortality (Birkun 2008).

Although the two common dolphins in this study were negative for DMV and we were not able to completely rule out the possibility of DMV infection as the cause of this unusual mass mortality, it is still important to report this unusual mortality in a high impacted anthropogenic area in order to call again the attention regarding the need to reinforce stranding networks in the Black Sea countries in order to be prepared for this kind of unusual mass mortality events in the future.

Acknowledgement

We would like to thank all contact persons in the TUDAV cetacean stranding network, local people and Şile Municipality for all information and help provided in the field; Dr. Raşit Bilgin for primers; Dr. Fernando Esperón Fajardo for the positive control of DMV, Dr. Tom Barrett (RIP) for PCR cycle conditions, Dr. Pavel E. Gol'din for his rapid feedback and input regarding the mass mortality events along the western Crimean coast; MARMAM and ECS-Talk members who shared all their knowledge and concerns; Onur Ozullu (Onser Motor Ltd.) for providing ATVs; Nilufer Tonay for reduction of this manuscript, the Permanent Secretariat of the Commission on the Protection of the Black Sea Against Pollution (BSC-ML/2008), Scientific Research Projects Coordination Unit of Istanbul University (Project number: T-924/061006) and Turkish Marine Research Foundation (TUDAV) for its financial support for the field studies (2007-2009).

2009 yaz mevsiminde, Türkiye'nin Batı Karadeniz kıyılarında deniz memelilerinin (Setase) olağandışı toplu ölümü

Özet

Batı Karadeniz Türkiye kıyılarında Temmuz ayı ortası ve Ağustos ayı başına kadar olağandışı toplu deniz memelileri (setase) ölümleri belirlenmiştir. 53 mutur, 9 tırtak, 7 afalina ve 45 tanımlanamayan olmak üzere toplam 114 setase bireyi ölü olarak bulunmuştur. 9 tırtak canlı olarak karaya vurmuş, 6'sı tekrar denize döndürülmüştür. Sadece 2 tırtak bireyi üzerinde nekropsi, örnekleme ve doku analizi yapılmıştır. Beyin, akciğer, dalak, karaciğer ve böbrek doku örnekleri formalinde fikse edilmiş ve parafine gömülmüştür. Histolojik analizler yapılmış, morbilivirüs antijeni tespiti için immünohistokimyasal teknik uygulanmıştır. Elde edilen RNA ve cDNA örnekleri morbilivirüs varlığının tespiti için geleneksel PCR ve geri transkripsiyon-PCR (RT-PCR) ile incelenmiştir. Başlıca histopatolojik bulgular olarak multifokal non purulent menenjit, akciğer alveollerinde ağır difüz ödem, karaciğerde multifokal periportal makrovasküler steotoz, böbrek medullasında intersitisyal ve tübüler dejenerasyonla beraber protein döküntüleri ve kalsifikasyon görülmüştür. Her iki bireyde de morbilivirüs enfeksiyonuna ait bir bulguya rastlanmadığından bu olağandışı toplu ölümlerin sebebi halen belirsizliğini korumaktadır.

References

Alekseev, A.Yu., Reguzova, A. Yu., Rozanova, E.I., Abramov, A.V., Tumanov, Yu. V., Kuvshinova, I.N., Shestopalov, A.M. (2009) Detection of specific antibodies to morbilliviruses, *Brucella* and *Toxoplasma* in the Black Sea Dolphin *Tursiops truncatus ponticus* and the Beluga Whale *Delphinapterus leucas* from the Sea of Okhotsk in 2002−2007. *Russian Journal of Marine Biology* 35(6): 494-497.

Birkun, A. Jr. (2002) Natural mortality factors affecting cetaceans in the Black Sea. In: Cetaceans of the Mediterranean and Black Seas: State of Knowledge and Conservation Strategies, (ed., G. Notarbartolo di Sciara), A report to the ACCOBAMS Secretariat, Monaco, February 2002. Section 16, 13 p.

Birkun, A. Jr. (2008) The state of cetacean populations. In: State of the Environment of the Black Sea (2001-2006/7), (ed., Temel Oğuz). The Commission on the Protection of the Black Sea Against Pollution (BSC), Istanbul, Turkey. pp. 365-395.

Birkun, A. Jr. (2009) The progress report on the implementation of the conservation plan for Black Sea Cetaceans November 2009 (October 2007-October 2009) Draft Doc.12.6.) BSC AG FOMLR Meeting in Istanbul.

Birkun, A., Jr., Kuiken, T., Krivokhizhin, S., Haines, D.M., Osterhaus, A.D.M.E., van de Bildt, M.W., Joiris, C.R., Siebert, U. (1999) Epizootic of morbilliviral disease in common dolphins (*Delphinus delphis ponticus*) from the Black Sea. *Vet. Res.* 144(4): 85-92.

Cowan, D.F. (2009) Pathology. In: Encyclopedia of Marine Mammals. Second Edition, (eds., W.F. Perrin, B. Würsig, J.G.M. Thewissen), Academic Press, USA, pp. 836-844.

Fernández, A., Esperón, F., Herraéz, P., Espinosa, A., Clavel, C., Bernabé, A., Sánchez-Vizcaino, J.M., Verborgh, P., DeStephanis, R., Toledano, F., Bayón, A. (2008) Morbillivirus and pilot whale deaths, Mediterranean Sea. *Emerging Infectious Diseases* 14(5): 792-794.

Gol'din, P.E. (2009) Harbour porpoises in the Sea of Azov: population structure, longterm dynamics and factors affecting them. In: Abstracts of 23rd Annual Conf. European Cetacean Society, Istanbul, Turkey, p. 20.

Hall, A., Harwood, J. (2009) Mass die-offs. In: Encyclopedia of Marine Mammals. Second Edition, (eds., W.F. Perrin, B. Würsig, J.G.M. Thewissen), Academic Press, USA, pp.709-712.

Müller, G., Wünschmann, A., Baumgärtner, W., Birkun, A., Komakhidze, A., Stanev, T., Joiris, C.R. (2002) Immunohistological and serological investigations of morbillivirus infection in Black Sea harbour porpoises (*Phocoena phocoena*). *Vet. Microbiol*. 87(2): 183-190.

Ozturk, B. (1996) Whales and Dolphins, Introduction of Cetology. Anahtar Kitapları Yayınevi, Istanbul. 136 pp (in Turkish).

Raga, J.-A., Banyard, A., Domingo, M., Corteyn, M., Van Bressem, M.-F., Fernández, M., Aznar, F.-J., Barrett, T. (2008) Dolphin morbillivirus epizootic resurgence, Mediterranean Sea. *Emerging Infectious Diseases* 14(3): 471-473.

Tonay, A. M., Dede, A., Ozturk, A.A. (2008) Cetacean strandings in the Turkish western Black Sea coast during spring months in 2003-2005. In: Proc. 22nd Annual Conf. European Cetacean Society, Egmond aan Zee, the Netherlands.

Tonay, A.M., Ozturk, B. (2003) Cetacean bycatches in turbot fishery on the western coast of the Turkish Black Sea. In: International Symposium of Fisheries and Zoology (In memory of Ord. Prof. Dr. Curt KOSSWIG in His 100th Birth Anniversary), (eds., I.K. Oray, M.S. Çelikkale, G. Özdemir), İstanbul, pp. 131-138.

Van Bressem, M.F. (2009) Emergency task force: guidelines for a coordinated Cetacean stranding response during mortality events caused by infectious agents and harmful algal blooms. ACCOBAMS Guidelines for a Coordinated Cetacean Stranding Response. 29pp.

Zaitsev, Y. (2008) An Introduction to the Black Sea Ecology. Smil Editing and Publishing Agency Ltd, Odessa, 228 pp.

Received: 05.12.2011
Accepted: 22.12.2011