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Paralytic shellfish poisoning and mortality of Southern right whales (Eubalaena australis) in Golfo Nuevo, Península Valdés, Argentina in 2022

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Paralytic shellfish poisoning and mortality of Southern right whales (*Eubalaena australis*) in Golfo Nuevo, Península Valdés, Argentina in 2022

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ABSTRACT

A total of 28 adult (22 females) and 2 juvenile Southern right whales (SRW, Eubalaena australis) died acutely between 24 September - 11 October 2022, in Golfo Nuevo, Península Valdés, Argentina. Six whales were necropsied and found in good body condition with recent food intake and no evidence of trauma. The neurotoxin producing Pseudo-nitzschia australis diatom and Alexandrium catenella/tamarense complex dinoflagellates were identified in gut content from 4/5 whales. Tissues and fluids from 8 whales including stomach and intestinal content, liver, aqueous and vitreous humor of the eye, kidney, milk, urine, and blood were tested for biotoxins. Domoic acid was not detected. Paralytic Shellfish Toxins (PST) were found in 19/24 samples tested, from all whales. Saxitoxin was the most frequent toxin (n=14), followed by neosaxitoxin, and gonyautoxins 3 and 2. Evidence of toxin metabolism following ingestion was found. The highest toxicity levels (ug STX eq.g⁻¹) were measured in intestinal content, blood, and feces. Whale mortality overlapped in time and space with a Harmful Algal Bloom (HAB) and unprecedented values of PST in shellfish and plankton in Golfo Nuevo. Adult females were the most affected group, coinciding with high energy demands from nursing newborn calves and urge to feed on spring plankton blooms. This is the largest known adult SRW mortality to date for the species. Although HABs are widespread along the Patagonian Shelf, their occurrence at Península Valdés raises concern for SRW conservation given increasing adult and juvenile mortality since 2019.

KEYWORDS: SOUTHERN RIGHT WHALE, MORTALITY, HARMFUL ALGAL BLOOM, PARALYTIC SHELLFISH POISON, PENINSULA VALDES.

INTRODUCTION

Harmful algal blooms (HABs) occur worldwide and have been increasingly linked to marine mammal mortality events (Fire and Van Dolah 2012, Fire et al. 2021). During HABs, several species of phytoplankton produce toxins that then accumulate and concentrate in the marine food webs. Marine mammals become exposed by consuming toxic prey. The most common toxins affecting marine mammals are neurotoxins, which cause disorientation, seizures, ataxia, muscle incoordination, and often death by respiratory paralysis (Cusick and Sayler 2013, Fire et al. 2021). Two major classes of HAB toxins are known to affect marine wildlife in South America and the Patagonian Sea, namely saxitoxins and domoic acid (Uhart et al. 2004, Uhart et al. 2008, Fire and Van Dolah 2012, Montoya 2019). Saxitoxins (STX) are one of the paralytic shellfish poisons produced by dinoflagellates in the *Alexandrium* genera, while domoic acid (DA) or amnesic shellfish poison is produced by diatoms of the *Pseudo-nitzchia* genera.

STX and DA have been detected in both mysticetes and odontocetes in North America, in some cases associated with mortality, but sublethal exposure also occurs (Doucette et al. 2006, Torres de la Riva et al. 2009, Fire et al. 2021). Lethal cases are often seen during HABs that are broadly spread, intense, and affecting numerous species (Torres de la Riva et al. 2009). Two baleen whale mass mortality events have been attributed to PSPs. One involved Humpback whales (*Megaptera novaeangliae*) in Cape Cod Bay, USA (Geraci et al. 1989). The event extended over five weeks and affected adult whales in good body condition which died acutely with fresh food in their gut. More recently, an impressively massive mortality of over 340 sei whales (*Balaenoptera borealis*) in Southern Chile was attributed to HABs in 2015-16 (Häussermann et al. 2017).

Exposure to DA and STX has been recorded in Southern Right Whales (SRW, *Eubalaena australis*) at the Península Valdés breeding ground (Wilson et al. 2016, D'Agostino et al. 2017). However, toxins have been detected in feces from apparently healthy living whales (D'Agostino et al. 2017) and links to calf mortality have remained inconclusive, despite spatial and temporal overlap with significant HABs in the area (Wilson et al. 2016).

The Southern Right Whale Health Monitoring Program studies the whales that die at Península Valdés, Argentina, since 2003. The researchers have documented unexplained increased adult and juvenile mortality since 2019 (see Sironi et al. this meeting). Here we report an event of unusual mortality of adult and juvenile SRW in 2022 at Golfo Nuevo, Península Valdés, attributed to PSPs within the context of an unprecedented HAB in the area during the peak of whale breeding season.

Adult and juvenile southern right whale mortality event

Between 24 September and 11 October 2022, 30 adult and juvenile whale deaths were recorded in Península Valdés, 29 in Golfo Nuevo, the southern gulf and 1 in Golfo San José, the northern gulf (Figure 1). One additional juvenile was found in Golfo San José on November 2. The total count for the event was 28 adults (22 females, 2 males, 4 unknown) and 3 juveniles (1 male, 2 unknown).

The event lasted approximately three weeks, and carcasses were found between the end of September (n=8; 22%) and mid-October (n=22; 71%). However, half of the dead whales (n=12) found in October were sighted during a survey flight performed on October 11. During the survey, the carcasses were scattered around the peninsula and in a similar advanced state of decay, indicating that the deaths occurred earlier (1-2 weeks prior). The highest number of strandings of adults and juveniles (89 %) occurred on the northern and western shores of Golfo Nuevo where mothers with calves congregate at that time of year.

At the time of whale deaths, a HAB was detected in Golfo Nuevo with record levels of PSP in shellfish, at 37410 mouse units in mussels near Puerto Pirámides (Direccion Provincial de Salud Ambiental, Ministerio de Salud, Chubut). A ban on shellfish consumption was imposed, since the limit for human toxicity of 400 mouse units was broadly exceeded (Código Alimentario Argentino).

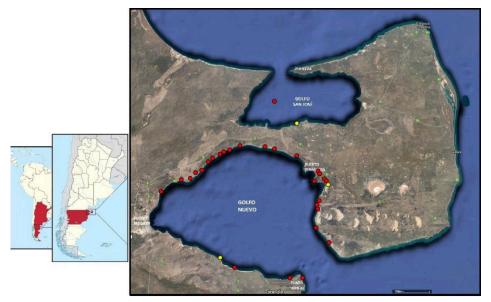


Figure 1. Location of dead whales during the mortality event at Península Valdés in 2022. Carcasses clustered along the northern and western shores of Golfo Nuevo. Red: adults, yellow: juveniles.

Investigation and preliminary results

Given the acute nature of whale deaths, the abrupt rise in mortality (8 deaths in 6 days), and the spatio-temporal overlap with the ongoing HAB with extreme levels of PSP, we investigated the event under the leading hypothesis of HAB poisoning.

The freshest and most accessible carcasses were selected for necropsies (n=9, 29%). Full necropsies and diagnostic sample collection (mainly gut, liver, kidney, urine, blood, eye fluids, milk) were performed on 6 whales (5 adults and 1 juvenile). Minimally invasive samples (milk, eye fluids) were collected from 3 additional whales which stranded in public areas where full necropsies are not allowed (these were later towed to secure beaches where additional samples, e.g. intestinal content, were collected at a later date, albeit in poorer condition). Diagnostic samples were not collected from the remaining 22 whales during the acute event, since they either stranded in public areas and were not towed to other beaches, were in an advanced state of decay when found, or were removed by the tides before we reached them. Response to carcasses stranded in accessible and public areas where concern was highest was prioritized over inaccessible areas. Notwithstanding, all carcasses that washed up on beaches (e.g., did not remain floating at sea or sunk) were examined to rule out trauma as a cause of death and collect relevant biological samples, e.g., for genetics. Baleen was collected from 21 (68%) carcasses.

Relevant findings

All the whales examined were in good body condition, with a thick blubber layer and abundant visceral fat. The 6 whales necropsied had contents in their digestive tract, indicating that they had fed prior to their death. None of the whales showed evidence of trauma or injuries that could explain their death.

Gut contents from 5 whales were recovered and analyzed locally by phytoplankton experts at Universidad Nacional de la Patagonia San Juan Bosco, Chubut. Findings included *Pseudo-nitzschia australis* diatoms in 2 whales, cells resembling the dinoflagellate *Alexandrium catenella/tamarense complex* in 2 whales, copepod fragments in 3, other unidentified diatoms in 3, and unidentified dinoflagellate cells in 1 whale (details in Table 1).

Tissues and fluids from 8 whales (n=24 specimens) including stomach and intestinal content, liver, aqueous and vitreous humor of the eye, kidney, milk, urine, and blood, were tested for biotoxins at the Argentina reference laboratory, Instituto Nacional de Desarrollo Pesquero, Buenos Aires. Specimens were analyzed for DA by HPLC and mass spectrometry and by HPLC for PSP (details in Montoya et al. 2022). All samples were negative for DA. However, quantifiable levels of PSP were found in 19/24 (70%) specimens analyzed from 7/8 whales tested (details in Tables 1 and 2), while trace levels were found in one adult female (092722PVEa34) for which only urine was available (Table 1). Quantifiable toxin types included STX (n=14), neosaxitoxin (NeoSTX) (n=8), gonyautoxin 2 (GTX2) (n=8), and gonyautoxin 3 (GTX3) (n=7) (Table 2). Only trace levels were found for Decarbamoylsaxitoxin (dcSTX) and Decarbamoylgonyautoxin3 (dcGTX3), and GTX1, GTX4, C1 and C2 were negative. Highest toxicity levels (ug STX eq.g⁻¹) were found in intestinal content, blood, and feces, followed by vitreous and aqueous humor of the eye, stomach content, and milk (Table 2).

At the time of this report, tissue samples remain to be analyzed by histopathology to identify potential lesions associated with gross necropsy and toxicological findings.

Discussion

The results demonstrate that the whales ingested and metabolized PSP toxins, with detectable levels found in 70% of specimens tested. This supports the hypothesis that the whales died during an extraordinary bloom of the dinoflagellate *Alexandrium tamarense/catenella* complex in Golfo Nuevo, which occurred between late September and early October 2022. Adult females were the age and sex class most affected, coinciding with high energy demands from nursing newborn calves and consequent urge to feed on spring plankton blooms (D'Agostino et al. 2023).

Paralytic Shellfish Poison toxin types and concentrations varied by whale and specimen tested. STX was the most frequent toxin found (n=14), and the highest toxin levels were measured in the first adult female to die (092422PVEa29) in both intestinal content and feces. Evidence of toxin metabolism, a mechanism aimed at making them more water soluble to facilitate shedding in urine, was observed. Moreover, the toxin profile detected in whale specimens differed from that of *A. tamarense/catenella complex* tested during the same event, further reinforcing that transformation of PSP occurred in the whales following ingestion (Montoya et al. 2022).

Lethal doses of PSP are unknown for right whales and mysticetes in general. PSP at concentrations as high as 0.5 µg STX equivalents g⁻¹ have been detected in feces of living, apparently healthy North Atlantic right whales (*Eubalaena glacialis*) (Doucette et al. 2006). In the only previously reported mass mortality of baleen whales attributed to PSP, 13 adult humpback whales were observed to die within a few hours of consuming toxic prey (Geraci et al. 1989). Toxin levels in that report are not comparable to our study given differences in analytical methods used (immunoassay versus HPLC, respectively), species, prey, and location. However, acute death and gross necropsy findings are similar, with whales found with partially digested prey in their gut and robust body condition, with a thick blubber layer. Likewise, PSP toxins were detected in high concentrations in the whale's prey and gut contents (Geraci et al. 1989).

Saxitoxins have been previously detected in two young whale calves (5.04 and 5.5 m length) that died in Golfo Nuevo in 2008 and 2009, respectively, during HABs (Wilson et al. 2016). In this case, transfer of toxins most likely occurred via nursing, and it is unknown whether these calves died from PSP. During the 2022 event, STX was found in the milk of two lactating females, though toxicity levels were relatively low. Ten calf deaths were recorded in Golfo Nuevo following the mortality event (after September 24 and through November 2), despite 22 adult females lost. While it may be that not all affected adult females had a calf, it is also possible that the rapid onset of toxicity and death in the mother hindered further toxin transfer to milk and to the calf. However, the fate of the calves that lost their mothers is unknown. Sightings of adult females with more than one accompanying calf

after the mortality event were reported.

Adult and juvenile right whale mortality at Península Valdés is rare in comparison with calf mortality (Rowntree et al., 2013; Sironi et al., 2018; Uhart et al., 2009). However, since 2019, with the caveat of no data in 2020 due to COVID-19 restrictions, higher than expected numbers of deaths in these categories have been recorded. In 2019, there were 6 dead adults and 1 juvenile (all females), and in 2021 there were 13 dead adults (12 females) and 7 juveniles (2 females). In these two seasons, however, mortalities were spread over several months, though concentrated in October and November, and occurred in both the northern and southern Península Valdés gulfs (see Sironi et al, this meeting). Prior to this recent 3-year cluster, since 2003, juvenile and adult deaths were unusually high in 2005 (4 juveniles and 7 adults) and 2010 (7 juvenile and 6 adult deaths) (Sironi et al., 2012). Analysis of samples collected in 2019 and 2021 is ongoing and may provide evidence of a longer-term shift in HAB exposure in Península Valdés southern right whales.

Conclusions

Findings from the ongoing investigation support the hypothesis that adult and juvenile southern right whales died from exposure to PSP toxins during a HAB event in Golfo Nuevo in 2022. Factors supporting this conclusion are:

(1) the first 8 dead whales were found close together and over the course of only 6 days,

(2) the deaths occurred acutely, and all whales examined showed evidence of recent food intake,

(3) the dead whales were in good body condition, with a thick blubber layer and abundant abdominal fat, and no evidence of external or internal traumatic injuries,

(4) the whales examined had ingested *Pseudo-nitzschia* spp. diatoms and/or dinoflagellates of the genus *Alexandrium catenella/tamarense complex*, producers of neurotoxins capable of causing the death of marine mammals, including large whales,

(5) biotoxins of the Shellfish Paralyzing group were found both in the gastrointestinal contents and in various tissues and fluids of the dead whales examined,

(6) the deaths affected mainly adult females, the age class with the highest energy demand while nursing (Christiansen et al. 2018) and which usually feed on spring plankton blooms in the gulfs of Península Valdés (D'Agostino et al. 2023),

(7) the death of the whales overlapped in time and space with a HAB and very high values of paralytic biotoxins in shellfish and plankton in Golfo Nuevo,

(8) simultaneously with whale mortality, deaths in seabirds and sea lions were recorded, in which the presence of PSP was also found in significant quantities (Uhart et al., in prep.),

(9) no adult whale deaths were recorded after October 11 in Golfo Nuevo, coinciding with a marked decline in the abundance of the paralytic toxin-producing species in plankton and in biotoxin levels in shellfish in this area,

(10) adult females were observed with more than one accompanying calf in the weeks that followed the mortality event, suggesting that some calves had lost their mothers.

This is the largest known adult southern right whale mortality to date. Although HABs are widespread along the Patagonian Shelf (Ramirez et al. 2022), their occurrence at Península Valdés raises concern for southern right whale conservation given increasing adult and juvenile mortality since 2019.

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Whale ID	Age class	Sex	Length (mts)	Date reported/necropsy	Carcass condition	Tissues and fluids positive for PSP	DA	Prey in whale grastrointestinal content
092422PVEa29	A	F	16.73	24/26-Sep	3	Feces, liver, intestinal content, aqueous humor	neg	Pseudo-nitzschia australis, cells compatible with Alexandrium catenella/tamarense complex, other non-identified diatoms, abundant copepod fragments
092622PVEa30	А	F	14.31	26/27-Sep	3	Liver, milk, aqueous humor	neg	Not analyzed
092722PVEa31	А	F	14.66	27/28-Sep	3	Intestinal content, blood, aqueous humor	neg	Centric and pennate non identified diatoms, copepod fragments
092722PVEa32	J	Μ	11.4	27/28-Sep	3	Aqueous humor, intestinal content	neg	Abundant athecate/nude cells compatible with Alexandrium catenella/tamarense complex
092722PVEa34	А	F	14.9	27-Sep/2-Oct	4	Urine	neg	Not analyzed
092822PVEa37	А	F	14.06	28/30-Sep	3	Stomach content, blood, kidney, urine	neg	Non identified spherical dinoflagellates compatible with athecate/nude cells
100222PVEa43	A	F	16.14	2/3-Oct	2	Blood, aqueous humor, vitreous humor	neg	Not analyzed
100322PVEa45	A	F	16.08	3/4-Oct	2	Milk, blood	neg	<i>Pseudo-nitzschia australis,</i> other centric and pennate non-identified diatoms, copepod fragments (sample collected Oct-7-22)

Table 1. Summary data for 8 Southern Right Whales sampled and analyzed for exposure to HAB toxins, including main findings for Paralytic Shellfish Poisons (PSP), Domoic Acid (DA), and prey items found in gastrointestinal content.

Whale ID	Age class	Sex	Specimens with highest PSP levels	Max toxicity ug STXeq.g ⁻¹	PSP toxins quantified
092422PVEa29	А	F	Intestinal content	0.6943	GTX3, GTX2, neoSTX, STX
			Feces	0.3696	neoSTX, STX
			Aqueous humor	0.0129	GTX3, GTX2
092622PVEa30	А	F	Milk	0.0609	STX
			Aqueous humor	0.0027	GTX3, GTX2
092722PVEa31	А	F	Blood	0.2304	neoSTX, STX, GTX4
			Aqueous humor	0.1969	neoSTX, STX
092722PVEa32	J	Μ	Aqueous humor	0.0334	STX
092822PVEa37	А	F	Blood	0.1022	GTX3, GTX2, neoSTX, STX
			Stomach content	0.0718	GTX3, GTX2, STX
100222PVEa43	А	F	Vitreous humor	0.0402	STX
			Blood	0.0736	GTX3, GTX2, neoSTX, STX
100322PVEa45	А	F	Blood	0.1321	GTX2, neoSTX, STX
			Milk	0.05	STX

Table 2. PSP toxins and maximum toxicity quantified in Southern Right Whale tissues and fluids.

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