SC/68A/E/08

Investigation of a mass stranding of 68 short-beaked common dolphins in Golfo Nuevo, Península Valdés, Argentina

MARCELA M. UHART, ENRIQUE A. CRESPO, MARÍA FLORENCIA GRANDI, ROCÍO LOIZAGA, MARIANA DEGRATI, et.al.

INTERNATIONAL WHALING COMMISSION

Investigation of a mass stranding of 68 short-beaked common dolphins in Golfo Nuevo, Península Valdés, Argentina

MARCELA M. UHART[1,2], ENRIQUE A. CRESPO[3], MARÍA FLORENCIA GRANDI[3], ROCÍO LOIZAGA[3], MARIANA DEGRATI[3], NESTOR A. GARCÍA[3], VALERIA D'AGOSTINO[3], DAMIÁN VALES[3], CRISTIAN DURANTE[3], FEDERICO HEREDIA[3], SANTIAGO FERNÁNDEZ[3], NICOLÁS SUEYRO[3], CARLA FIORITO[4], A. MARCELO SANTO[5], CONSTANZA MARCHESSI[3], MARIANO COSCARELLA[3], SILVANA DANS[3], GABRIELA NOVACOVSKY[4], AILÉN CHALCOVSKY[3], AILÍN SOSA DROUVILLE[3], MÓNICA BUONO[6], LUCÍA ALZUGARAY[7], AGUSTÍN LUZENTI[3], DÉBORA POLLICELLI[3], MATÍAS DI MARTINO[2], BREND KROCK[8], DENISE MCALOOSE[9], AGUSTINA RIMONDI[10], MARINA GALLO CALDERÓN[11], GASTÓN MORÉ[12]

[1]Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, USA

[2]Southern Right Whale Health Monitoring Program, Puerto Madryn, Argentina

[3]Laboratorio de Mamíferos Marinos, Centro para el Estudio de Sistemas Marinos (CENPAT-CONICET), Puerto Madryn, Argentina

[4] Grupo de Ecofisiología Aplicada, Centro para el Estudio de Sistemas Marinos (CENPAT-CONICET), Puerto Madryn, Argentina

[5] Servicio Centralizado de Histología (CENPAT-CONICET), Puerto Madryn, Argentina

[6] Instituto Patagónico de Geología y Paleontología (CENPAT-CONICET), Puerto Madryn, Argentina

[7] Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, Argentina

[8] Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany[9] Wildlife Conservation Society, Zoological Health Program, New Yok, USA.

[10] Instituto de Virología, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina

[11] Instituto Cesar Milstein (CONICET), Buenos Aires, Argentina

[12]Laboratorio de Inmunoparasitología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina

ABSTRACT

We report on the investigation of a mass stranding of 68 short-beaked common dolphins (*Delphinus delphis*) that occurred in Golfo Nuevo, Península Valdés, Argentina in March 2018. Twenty-one of the stranded dolphins were returned alive to the sea, while 47 animals died. Dead dolphins included all ages, with more males than females (29 males and 18 females). The cause of death investigation reported here is restricted to 15 adult individuals and one fetus on which a full set of diagnostics was prioritized due to limited funding. Our results demonstrate that the death of 16 dolphins assessed in this study was not due to obvious human effects (e.g. bycatch) or underlying pathologies, as all animals were in good body condition and had no external evidence of injuries. Infections by Morbillivirus, Influenza A virus, *Sarcocystis* spp., *Toxoplasma gondii*, or *Neospora caninum,* as well domoic acid (DA) toxicity were ruled out as ethiologies in this event. Notably, results on exposure to paralytic shelfish toxins (PSP) were the only investigated cause of death found positive. This is the first documentation of exposure to PSP toxins in short-beaked common dolphins from the Argentine Sea. At present our results are insufficient to assess whether PSP toxin exposure played a role in the death of the stranded dolphins. Notwithstanding, the full documentation and investigation of the most commonly reported pathogens and toxins involved in cetacean mass strandings allowed us to clear the most relevant health differentials and suggests areas for future study. Additional potential hypothesis related to factors known or speculated to cause cetacean mass strandings are currently being explored within the ecological context at the time of the event.

INTRODUCTION

A mass stranding of 68 short-beaked common dolphins (*Delphinus delphis*) occurred in El Doradillo, Golfo Nuevo, Península Valdés, Argentina (42° 38' S, 64° 58' W;Fig. 1), on Sunday 25th March 2018.

The short-beaked common dolphin is a species of cosmopolitan distribution found in all the temperate and tropical seas (Heyning and Perrin, 1994; Perrin, 2002; Jefferson et al., 2008, 2009) with presence of local populations on the shores of the Argentine Sea (Crespo and Dans, 2008). It is a very common species on the coasts of Buenos Aires and the north of the Patagonian coast and suffers incidental capture in trawl and purse seine fisheries off Argentina (Corcuera et al., 1994; Crespo et al., 2000). The southernmost records include sightings in Golfo Nuevo (Fig. 1), where the stranding event reported here occurred, and an incidental catch in Golfo San Jorge (44º-47ºS, 65º-67º W) (Crespo et al., 2007).

Short-beaked common dolphins form fission-fusion groups which can reach several hundred individuals (although the basic nucleus is about 30 individuals) as seen in Golfo San Matías and Buenos Aires Province, Argentina. The total population of short-beaked common dolphins in Argentina was estimated in 14,200 individuals (LAMAMA unpublished information). The most important conservation problem for the species is bycatch in mid-water fisheries for anchovy (*Engraulis anchoita*). This conflict affects about 0.66% to 3.76% of the stock (Crespo et al., 1997, 2000; LAMAMA unpublished information).

No previous mass stranding of short-beaked common dolphins has been recorded in Argentina. However, there are several events reported elsewhere, notably two events in the UK in 2002 and 2008 (Viricel et al., 2008; Jepson et al., 2013), many in Cape Cod, USA, since the 2000s (the largest in 2012, NOAA), one in New Zealand in 2008 (Stockin, 2008) and another in Brazil in 2012 (Brownell et al., 2012).

The cause of death investigation reported here is restricted to 15 adult individuals and one fetus on which a full set of diagnostics was prioritized due to limited funding and the need for a rapid response (both locally and internationally). Specifically, our assessment targeted identifying evidence of human interaction, overall health condition and underlying pathologies, pathogens previously found responsible for cetacean mass strandings (*Morbillivirus*, Influenza A virus, protozoans such as *Sarcocystis* spp. and *Toxoplasma gondii*), and biotoxins.
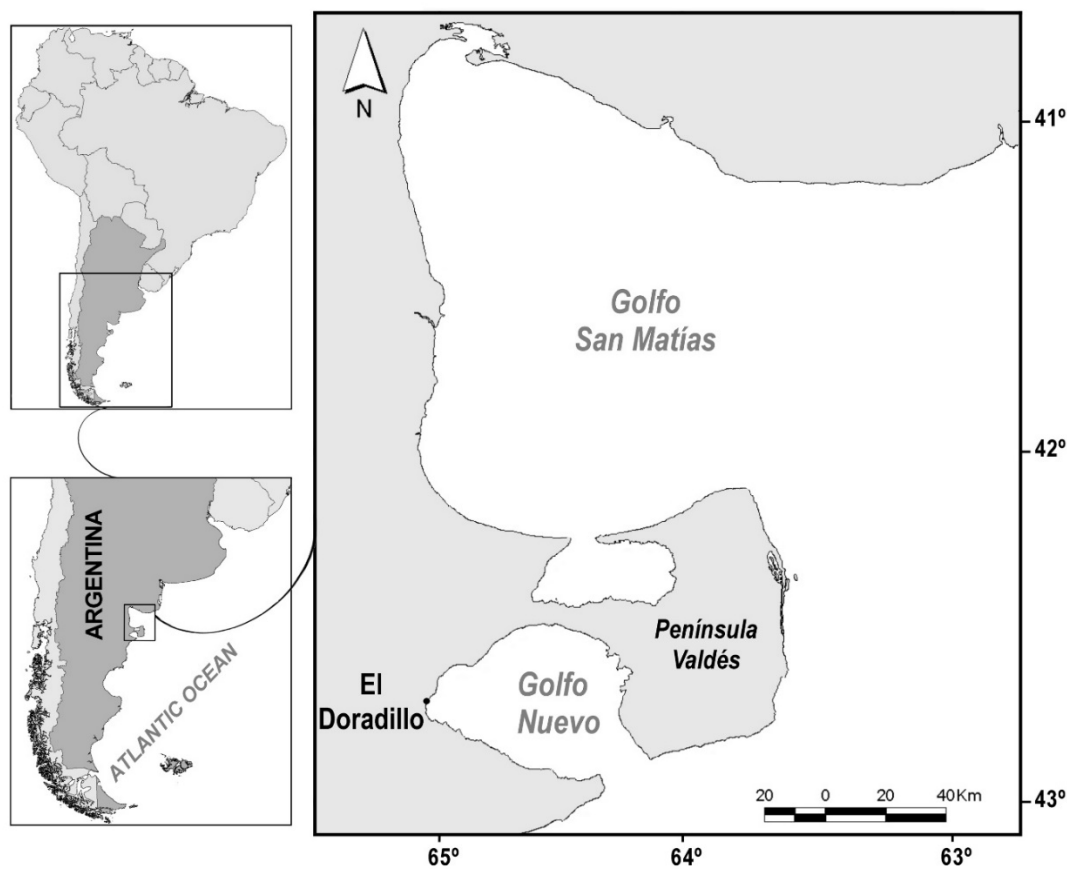
Figure 1. Map of study area showing the site where the mass stranding occurred (El Doradillo).

METHODS

Between 8 and 12 hours post mortem, individual dolphins were transported from the beach and stored frozen at a local fish processing plant (-20°C) until 48 hours pre-necropsy. Dolphins were thawed at room temperature. Necropsies were performed between April 24th and May 4th at the *Centro para el Estudio de Sistemas Marinos* (CESIMAR-CONICET) facilities. Two to three animals were necropsied per day.

External examination with collection of morphometric data (Norris, 1961), gross necropsy examination, tissue sample collection for histology and ancillary diagnostics, and photographic documentation were performed on each animal. Gross necropsy examination was performed using available dolphin necropsy protocols "Odontocete Salvage, Necropsy, Ear Extraction, and Imaging Protocols" compiled and edited by Nina M. Young et al. (2007) and "Marine Mammal Necropsy: An introductory guide for stranding responders and field biologists" (Pugliares

et al., 2007). Carcass decomposition was not graded since animals were frozen fresh. However, variations in carcass preservation were noted after thawing, with tissues often disintegrating on defrost. Age class was determined by body length and body weight (Table 1). Estimation of age will be performed at a later stage based on reading growth layer groups on dentine from teeth at Marine Mammal Laboratory (CESIMAR-CONICET).

A full set of tissue samples (all tissues and organs and any obvious abnormalities) was collected, preserved in 10% neutral buffered formalin and stored at room temperature. Formalin was replaced after 48 hours, and in some cases a third time ~7 days later (until clear). Tissues for histologic examination were then submitted to a local specialized laboratory (Laboratorio Veterinario Dra. Duchene[1]) for processing by routine methods, embedded in paraffin blocks, sectioned at 5 μm, and stained with hematoxylin and eosin (HE). Histopathology was performed in Argentina by board-certified pathologist Denise McAloose from the Wildlife Conservation Society.

Duplicates and triplicates of all major tissues, organs and fluids (urine, milk, semen, blood) were collected and stored in viral transport media (BD™ Universal Viral Transport System), lysis buffer (RNA*later*™ Storage Solution, Ambion, Inc., Austin, Texas) or without preservative in cryovials and/or whirlpack bags. These samples were kept on ice packs during necropsy (approximately 4-6 hours) and then stored at -70°C until submission to diagnostic laboratories. Samples were shipped frozen on dry ice within a month from collection. Ears were dissected, placed in formalin for approximately 2 weeks (formalin was replaced several times until clear) and then transferred to 70% ethanol. Samples not submitted for diagnostics remain in a tissue bank at the National Research Council's CESIMAR -CONICET[2] in Puerto Madryn.

For Influenza A virus diagnostics, samples of brain, lung, pulmonary, bronchial and prescapular lymph nodes and rectal swabs were submitted to the *Virology Institute of the Instituto Nacional de Tecnología Agropecuaria* (INTA[3]) in Buenos Aires. Briefly, homogenates of lung, lung lymph node, pericardial lymph node and prescapular lymph node were prepared. Then, pools of 4-5 samples were made and each pool was subjected to viral RNA extraction using the commercial kit QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. Subsequently, a retro-transcription of RNA to DNA copy (cDNA) was performed using the High Capacity cDNA Archive kit (Applied Biosystems) to finally submit the cDNA to a real-time PCR that determines the presence of Influenza A Virus (IAV). For the real-time PCR the primers InfA Fw, InfA Rv and the InfA probe previously described in the WHO / CDC protocol were used (CDC protocol of real time RTPCR for swine influenza A (H1N1) and 8 April 2009 revision 1 30 April 2009).

For *Morbillivirus* diagnostics, samples from brain, lung and lung lymph nodes were submitted to *Cesar Milstein Institute*[4] (CONICET) in Buenos Aires. Each sample was analyzed individually. After mechanical lysis on a mortar, RNA was extracted with TRI Reagent®, according to manufacturer's instructions. The RNA obtained was quantified using a NanoDrop 2000® and approximately 1.6 μg of RNA was used as a template in each RT-PCR reaction. The reactions were carried out in a volume of 30 μl, in the presence of specific oligonucleotides for the detection of a region of 429 base pairs of the P gene of the DMV (Dolphin Morbillivirus, Barret et al., 1993), with the One Step RT-PCR kit (Qiagen®), in a thermocycler (Eppendorf) Master Cycler® Gradient. In addition, in all cases, the amplification of the GAPDH gene was carried out, to evaluate the integrity of the obtained RNA. RNA obtained from the blood of a dog with molecular diagnosis positive for Canine Distemper Virus was used as a positive control.

For protozoan diagnostics, samples from brain, heart, tongue, masseter muscle, diaphragm and dorsal and/or ventral muscle were submitted to the *Immunoparasitology Lab* (LAINPA)*, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata*[5] in La Plata. Two diagnostic methods were applied. First samples were microscopically assessed. For this, samples of heart and skeletal muscles (processed as a pool for each animal) were homogenized in a mixer with PBS and analysed for the presence of cysts of *Sarcocystis* spp. as previously described (Moré et al., 2011, 2014). In a second phase, samples of brain, homogenate of heart and pool of skeletal muscles and cystic structures were subjected to DNA extraction with the commercial kit Wizard genomics (Promega) according to manufacturer's instructions. All samples were processed by conventional PCR using the Tox5-Tox8 primers, specific for *T. gondii* and the primers SarcoFext and SarcoRext that amplify fragments of the gene for the 18S rRNA of species in the genus *Sarcocystis*. Protocols previously described were used (Moré et al., 2013; Pardini et al., 2014). Additionally, the SNC DNA samples were analysed by specific PCR for *Neospora caninum* using the primers Np6 + and Np21 +, proceeding according to what was previously described by Moré et al. (2008).

---

[1] http://laboduchene.com/wp/

[2] http://www.cenpat-conicet.gob.ar/

[3] https://inta.gob.ar/

[4] https://milstein.conicet.gov.ar/

[5] http://www.fcv.unlp.edu.ar/index.php?option=com_content&view=article&id=1938&Itemid=1811

Samples for biotoxin detection were exported and analyzed at the *Alfred Wegener Institute for Polar and Marine Research*[6] (AWI, Bremerhaven, Germany). Sample processing and diagnostics were performed by LAMAMA scientists Dr. Valeria D'Agostino and Dr. Mariana Degrati, under the supervision of Dr. Bernd Krock. Targeted biotoxins included amnesic shellfish toxins (domoic acid) and paralytic shellfish toxins. Samples from liver, kidney, blood and muscle were lyophilized in Argentina to eliminate water content without altering sample composition. Samples were then grounded, aliquoted in halves, and stored in ziploc bags at -20 °C until shipment to Germany. At the lab, samples of liver, kidney, muscle and blood were processed as described in Luckas et al. (2015) for domoic acid (DA) and paralytic shellfish poisoning (PSP) toxins extraction. Samples were weighed (4.0 ± 0.1 g) into a centrifuge tube. After adding 5 mL of methanol for DA and 0.2 M hydrochloric acid (HCl) for PSP toxins extraction, the sample was homogenized for 1 min with a vortex, and then placed in an ultrasonic bath for 5 min. After homogenization, samples were centrifuged at $2,500 \times g$ for 5 min and the supernatant was transferred to a centrifuge tube. The residues were re-extracted with 3 mL extraction solvent two times. The third centrifugation runs were at $3220 \times g$ for 10 min. The supernatant was transferred to the centrifuge tube, the final volume was recorded, and 0.5 mL of the extract filtered through a spin filter (0.45 mm pore-size) and centrifuged at $16,100 \times g$ at 4 °C for 2 min, followed by transfer to autosampler vials. Analysis of DA and PSP toxins were performed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), as described in Krock et al. (2008) and Boundy et al. (2015), respectively.

RESULTS

Demography and necropsy findings

Of the 68 stranded dolphins, 21 were returned alive to the sea, while 47 animals died on the beach and were recovered for post-mortem examination. Dead dolphins included all ages, with more males than females (29 males and 18 females). Most of the adult females were pregnant or lactating. All animals appeared in good overall condition. No animals showed signs of potential human interaction (i.e. net markings, amputations).

The fifteen animals examined for this investigation included eight adult males, seven adult females and one unborn fetus (Table 1). An additional unborn fetus was recovered and preserved frozen for anatomical studies. Length varied from 191 to 200 cm in adult females (mean 195.5 cm), and from 204 to 219 in adult males (mean 212.7 cm). Weights of adult females ranged between 82.5 and 101.5 kg (mean 92kg), and adult males between 103 and 130 kg (mean 120kg) (Figures 2 and 3). Three adult females were pregnant (two of them near term, and another one with a fetus about 6.5cm length); two were lactating but not pregnant, and two were resting (neither pregnant nor lactating).

No significant lesions were noted during gross post-mortem examination. All animals were in good body condition, with expected body fat and musculature. Some of the larger males were missing several teeth in the upper and lower jaws. Suspect skin lesions or abnormalities were collected for ancillary histological and virological examination by C. Fiorito (CESIMAR-CONICET). Nematodes were observed in the ears of 2 individuals, a trematode cyst in the caudal blubber of one individual, and undetermined parasites were recorded in the liver of two individuals and in the lung and kidney of others. More in depth studies of these incidental findings are in process. Macroscopic assessment was in many cases limited by carcass condition, with internal organs partially frozen or quickly decomposing upon thawing. Gastrointestinal tracts were not examined at necropsy and were preserved whole for later processing for dietary contents, parasites, etc. Skulls were not opened, and brain samples were collected with a spoon-like tool through the foramen magnum at the base of the skull.

A full set of tissue samples (all tissues and organs and any obvious abnormalities) was collected from 15 adult dolphins and 1 fetus. Ears were dissected from 9 adult individuals.

Table 1. Sex, age, morphometrics and reproductive status of seventeen short-beaked common dolphins (*Delphinus delphis*) included in full cause of death investigation.

ID	Sex	Weight (kg)	Standard length (cm)	Age category	Necropsy date	Reproductive status
Dd 085	M	121	219	adult	24-04-18	Mature*
Dd 086	M	124	219	adult	25-04-18	Mature*
Dd 087	M	117.5	215	adult	25-04-18	Mature*

Dd 088	F	91	198	adult	26-04-18	Early pregnancy (fetus ~6.5cm)
Dd 089	M	113	210	adult	26-04-18	Mature*
Dd 090	M	103	204	adult	27-04-18	Mature*
Dd 091	M	124	204	adult	27-04-18	Mature*
Dd 092	F	99.5	197	adult	03-05-18	Pregnant near full term. Fetus Dd 095. Non-developed mammary gland. No milk.
Dd 093	F	82.5	197	adult	03-05-18	Not pregnant, not nursing. Non-developed mammary gland
Dd 094	F	101.5	200	adult	03-05-18	Not pregnant but nursing. Mammary glands developed with much milk.
Dd 095	F	8.7	99 (curved)	Unborn fetus	03-05-18	
Dd 096	F	85.5	191	adult	04-05-18	Not pregnant, but nursing
Dd 097	F	100	194	adult	04-05-18	Pregnant, advanced. Fetus Dd 098. Little-developed mammary gland. No milk.
Dd 099	F	85	192	adult	04-05-18	Not pregnant, but nursing
Dd 100	M	128.5	215	adult	05-05-18	Mature*
Dd 101	M	130	216	adult	05-05-18	Mature*

* presence of semen in testis and/or epididymis
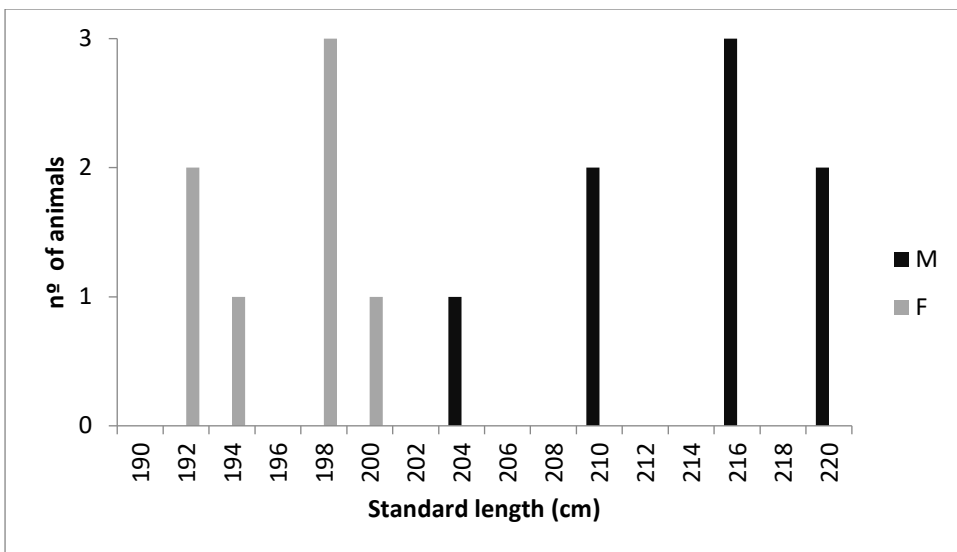
Figure 2. Frequency of examined individual dolphins, females and males, by length.

Date
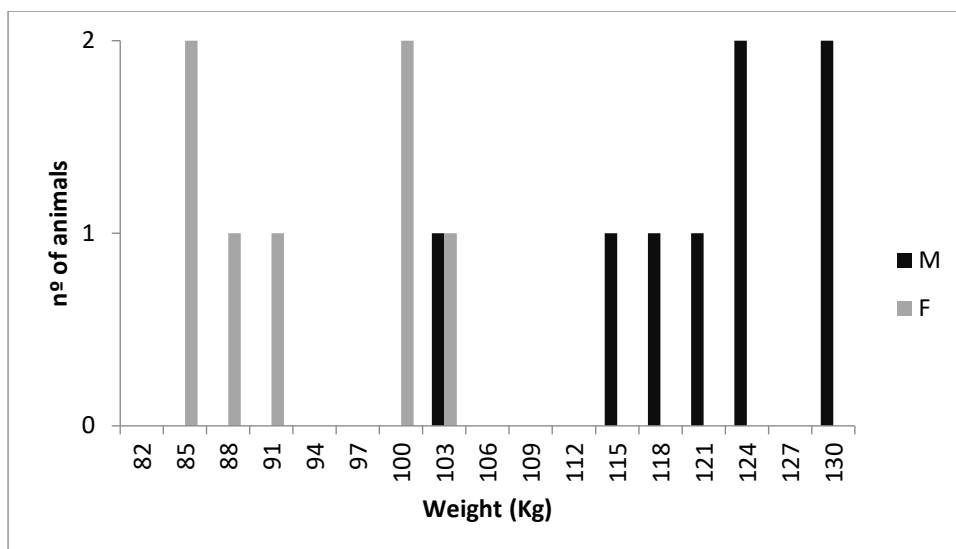
Figure 3. Frequency of examined individual dolphins, females and males, by weight.

Diagnostic findings

Influenza A virus: Samples analysed belonged to 16 dolphins, of which 8 were adult males, 7 were adult females, and 1 was a full-term female fetus. Types of samples analysed included lung (N = 16), pulmonary lymph node (N = 16), pericardial lymph node (N = 1), prescapular lymph node (N = 13), and rectal swab (N = 14). All samples were negative for Influenza A virus by real-time PCR.

*Morbillivirus***:** Samples from 16 dolphins were analyzed, including brain (N = 16), lung (N = 15), and pulmonary lymph node (N = 15). In all cases the GAPDH gene was amplified (confirming the integrity of RNA), but no samples were positive for Morbillivirus by RT-PCR.

*Protozoans***:** Brain samples, heart, tongue and other muscles (diaphragm, masseter, dorsal and/or ventral) of 16 dolphins were processed. In addition, a vesicular structure found in the diaphragm of 1 of the dolphins (Dd87) was analysed. Microscopic observations of fresh tissue revealed only one suspect cystic structure in Dd94. No other cysts were found in either heart of muscle pools. Pigmented cells were observed in 5 of 16 heart samples and 7 of 16 muscle pools. The significance of this finding is unknown. Arthropod eggs, suggestive of contamination during thawing and necropsy were recorded in two muscle pool samples. The macroscopic and microscopic observation of the cystic structures of Dd87 (collected at necropsy) and Dd94 (found during microscopic examination), were negative for the identification of structures produced by protozoa. Additionally, samples from heart, skeletal muscles and cystic structures were all negative for *T. gondii, Sarcocystis* spp. and *Neospora caninum* by conventional PCR.

Biotoxins: Domoic acid levels in dolphin samples were below the detection limit (LOD) (LOD liver = 4.15 – 5.40 ng/g; LOD kidney = 1.66 – 5.60 ng/g and LOD muscle = 3.32 – 5.19 ng/g). Paralytic shellfish toxins were detected in blood samples of 11 of the 17 dolphins tested, with levels between 0.43 and 1.64 pg µL (Table 2). Liver, kidney, and muscle samples from 17 dolphins analyzed in the present study were negative for PSP toxins. However, PSP toxins were detected in 23 kidney samples of additional common dolphins stranded in the same event but not included in the cause of death investigation and reached a maximum level of 9.70 pg/µL (data not shown).

Table 2. Paralytic shellfish toxin (C3/C4) levels in blood samples from 11/16 stranded common dolphins included in this study

ID	C3/C4 pg/µL
Dd 087	1.64
Dd 089	0.43
Dd 091	1.11
Dd 092	0.00
Dd 093	0.86
Dd 094	0.00
Dd 095	0.74
Dd 096	1.11

Dd 097	1.09
Dd 099	0.48
Dd 100	0.82

Histopathology: case materials from a total of 16 adult common dolphins, including 8 males and 8 females (including a fetus) were examined histologically. Females included 3 pregnant females (Dd88 -fetus not available for histology; Dd92 -fetus Dd95; and Dd97 (fetus Dd98 - not available for histology); and 1 lactating female, Dd94. A total of 326 slides were reviewed, with an average number of slides per case of 20, and an average number of tissue types per case of 17. Tissue types were: heart (16), trachea (10), lung (16), liver (16), kidney (16), urinary bladder (14), spleen (11), lymph node (16), tongue (13), oesophagus (9), stomach (0), small intestine (2), large intestine (0), pancreas (13), skin (13), skeletal muscle (16), connective tissue (12), uterus (7), mammary gland (5), umbilicus (2), testis (8), epididymis (8), penis (1), artery (11), adipose (5), adrenal gland (14), gland (not otherwise specified (1), brain (10), spinal cord (4), peripheral nerve (4). Histologic examination was performed on a total of 28 different tissue types (average number of tissue types/animal = 17) including brain (10), lung (16), heart (16), liver (16), kidney (16) and spleen (11).

In utero death of a fetal dolphin (Dd95) due to the death of the dam was the only dolphin of the 16 in which a cause of death was determined. Significant infectious, inflammatory or other pathologic processes that would have contributed to or caused the stranding event were not identified in tissues from the fetus or the other 15 dolphins. Samples of small intestine from two dolphins were the only samples from the gastrointestinal tract available for histologic review; histologic lesions were not seen in the small intestinal samples from these two dolphins. Mild multifocal metazoan parasite infection with associated chronic inflammation was seen in the pancreas of one adult female dolphin. It was considered an incidental finding and not severe enough to have been significant in the stranding or death of the animal.

DISCUSSION

Our results indicate that the death of 16 short-beaked common dolphins assessed in this study was not due to obvious human effects (e.g. bycatch) or underlying pathologies, as all animals were in good body condition and had no external evidence of injuries. Infections by Morbillivirus, Influenza A virus, *Sarcocystis* spp., *Toxoplasma gondii*, or *Neospora caninum,* as well domoic acid (DA) toxicity were ruled out as ethiologies in this event. Notably, results on exposure to paralytic shelfish toxins (PSP) were the only investigated cause of death found positive. This is the first documentation of exposure to PSP toxins in short-beaked common dolphins from the Argentine Sea.

PSP are potent neurotoxins produced by natural, environmentally-driven populations of some marine dinoflagellates (Landsberg, 2002). In Golfo Nuevo, the dinoflagellate *A. catenella* (formerly named *A. tamarense*) is the recognized source of PSP toxins. In this gulf, the blooms of *A. catenella* as well as the detection of PSP toxins are recurrent phenomena. Consequently, PSP toxins are regularly monitored and shellfisheries have been closed annually when PSP toxins have exceeded the regulatory limit of 80 µg saxitoxin (STX) eq 100 g^{-1} of mussel tissue (HAB and Shellfish Toxicity Monitoring Program; Wilson et al., 2015). Several seabird mortalities linked to PSP toxins have been reported in the area since the 2000s, though some remain unconfirmed (Quintana et al., 2000; Uhart et al., 2008). More recently, Wilson et al. (2015) described PSP toxins in two dead southern right whales (*Eubalaena australis*) calves in Golfo Nuevo (feces, stomach tissue and liver).

We found PSP toxins in 34 samples (11 blood and 23 kidney samples) from 47 dolphins. The detection of toxins in the blood and kidney suggest acute exposure, with the toxins recently absorbed into the bloodstream from the gut and initial kidney filtration. In an experimental study in cats (*Felis catus*), Andrinolo et al. (2002) described toxic levels of PSP in blood within 60 minutes of ingestion, and in urine at 150 minutes. These authors also noted that toxic levels in blood were accompanied by a fall in arterial pressure and reduction in urinary flow, which would explain toxin detection in kidneys yet absence of toxin in urine in our study.

Most dolphins examined in our study had full stomachs, and many had fish in their oesophagus, suggesting active feeding near to the time of death. PSP toxins were found in anchovies collected two days after the event near the stranding location (data not shown). While gut content of stranded dolphins has not yet been analyzed for PSP toxins, the presence of these neurotoxins in a common prey item suggests a potential connection between toxic algal producers and dead dolphins. Further work is underway to explore this hypothesis.

Previous reports of PSP toxin-linked death in dolphins include two captive Atlantic dolphins (species not specified) from an artificial lagoon in a hotel in Hawaii in 1989 (Hokama et al., 1990). Also, Fire et al.

(unpublished[7]) described saxitoxin in apparently healthy and stranded bottlenose dolphins (*Tursiops truncatus*) from Indian River Lagoon, Florida between 2001- 2012. Recently, Starr et al., (2017) reported a multi-species PSP toxic event from 2008 in St. Lawrence Estuary, Canada. This event affected belugas (*Delphinapterus leucas*), a fin whale (*Balaenoptera physalus*), harbour porpoises (*Phocoena phocoena*) and seals (mostly Grey seals, *Halichoerus grypus*), as well as large numbers of several seabird species. To the best of our knowledge, no other species were affected in the Península Valdés area at the time of the dolphin mass stranding reported here.

Unlike human PSP toxicity, there are no reference toxin cut-off values in wildlife, and different taxa seem to have distinct tolerance levels (Landsberg, 2002; Lefebvre et al., 2016). In the study conducted by Fire et al. (unpublished), saxitoxin was found both in stranded and live dolphins showing no signs of illness. The values reported for both groups are higher than the ones found in our study. Starr et al., (2017) reported PST >2.2 μg 100 g-1 in the liver and/or gut contents and tissues of about half the animals they examined (>300). However, they found great variations in positive tissues and toxin levels across taxa examined. Exposure to saxitoxin has been reported in living North Atlantic right whales (*Eubalaena glacialis*) (Durbin et al., 2002; Doucette et al., 2012). In a study spanning 2001-2006, the highest mean value was 0.793mg STX eq./g feces, and a maximum of 1.763mg STX eq./g, exceeding by far the toxicity threshold for humans (Doucette et al., 2012). Negative direct (neurotoxicity) and indirect (behavioural, physiological) impacts from these toxins are assumed to be significant for this endangered species yet remain uncharacterized.

In our study toxin levels were quantified via LC-MS, which is considered the best available technique for marine toxins (Cusick et al., 2013). Whether the levels found were enough to cause death of all deceased dolphins is unclear. A concentration of toxin above a certain threshold may be lethal, whereas lower doses may cause only a mild physiological, pathological, or behavioral response (Landsberg, 2002). In 1997, over 100 Mediterranean monk seals (*Monachus monachus*) were thought to have died from PSP on coastal western Sahara, Africa. Dead seals were in good nutritional condition and only congestion of the lungs was observed upon necropsy (Hernández et al., 1998). Terminally ill animals were described to be lethargic, lacking motor coodination, and appearing to be paralyzed in the water. The authors state that the period between the onset of clinical signs and death was short, and the animals were asumed to have drowned from paralysis caused by the toxins (Hernández et al., 1998). A similar pattern was described by Geraci et al., (1989) in 14 humpback whales (*Megaptera novaenglae*) stranded over five weeks on Cape Cod Bay in 1987-88, also attributed to PSP poisoning.

At present our results are insufficient to assess whether PSP toxin exposure played a role in the death of the stranded dolphins. Notwithstanding, the full documentation and investigation of the most commoly reported pathogens and toxins involved in cetacean mass strandings allowed us to clear the most relevant health differentials and suggests areas for future study.

Additional potential hypothesis related to factors known or speculated to cause cetacean mass strandings, such as social structure and behavior, extreme weather, extreme tides, predator avoidance, seismic activity or other sonar disruptions, and feeding-frenzy are currently being explored within the ecological context at the time of the event.

REFERENCES

Andrinolo, D., Iglesias, V., García, C., & Lagos, N. (2002). Toxicokinetics and toxicodynamics of gonyautoxins after an oral toxin dose in cats. Toxicon, 40(6), 699-709. https://doi.org/10.1016/S0041-0101(01)00263-X.
Barett, T., Visser, I.K., Mamaev, L., Goatley, L., Van Bressem, M.F., & Osterhaust, A.D. (1993). Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology, 193(2), 1010–1012. https://doi.org/10.1006/viro.1993.1217.

---

[7] https://conference.ifas.ufl.edu/marinemammal18/presentations/Wednesday/0945%20Spencer%20Fire.pdf

Boundy, M.J., Selwood, A.I., Harwood, D. T., McNabb, P. S., & Turner, A.D. (2015). Development of a sensitive and selective liquid chromatography–mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction. Journal of Chromatography A, 1387, 1-12. https://doi.org/10.1016/j.chroma.2015.01.086.

Brownell, R.L., Siciliano, S., & de Moura, J.F. (2012). Mass stranding of Short-beaked common dolphins, *Delphinus delphis*, at Arraial do Cabo, Brazil: Natural or Human Induced? International Whaling Commission, SC/65a/SM27.

Corcuera, J., Monzón, F., Crespo, E.A., Aguilar, A., & Raga, J.A. (1994). Interactions between marine mammals and coastal fisheries of Necochea and Claromecó Buenos Aires Province, Argentina. 1994 Gillnets and Cetaceans. In: Perrin, W.F., Donovan G. P., Barlow J. (Eds.) Rep. Int. Whal. Comm., Special Issue 15, 283–290.

Crespo, E.A., Pedraza, S.N., Dans, S.L., Koen Alonso, M, Reyes, L.M., Garcia, N.A., Coscarella, M. & Schiavini, A.C.M. (1997). Direct and Indirect Effects of the High seas Fisheries on the Marine Mammal Populations in the northern and central Patagonian Coast. Journal Northwest Atlantic Fisheries Science, 22, 189-207.

Crespo, E.A., Koen Alonso, M., Dans, S.L., García, N.A., Pedraza, S.N., Coscarella, M.A. & González, R. (2000). Incidental Catch of Dolphins in Mid-Water Trawls for Southern Anchovy Off Patagonia. Journal of Cetacean Research and Management, 2(1), 11-16.

Crespo, E.A., Lewis, M.N., & Campagna, C. (2007). Mamíferos marinos: pinnipedios y cetáceos. In: Carreto, J.I., & Bremec, C. (Eds.). El Mar Argentino y sus recursos pesqueros, Tomo 5. El Ecosistema Marino. INIDEP. Pp 127-150.

Crespo, E.A., & Dans, S.L. (2008). Bases para el monitoreo y herramientas de gestión para el manejo de las poblaciones de mamíferos marinos afectadas por las actividades turísticas y recreativas en el litoral Patagónico. Tech. Report FMAM/BIRF 28385-AR. Proyecto PNUD ARG 02/018. 16 pp.

Cusick, K.D., & Sayler G.S. (2013). An Overview on the Marine Neurotoxin, Saxitoxin: Genetics, Molecular Targets, Methods of Detection and Ecological Functions. Marine Drugs, 11, 991-1018. doi:10.3390/md11040991

Doucette, G.J., Mikulski, C.M., King, K.L., Roth, P.B., Wang, Z., Leandro, L.F., DeGrasse, S.L., White, K.D., De Biase, D., Gillett, R.M., & Rolland, R.M. (2012). Endangered North Atlantic right whales (*Eubalaena glacialis*) experience repeated, concurrent exposure to multiple environmental neurotoxins produced by marine algae. Environmental Research, 112, 67-76, ISSN 0013-9351, https://doi.org/10.1016/j.envres.2011.09.010.

Durbin, E., Teegarden, G., Campbell, R., Cembella, A., Baumgartner, M.F., & Mate, B.R. (2002). North Atlantic right whales, *Eubalaena glacialis*, exposed to paralytic sellfish poisoning (PSP) toxins via zooplankton vector, *Calanus finmarchicus*. Harmful Algae, 1, 243-251. https://doi.org/10.1016/S1568-9883(02)00046-X.

Geraci, J.R., Anderson, D.M., Timperi, R.J., St. Aubin, D.J., Early, G.A., Prescott, J.H., & Mayo, C.A. (1989). Humpback whales (*Megatpera novaengliae*) fatally poisoned by dinoflagellate toxin. Canadian Journal of Fisheries and Aquatic Science, 46, 1895-1898.

Hernández, M., Robinson, I., Aguilar, A., González, L.M., López-Jurado, L.F., Reyero, M.I., Cacho, E., Franco, J., López-Rodas, V., & Costas, E. (1998). Did algal toxins cause monk seal mortality? Nature, 393, 28–29.

Heyning, J.E., & Perrin, W.F. (1994). Evidence for two species of common dolphins genus *Delphinus* from the eastern North Pacific. Nat. Hist. Mus. Los Angel. Cty. Contrib. Sci. 442, 1–35.

Hokama, Y., Asahina, A.Y., Hong, T.W.P., Katsura, K., Shang, E., Miyahara, J.T., Sweeney, J.C., Stone, R. (1990). Causitive Toxin(s) in the Death of Two Atlantic Dolphins. Journal of Clinical Laboratory Analysis, 4, 474-478. https://doi.org/10.1002/jcla.1860040615.

Jefferson, T.A., Webber, M.A., & Pitman, R.L. (2008). Marine Mammals of the World: A Comprehensive Guide to Their Identification. Academic Press, Elsevier, San Diego.

Jefferson, T.A., Fertl, D., Bolaños-Jiménez, J., & Zerbini, A.N. (2009). Distribution of common dolphins *Delphinus* spp. in the western Atlantic Ocean: a critical re-examination. Marine Biology, 156, 1109–1124. Doi:10.1007/s00227-009-1152-y.

Jepson, P.D., Deaville, R., Acevedo-Whitehouse, K., Barnett, J., Brownlow, A., Brownell Jr., R.L., Clare, F.C., Davison, N., Law, R.J., Loveridge, J., Macgregor, S.K., Morris, S., Murphy, S., Penrose, R., Perkins, M.W., Pinn, E., Seibel, H., Siebert, U., Sierra, E., Simpson, V., Tasker, M.L., Tregenza, N., Cunningham, A.A., & Fernández, A. (2013). What Caused the UK's Largest Common Dolphin (*Delphinus delphis*) Mass Stranding Event? PLoS ONE, 8(4), e60953. https://doi.org/10.1371/journal.pone.0060953

Krock, B., Tillmann, U., Selwood, A.I., & Cembella, A. (2008). Unambiguous identification of pectenotoxin-1 and distribution of pectenotoxins in plankton from the North Sea. Toxicon, 52, 927–935. https://doi.org/10.1016/j.toxicon.2008.09.006.

Landsberg, J.H. (2002). The effects of harmful algal blooms on aquatic organisms. Reviews in Fisheries Science, 10, 113-390. https://doi.org/10.1080/20026491051695.

Lefebvre, K.A., Quakenbush, L., Frame, E., Huntington, K.B., Sheffield, G., Stimmelmayr, R., Bryan, A., Kendrick, P., Ziel, H., Goldstein, T., Snyder, J.A., Gelatt, T., Gulland, F., Dickerson, B., & Gill, V. (2016). Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment. Harmful Algae, 55, 13-24. https://doi.org/10.1016/j.hal.2016.01.007.

Luckas, B., Erler, K., & Krock, B. (2015). Analysis of Marine Biotoxins using LC MS/MS. In: Stengel, D., Connan, S. (editors). Natural Products from Marine Algae Methods and Protocols, Methods in Molecular Biology, New York, Humana Press, 21 p., ISBN: 978 1 4939 2683 1.

Norris, K.S. (1961). Standarized methods for measuring and recording data on the smaller cetaceans. Journal of Mammalogy, 42(4), 471-476.

Moré, G., Basso, W., Bacigalupe, D., Venturini, M.C., & Venturini, L. (2008). Diagnosis of *Sarcocystis cruzi*, *Neospora caninum*, and *Toxoplasma gondii* infections in cattle. Parasitology Research, 102, 671–675. Doi:10.1007/s00436-007-0810-6.

Moré, G., Abrahamovich, P., Jurado, S., Bacigalupe, D., Marin, J.C., Rambeaud, M., Venturini, L., & Venturini, M.C. (2011). Prevalence of Sarcocystis spp. in Argentinean cattle. Veterinary Parasitology, 177(1-2), 162-5. doi:10.1016/j.vetpar.2010.11.036.

Moré, G., Scharesa, S., Maksimova, A., Conrathsa, F.J., Venturini, M.C., Scharesaa, F.G. (2013). Development of a multiplex real time PCR to differentiate Sarcocystis spp. affecting cattle. Veterinary Parasitology, 197(1–2), 85-94. https://doi.org/10.1016/j.vetpar.2013.04.024

Moré, G.A., Pantchev, N., Herrmann, D., Globokar Vrhovec, M., Öfner, S., Conraths, F.J., & Schares, G. (2014). Molecular identification of Sarcocystis spp. helped to define the origin of green pythons (*Morelia viridis*) confiscated in Germany. Parasitology, 141, 646–651. doi:10.1017/S0031182013001960

NOAA. http://www.nmfs.noaa.gov/pr/health/commondolphins_massachusetts2012.htm (accessed 04042018).

Pardini, L., Carral, A., Bernstein, M., Gos, M.L., Olejnik, P., Unzaga, M.J., Kaufer, F.J., Freuler, C.B., Durlach, R.A., & Venturini, M.C. (2014). First isolation and molecular characterization of *Toxoplasma gondii* from a human placenta in Argentina. Parasitology International, 63, 470–472. https://doi.org/10.1016/j.parint.2013.10.011

Perrin, W.F. (2002). Common dolphins *Delphinus delphis*, *D. capensis*, and *D.tropicalis*. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals. Academic Press, San Diego, pp. 245–248.

Pugliares, K.R., Bogomolni, A., Touhey, K.M., Herzig, S.M., Harry, C.T., & Moore, J.M. (2007). Marine Mammal Necropsy: An introductory guide for stranding responders and field biologists Adapted and Excerpted from WHOI-2007-06, Woods Hole Oceanographic Institution.

Quintana, F., Uhart, M., & García Borboroglu, P. (2001). Causa de mortandad masiva de aves marinas en las costas de Chubut. Unpublished Report, 18 January 2001, pp. 1–3.

Starr, M., Lair, S., Michaud, S., Scarratt, M., Quilliam, M., Lefaivre, D., Robert, M., Wotherspoon, A., Michaud, R., Ménard, N., Sauvé, G., Lessard, S., Béland, P., & Measures, L. (2017). Multispecies mass mortality of marine fauna linked to a toxic dinoflagellate bloom. PLoS ONE, 12(5), e0176299. https://doi.org/10.1371/journal.pone.0176299

Stockin, K.A. (2008). The New Zealand common dolphin (*Delphinus sp.*) – Identity, ecology and conservation. PhD thesis, Massey University, Auckland, 235pp.

Uhart, M., Montoya, N., Lisnizer, N., García Borboroglu, P., Gatto, A., Svagelj, W., Mauco, L., & Carreto, J.I. (2008). Mortalidad de gaviota cocinera (*Larus dominicanus*) por toxina paralizante de los moluscos en Chubut, Argentina. XII Reunión Argentina de Ornitología. Neuquén, Argentina, Pp. 60.

Viricel, A., Strand, A., Rosel, P., Ridoux, V., & Garcia, P. (2008). Insights on common dolphin (*Delphinus delphis*) social organization from genetic analysis of a mass-stranded pod. Behavioral Ecology and Sociobiology, 63, 173–185. https://doi.org/10.1007/s00265-008-0648-7.

Wilson, C., Sastre, A.V., Hoffmeyer, M., Rowntree, V.J., Fire, S.E., Santinelli, N.H., Díaz Ovejero, S., D'Agostino, V.C., Maron, C., Doucette, G., Broadwater, M., Wang, Z., Montoya, N., Seger, J., Adler, F., Sironi, M., & Uhart, M. (2015). Southern right whale (*Eubalaena australis*) calf mortality at Península Valdés, Argentina: Are harmful algal blooms to blame? Marine Mammal Science, 32(2), 423-451. https://doi.org/10.1111/mms.12263.

Young, N. M., Arruda, J., Costidis, A., Cramer, S., Ketten, D.R., McLellan, W., Montie, E.W., Moore, M.J., & Rommel, S. (2007). Odontocete Salvage, Necropsy, Ear Extraction, and Imaging Protocol. Ocean Research, Conservation and Solutions [ORCAS] and ONR, 1-171.