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Review of historical unusual mortality events (UMEs) in the Gulf of Mexico (1990–2009): providing context for the multi-year northern Gulf of Mexico cetacean UME declared in 2010

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ABSTRACT: An unusual mortality event (UME) was declared for cetaceans in the northern Gulf of Mexico (GoM) for Franklin County, Florida, west through Louisiana, USA, beginning in February 2010 and was ongoing as of September 2014. The 'Deepwater Horizon' (DWH) oil spill began on 20 April 2010 in the GoM, raising questions regarding the potential role of the oil spill in the UME. The present study reviews cetacean mortality events that occurred in the GoM prior to 2010 (n = 11), including causes, durations, and some specific test results, to provide a historical context for the current event. The average duration of GoM cetacean UMEs prior to 2010 was 6 mo, and the longest was 17 mo (2005–2006). The highest number of cetacean mortalities recorded during a previous GoM event was 344 (in 1990). In most previous events, dolphin morbillivirus or brevetoxicosis was confirmed or suspected as a causal factor. In contrast, the current northern GoM UME has lasted more than 48 mo and has had more than 1000 reported mortalities within the currently defined spatial and temporal boundaries of the event. Initial results from the current UME do not support either morbillivirus or brevetoxin as primary causes of this event. This review is the first summary of cetacean UMEs in the GoM and provides evidence that the most common causes of previous UMEs are unlikely to be associated with the current UME.

KEY WORDS: Bottlenose dolphin · *Tursiops truncatus* · Strandings · Brevetoxin · Morbillivirus · 'Deepwater Horizon' oil spill · *Karenia brevis*

INTRODUCTION

Every year thousands of marine mammals strand along US coastlines, and related information from these strandings is tracked by the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) Marine Mammal Health and Stranding Response Program (MMHSRP) and the US Fish and Wildlife Service (USFWS). The Southeastern US (SEUS) Marine Mammal Stranding Network (MMSN) was first organized in 1977 following the first Marine Mammal Stranding Workshop (Odell 1991). The SEUS MMSN organizations and personnel are authorized by either NMFS (cetaceans

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and pinnipeds) or the USFWS (manatees) to respond to and collect data from stranded marine mammals. Routine data collection from strandings enables the critical development of baselines that are necessary to accurately detect and investigate emerging or unusual events.

Unusual mortality events (UMEs) are defined under the Marine Mammal Protection Act (16 U.S.C. 1421h) as stranding events that are unexpected, involve a significant die-off of any marine mammal population, and demand immediate response. Seven criteria are used to determine whether a mortality event is 'unusual,' including higher than expected numbers of stranded marine mammals in a given time and location (NMFS OPR 2013a). For strandings in the Gulf of Mexico (GoM), 'higher than expected' is typically defined as 2 standard deviations above the mean stranding rate for the time period of interest (e.g. monthly or annual). UMEs are declared based upon comparisons to historical data, review and recommendation by a federally appointed Working Group for Marine Mammal Unusual Mortality Events (WGMMUME), and input from NMFS, USFWS, and the Marine Mammal Commission. The Working Group has been operational and the review and recommendation process has been in place since 1991 (Gulland 2006).

On 13 December 2010, a UME was declared for cetaceans in the northern GoM (hereafter abbreviated as NGUME) with a retrospective start date of February 2010 (NMFS OPR 2013b). At the time of declaration, boundaries for this event were defined as Franklin County, Florida, west to the Louisiana/Texas state line (Fig. 1). The majority (87%) of stranded cetaceans recovered during this UME have been common bottlenose dolphins *Tursiops truncatus*, hereafter referred to as bottlenose dolphins. The 'Deepwater Horizon' (DWH) oil rig explosion occurred off the coast of Louisiana on 20 April 2010, leading to the largest oceanic oil spill in US history and raising questions as to whether the spill and/or response activities contributed to the magnitude and duration of the NGUME. As part of the investigation into the causes of the NGUME, a review was conducted of 10 previously declared cetacean UMEs in the GoM plus a well-documented 1990 mortality event in the GoM (Hansen 1992). While the 1990 mortality event was technically not a UME due to its pre-dating the formal UME program, it is treated as a historical UME throughout the manuscript. Although the NGUME was still ongoing as of September 2014, comparisons of the first 4 yr of the NGUME to historical events provide useful insights to inform the ongoing NGUME investigation.

METHODS

Data sources

For each stranding reported to the Stranding Network, the authorized responding agency is required to complete a Marine Mammal Stranding Report – Level A data form (NOAA Form 89-864; OMB No. 0648-0178; form available at www.nmfs.noaa.gov/pr/pdfs/health/levela.pdf) for each stranded animal. Level A data include details of each stranding (e.g. species, date, stranding location, carcass condition, sex, length, examiner, signs of human interaction). For some of the events, particularly earlier ones, a complete listing of specific individual animals included in the event is not available. In these cases, Level A stranding records were extracted from the NOAA MMHSRP National Database (1996–2009) or the SEUS Historical Stranding Database (1990–1995) based on the temporal and geographic boundaries described for the event. In addition to the NOAA MMHSRP database, data from the NGUME are also maintained in a separate, more detailed, Microsoft Access database housed at the NMFS Southeast Fisheries Science Center (SEFSC) in Miami, Florida.

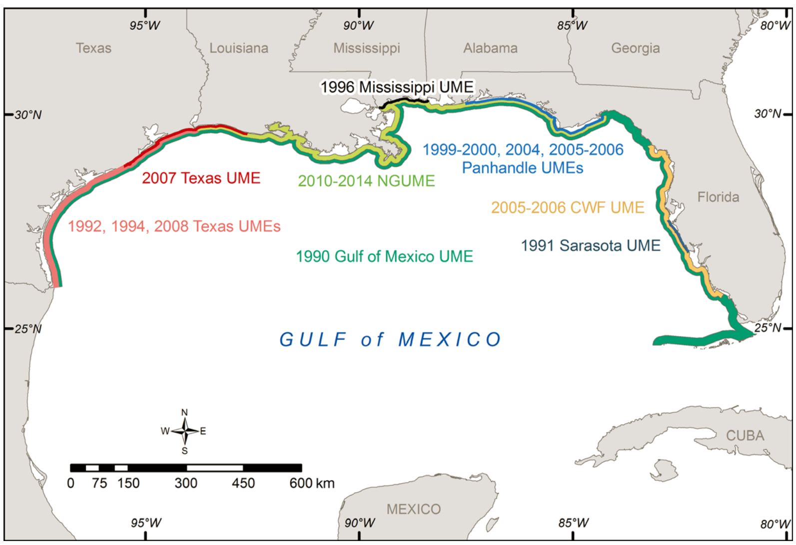

Fig. 1. Historical Gulf of Mexico mortality events (1990–2009) and the northern Gulf of Mexico unusual mortality event (NGUME). The geographic extent of each event is color coded to match the event title. CWF: central west Florida

The final data used in this paper were extracted from the databases on 2 September 2014 and data up through June 2014 are included.

In addition to Level A data, peer-reviewed publications and published reports related to GoM cetacean UMEs were reviewed (e.g. Hansen 1992, Lipscomb et al. 1996, NOAA 2004, Gulland 2006, Fire et al. 2011, Twiner et al. 2012). Unpublished data (including draft UME reports) housed at the NMFS SEFSC were also reviewed. Information gathered from these sources include the total number of reported strandings per event, geographic distribution, concurrent die-offs of other species, event duration, key diagnostic test results, and confirmed or suspected etiologies.

A Texas UME involving 126 bottlenose dolphins that occurred from November 2011 to March 2012 (concurrent with a portion of the NGUME) was excluded from our review. Due to epidemiology, short duration, and information provided from the network at the time of consultation, the WGMMUME recommended that the Texas event be considered a separate event (NMFS OPR 2013c). Therefore, the data on the Texas event were not included in either the historical (up to 2009) or NGUME datasets for the analyses described here. The investigation into the Texas event was in progress at the time of this publication.

For all events, Level A records were used to calculate the percent females (defined as total number of females divided by total number of animals with known sex, unknown sex excluded) and percent perinates in season (Table 1). Percent perinates was defined as number of bottlenose dolphins with a total body length of less than 115 cm that stranded between January and April divided by the total number of bottlenose dolphins with a known total length stranded during the same time period. Data from 2007 on included a field for partial carcasses, allowing us to exclude partial carcasses from the percent perinate calculation. Data prior to 2007 did not have this field available. The percent perinates represents the proportion of mortalities occurring during the final stages of pregnancy and immediately following birth. The length used to define perinates (115 cm) was selected based on previously established length at birth estimates (98–103 cm for females, 100–107 cm for males) from the north-central GoM (Mattson et al. 2006). Historically, there is a peak in perinate strandings in the GoM between January and April (Hansen 1992, Mattson et al. 2006). From 1990 to 2009, an average of 85% of annual perinate strandings occurred between January and April of each year. Therefore, we restricted the perinate calculation to January to April to normalize for events that extended year round and therefore would have a lower prevalence of perinates.

Diagnostic tests for detecting marine biotoxins and dolphin morbillivirus

This manuscript includes results from past UME investigations using analytical methods that may have changed over the years. Citations for results and methods used for both biotoxin screening and morbillivirus testing for each historical event are included in Table S1 in the Supplement (www.int-res.com/articles/suppl/d112p161_supp.pdf). For those events where morbillivirus or biotoxin results were available, the percent of positive cases was calculated as the number of positive cases divided by the total number of cases tested (Table 1). Methods for biotoxin analyses historically have included enzyme-linked immunosorbent assays (ELISA), receptor binding assays (RBA), and/or liquid chromatography/mass spectrometry (LC-MS) (Naar et al. 2002, Flewelling et al. 2005, Maucher et al. 2007, Fire et al. 2008). For the NGUME, brevetoxin (PbTx) was detected using the ELISA method according to Maucher et al. (2007) and Fire et al. (2011) and/or the LC-MS method according to Fire et al. (2008). Samples were analyzed for the presence of domoic acid by tandem mass spectrometry coupled with LC-MS/MS (Fire et al. 2011). Analysis for okadaic acid and its 2 congeners also followed methods outlined by Fire et al. (2011). Screening for saxitoxins was conducted using both RBA and LC-MS (Fire et al. 2012).

Methods for morbillivirus analysis have also changed during the years covered in this evaluation. Stranded bottlenose dolphins from historical UMEs were tested for morbillivirus using the polymerase chain reaction (PCR), immunohistochemical staining (IHC), and/or ELISA (Duignan et al. 1996, Lipscomb et al. 1996, Saliki & Lehenbauer 2001). Samples from paraffin blocks or frozen tissues were tested via PCR and IHC using the methods of Lipscomb et al. (1994). Morbillivirus screening for the NGUME was performed using a nested PCR method as described by Sierra et al. (2014).

Identification of *Tursiops truncatus* ecotypes

Two distinct ecotypes of bottlenose dolphins have been identified in the western North Atlantic and GoM, viz. a smaller coastal ecotype and a larger offshore ecotype (Mead & Potter 1995, Vollmer 2011).

Table 1. Summary of historical (1990–2009) unusual mortality events (UMEs) in the US Gulf of Mexico compared to the on-going northern Gulf of Mexico unusual mortality event (NGUME), including duration, total number of stranded cetaceans included, geographic distribution, key demographics, and suspected or confirmed etiology. Percent (%) females was calculated for bottlenose dolphins *Tursiops truncatus* (Tt) as the number of females divided by the total number of known sex. Percent (%) perinates in season (January to April) was calculated as the number of bottlenose dolphins with an estimated or actual whole body length of <115 cm divided by the total number of bottlenose dolphins with a known whole body length. 'Unknown' is listed when data could not be found and it was unclear whether data existed or were never collected. TX: Texas; AL: Alabama; MS: Mississippi; FL: Florida; LA: Louisiana; na: not applicable (outside of season); HAB: harmful algal bloom

Year	Reported duration	Total	Geographic distribution	Female Tt (%)	Perinate Tt (%)	Concurrent die-off of other species	% morbillivirus positive (no. positive/total)	% brevetoxin positive (no. positive/total)	Suspected or confirmed cause
1990	Jan–May (5 mo)	344	Gulf of Mexico	48	17	No	Not tested	Brevetoxin assay on water inconclusive	Undetermined, cold weather & morbillivirus suspected
1991	July–Dec (6 mo)	31	Charlotte – Manatee County, FL	42	na	Unknown	Not tested	100 (15 archived samples were positive)	Undetermined, biotoxin suspected
1992	Jan–May (5 mo)	119	Matagorda Bay area, TX	42	4	Yes (birds and fish)	24 (serology- post event live capture)	Water and fish tested were negative	Undetermined, low salinity, agricultural runoff, & morbillivirus suspected
1994	Dec 93–May 94 (6 mo)	240	TX	36	26	No	TX: 62 (29/47); AL & MS: 60 (6/10)	Unknown	Confirmed morbillivirus
1996	Nov–Dec 1996 (2 mo)	31	MS	25	na	Yes (birds and fish)	Not tested	Not tested but blooms present in the area	Undetermined biotoxin suspected
1999–2000	Aug 99–May 00 (9 mo)	162	FL Panhandle	42	9	Yes	0 (0/11)	52 (13/25)	Confirmed biotoxin
2004	Mar–Apr (2 mo)	107	FL Panhandle	44	6	Yes	0 (0/24)	100 (39/39)	Confirmed biotoxin
2005–2006	July 05–Nov 06 (17 mo)	190	Central west FL	47	3	Yes	0 (0/28)	53 (42/79)	Confirmed biotoxin
2005–2006	Sept 05–Apr 06 (8 mo)	93	FL Panhandle	53	4	Yes	1 negative by PCR and 1 with negative serology	93 (38/41)	Confirmed biotoxin
2007	27 Feb–27 Mar (1 mo)	66	Northeast TX (61), west LA (5)	24	47	Yes (fish)	0 (0/7)	0 (0/8)[a]	Undetermined
2008	1 Feb–31 Mar (2 mo)	113	TX	38	44	Shellfish bed closures	Not tested	17 (1/6)[a]	Undetermined
2010–2014	Feb 2010–ongoing (48 mo +)	>1000	FL Panhandle, AL, MS, LA	40	23 (2010) 49 (2011) 36 (2012) 34 (2013) 45 (2014)	Fish kills early in 2010	15 (7/46 by PCR)	12 (6/50)[a] at low levels	Undetermined

[a]See the 'Results' section for other HAB results

Stranded bottlenose dolphins from the NGUME and several previous UMEs were identified to ecotype using DNA sequencing of the mitochondrial DNA control region following Rosel et al. (2009) using primers L15824 (Rosel et al. 1999) and H16498 (Rosel et al. 1994). For highly decomposed animals that yielded very poor quality and quantity DNA, 2 smaller, overlapping fragments were amplified and sequenced using the primers L15824 paired with H16081 (Vollmer 2011) and L16061 (Tolley & Rosel 2006) paired with H16498, or through nested PCR reactions. All resultant sequence data were compared to reference sequences to identify each sample to species (e.g. Ross et al. 2003).

RESULTS

Historical review of cetacean GoM UMEs

The geographic extent of each event is shown in Fig. 1. Key demographic, brevetoxin, and morbillivirus data are provided for each of the historical GoM UMEs (1990–2009) and the NGUME (Table 1). Brevetoxin results are included in the table because of their role in past GoM UMEs. Results of other biotoxin testing when available are listed in the summaries of each event below.

1990 GoM bottlenose dolphin die-off

Prior to the official start of the UME program in 1991, a large die-off of bottlenose dolphins (n = 344; Fig. 1) occurred in the GoM (Florida through Texas) from January through May 1990 (Hansen 1992). Proportionally fewer young animals (<140 cm body length) stranded in 1990 than in previous years (Hansen 1992). While the majority of carcasses were in moderate to advanced states of decomposition, 32 carcasses were suitable for complete necropsies, 13 clinical necropsy reports were available, and histopathology was completed for 5 animals (Hansen 1992). Forty liver samples were analyzed for brevetoxin using assays available at the time; however, those assays were in the early stages of development and results were considered inconclusive (Hansen 1992). Morbillivirus testing was not conducted for this event. Additionally, cold weather-related fish kills in Texas preceded the event (Miller 1992), but examinations of 38 whole dolphin stomachs showed no significant difference in food habits (stomach fullness and prey type) compared to a previous study in the same area in 1986–1987 (Hansen 1992). Using only the Texas data, a significant correlation was found between abnormally low December and January sea surface temperatures (1986–1990) and the following spring stranding peaks. The importance of this relationship could not be determined due to the short duration of the available dataset (Hansen 1992). No conclusive cause was identified for this event (Hansen 1992), but based on serum neutralization tests conducted in the GoM after this event, there was strong suspicion that it was related to the emergence of morbillivirus on the east coast of the US (Duignan et al. 1996). In addition, a review of available histopathology reports found pathologies suggestive of morbillivirus, including pan-lymphoid depletion, opportunistic bacterial and fungal septicemia, and disseminated infections (R. Ewing pers. comm.), which also suggest that morbillivirus may have contributed to this mortality event.

1991 Sarasota, Florida UME

Thirty-one bottlenose dolphins stranded along the central west coast of Florida (Charlotte, Sarasota, and Manatee Counties) from July to December 1991. The event peaked in September when 11 dolphin strandings were reported. There was a concurrent *Karenia brevis* bloom along the Florida coast. Archived tissues from 15 animals were tested retrospectively for brevetoxin, and all were above the limit of detection (>5 ng g^{-1} PbTx), and for 9 cases, red tide intoxication was determined to be contributory to or the primary cause of death (Fauquier et al. 2007). No dolphins were tested for morbillivirus. Although the official cause of the event was undetermined (Gulland 2006, NMFS OPR 2013a), the high number of cases that retrospectively tested positive for brevetoxin and had indications of red tide intoxication suggest that biotoxins may have contributed to the event.

1992 Texas UME – Matagorda Bay area

More than 260 cetaceans stranded in Texas in 1992, 119 of which were bottlenose dolphins that stranded in the Matagorda Bay area (Matagorda, Calhoun, and Aransas Counties) between January and May 1992. More than 94 % of the animals had moderate or advanced decomposition, preventing analysis for virology, microbiology, histopathology, and organic contaminants. Skin lesions in the form of ‘a gray, pasty substance and severe skin blisters’ were found on some of the animals (Colbert et al. 1999, p. 8). No dolphin samples were tested for biotoxins or morbillivirus. However, analysis of surface water and finfish were negative for brevetoxins and domoic acid, icthyotoxicity tests exposing sheepshead minnows to extracts of water and finfish were negative, oysters tested for domoic acid were negative, and phytoplankton tows in the area found no evidence of harmful phytoplankton species (Colbert et al. 1999). A live capture health assessment involving 36 dolphins was conducted in July 1992 in the area where the mortalities occurred (Sweeney 1992). A retrospective study found that 24 % of the captured dolphins were seropositive for morbillivirus antibodies, indicating previous exposure (Duignan et al. 1996). Additionally, the strandings occurred mainly in an inshore bay system and coincided with a fish and bird die-off. The dolphin mortalities also coincided with record rainfall from December 1991 to April 1992. This rainfall reduced salinity in the bays and contributed to elevated concentrations of pesticides, presumably from increased runoff (Colbert et al. 1999). While the cause of the event was undetermined (NMFS OPR 2013a), the record rainfall leading to low salinity combined with pesticide runoff and morbillivirus exposure were thought to be possible contributing factors (Duignan et al. 1996, Colbert et al. 1999).

1994 Texas UME

A total of 236 bottlenose and 4 unidentified dolphin strandings were reported in Texas from December 1993 through May 1994, with more than 165 stranded in March and April 1994 (Table 1). The majority of the strandings were reported between the beaches of Matagorda Bay and the Texas/Louisiana state line (Worthy 1998). A high proportion of carcasses was severely decomposed (50–70 % per month compared to 35 % typically; Worthy 1998). Some of the decomposed carcasses were found on the day after a beach survey, suggesting that animals were dying offshore and then floating for days before stranding (Worthy 1998). Biotoxin testing was not conducted. Complete necropsies were not performed due to advanced decomposition; however, samples of frozen lung collected from 57 dolphins were tested for morbillivirus by RT-PCR (Lipscomb et al. 1996). Morbilliviral RNA was detected in 29 lung tissues tested, while nucleic acids from 10 could not be amplified due to decomposition. Therefore, 62 % (29/47) of the Texas dolphins that could be evaluated for morbillivirus using RT-PCR were positive (Lipscomb et al. 1996). In addition, 10 dolphins from Alabama and Mississippi were examined histologically, and 6/10 had lesions highly characteristic of morbillivirus. Five of those were positive by IHC and all 6 were PCR positive (Lipscomb et al. 1996). Lipscomb et al. (1996) concluded there was evidence for an extensive morbilliviral epizootic of bottlenose dolphins in the GoM with a westward spread of the virus. Infectious disease, specifically morbillivirus, was determined to be the cause of this event (Gulland 2006, NMFS OPR 2013a).

1996 Mississippi UME

Thirty-one bottlenose dolphins stranded in Mississippi during November through December 1996. This event may have started in the Panhandle of Florida on 30 October 1996 and also included several dead dolphins reported in Alabama and Louisiana (MMC 1996). No common pathological conditions were found in the necropsied dolphins (MMC 1996). Samples were not tested for biotoxins or morbillivirus. However, stranding records and WGMMUME meeting notes mentioned a red tide in the area with *K. brevis* cell counts >13 million cells l^{-1} on 20 November at Cat Island, Mississippi. The largest number of strandings occurred a few days after the bloom peak in late November. Additionally, oyster beds were closed to harvesting, and fish and bird mortalities were also reported during this event (MMC 1996). While the cause of this event was undetermined, these mortalities were suspected to be related to brevetoxicosis (MMC 1996, NOAA 2004, Gulland 2006, NMFS OPR 2013a).

1999–2000 Panhandle of Florida UME

Between 5 August 1999 and 20 May 2000, 162 cetaceans (150 bottlenose dolphins) stranded in the Florida Panhandle between Franklin County and Escambia County. All 68 bottlenose dolphins examined using mitochondrial DNA sequence data were of the coastal ecotype. Gross necropsy and histological data were available for 60 bottlenose dolphins. No consistent gross or microscopic lesions were detected, and most dolphins were in good nutritional condition. Lung samples from 11 dolphins tested for morbillivirus by RT-PCR were negative. Samples from 52 % (13/25) of dolphins analyzed for brevetoxins had detectable levels of toxins (Twiner et al. 2012). The majority of tissues tested were liver and kidney. Stomach content samples from 4 dolphins were tested and had the highest brevetoxin concentrations ranging from 53 to over 500 ng g^{-1}. High-density *K. brevis* blooms were consistently detected in the Panhandle bays and coastal waters between August 1999 and January 2000, and fish kills were also reported in the Panhandle between August and December 1999 (Twiner et al. 2012). The bloom was first detected in the east (Apalachicola Bay then St. Joseph Bay) before moving west into St. Andrew Bay and Choctawhatchee Bay (Twiner et al. 2012). Similarly, there appeared to be a temporal east to west spread in dolphin mortalities, with most strandings around St. Joseph Bay in August and September, followed by an increasing number of strandings in Choctawhatchee Bay by December 1999 and an increase around Pensacola Bay towards the end of the UME. The determined cause of this UME was brevetoxicosis (Gulland 2006, NMFS OPR 2013a).

2004 Panhandle of Florida UME

The 2004 Panhandle UME differed from the 1999–2000 event in that it spanned just 34 d (10 March to 13 April) and included 107 dolphins (105 bottlenose dolphins, 2 unidentified dolphins). Thirty-one dead bottlenose dolphins stranded within the first 4 d. While the geographic boundaries of the declared UME were the same as the 1999–2000 UME (Franklin to Escambia Counties), this event was much shorter in duration, and 66% of the strandings occurred in Gulf County primarily in St. Joseph Bay. Of 65 bottlenose dolphins examined using mitochondrial DNA sequence data, all were of the coastal ecotype. Necropsies were conducted on 46 animals, and 35 were examined histologically. No consistent lesions or evidence of infectious disease were identified (Twiner et al. 2012, NMFS unpubl. data). Most of the animals were considered to be in good nutritional condition; however, a few were considered to be thin (Gaydos 2006). The majority of stomachs were distended with undigested or partially digested contents, suggesting that the dolphins had gorged prior to death. Menhaden was the dominant prey species in at least 50% of the 28 stomachs examined (NOAA 2004). Viral testing, including tests for morbillivirus, was negative (NOAA 2004, Gaydos 2006). Tissues from 39 dolphins were analyzed for brevetoxins using multiple methods, and 100% of dolphins tested had high levels of brevetoxin in their stomach contents (54 to 29126 mg PbTx-3 ml^{-1} gastric fluid; Twiner et al. 2012). In addition, trace levels of domoic acid were found in the stomach contents, blood, and urine samples of 88% (8/9) of dolphins tested; however, the significance of these trace levels is unclear (Twiner et al. 2012). Satellite imagery indicated elevated chlorophyll levels in the UME area from 9 to 11 March 2004, but a *K. brevis* bloom was not detected. It was suggested that there may have been a bloom, possibly sub-surface, prior to the event that went undetected (NOAA 2004, Twiner et al. 2012). Several fish kills were reported in the area between March and early May, and fish collected (both live and from fish kills) from St. Joseph Bay throughout March tested positive for brevetoxin (NOAA 2004, Flewelling et al. 2005). While no *K. brevis* bloom was detected, the presence of toxin in fish and results of the dolphin stomach content analysis suggest there was an undetected bloom. The determined cause of this UME was brevetoxicosis (Gulland 2006, NMFS OPR 2013a).

2005–2006 central west Florida (CWF)

The 2005–2006 CWF multi-species UME (CWF UME) included 132 manatee strandings between March 2005 and December 2006 and 190 dolphin strandings (166 bottlenose dolphins, 23 unidentified dolphins, 1 *Stenella frontalis*) between July 2005 and November 2006. The area of the UME was defined as Collier County north to Levy County, Florida. As this paper is concerned with cetaceans that were affected during this event and not manatees, the duration is defined as the 17 mo (July 2005 through November 2006) during which dolphin strandings were high. Of 87 bottlenose dolphin samples examined using mitochondrial DNA sequence data, 6 were of the offshore ecotype and the rest were the coastal ecotype. Full necropsies were performed on 79 fresh dead or moderately decomposed dolphins, and histopathology was performed on 39 dolphins. Overall, there were no consistent necropsy findings. Tissues from 53% (42/79) of dolphins tested were positive for brevetoxin, with the highest concentrations of brevetoxin found in stomach contents (range 6 to 19000 ng g^{-1}; Florida Fish and Wildlife Conservation Commission and National Ocean Service unpubl. data). Twenty-eight dolphins were PCR-negative for morbillivirus, and 3 live dolphins admitted into rehabilitation were sero-negative. The CWF UME was associated with a *K. brevis* bloom that began in January of 2005 and persisted into February 2006 along the southwest Florida coast. A subsequent red tide bloom was detected in late June 2006 immediately southwest of Sanibel Island, Lee County, and persisted through 2006 into 2007. Sea turtle strandings also increased in this area during the UME, and die-offs of fish and birds were reported (Gannon et al. 2009, Fauquier et al. 2013a,b).

Cetacean mortalities showed 4 peaks. The first peak from July to November 2005 was characterized by high levels of brevetoxin in the dolphin tissues tested. During the second peak (December 2005 to March 2006), brevetoxin was detected in fewer tissues and at lower concentrations than the first peak, and several animals had skin lesions (n = 9). The third peak (July to early August 2006) was characterized by a high prevalence of human interactions, primarily involving ingestion of fishing gear (Powell & Wells 2011). It is hypothesized that secondary effects such as decreased prey availability post-bloom may have negatively impacted animals already in physiological stress (second mortality peak) and may have led to increased human interactions during the third peak as evidenced by alterations in fish communities and human interaction rates (Gannon et al. 2009, Powell & Wells 2011). The last peak (October 2006) was concentrated around Lee County, concurrent with the bloom, and the dolphins tested from this peak again had high brevetoxin concentrations in the tissues tested. The absence of unifying infectious diseases on a population level, results of brevetoxin tissue and gastric content analyses, and spatial and temporal association with the red tide event(s) all support the conclusion that brevetoxin was the primary factor in the increase in dolphin mortalities. The determined cause of this UME was brevetoxicosis (NMFS OPR 2013a).

2005–2006 Panhandle of Florida UME

This mortality event included 93 cetaceans (88 bottlenose dolphins and 5 unidentified species) stranded between September 2005 and April 2006. Similar to previous Panhandle UMEs, the strandings were spread throughout the Panhandle region (Franklin to Escambia Counties); however, unlike the previous events which began around St. Joseph Bay, 54% of the strandings in this event occurred in and around Choctawhatchee Bay (Walton and Okaloosa Counties) to the west. Of 64 bottlenose dolphins examined using mitochondrial DNA sequence data, 2 were of the offshore ecotype and the rest were the coastal ecotype. Necropsies and histopathology were conducted on 44 animals including 3 fetuses. The majority of animals were in good nutritional status except for 6 that were emaciated. There were no consistent findings, although interpretation was hampered by autolysis. Fish remains were present in all 17 stomachs examined but were mostly digested. Unlike the 2004 UME, dolphin stomachs examined in this event had moderate amounts of contents and a low incidence of clupeids. Brevetoxin was detected in 93% (38/41) of the dolphins tested. Gastric fluids had the highest concentrations (range 20 to 2724 PbTx-3 ng ml^{-1}; Twiner et al. 2012). Most dolphins had at least 1 food item in the stomach that tested positive for brevetoxin, although the concentrations were lower than those seen during the 2004 event (Twiner et al. 2012). None of the animals tested were positive for domoic acid (Twiner et al. 2012). Two live-stranded dolphins were sero-negative for morbillivirus (titers less than 4 and less than 8). One of these dolphins also had lung and brain tested by PCR for morbillivirus and was negative. A *K. brevis* bloom was detected as early as September in the Panhandle, with levels in some areas remaining elevated through December 2005 (FWCC 2005, Twiner et al. 2012). Fish kills were also reported in the entire UME area from August 2005 to April 2006, including a concentration of fish mortalities in the Choctawhatchee Bay around Garnier Bayou (FWCC 2013). The determined cause of this UME was brevetoxicosis (NMFS OPR 2013a).

2007 Texas and Louisiana UME

An increase in bottlenose dolphin strandings began on 27 February 2007 and lasted through March 2007 and included 66 dolphins (64 bottlenose and 2 unidentified dolphins) in northeastern Texas and western Louisiana (Brazoria County, Texas, through Cameron Parish, Louisiana). The majority of carcasses were reported in Galveston and Jefferson Counties in Texas. All age classes were represented; however, 47% of strandings were under 115 cm and classified as perinates. Over 90% of the carcasses were moderately to severely decomposed and only 2 were fresh dead. Of 15 bottlenose dolphins examined using mitochondrial DNA sequence data, all were of the coastal ecotype. Tissues submitted for biotoxin analysis included liver, stomach contents, and gastric fluid. All dolphins tested were negative for saxitoxin (n = 5) and brevetoxin (n = 8). Domoic acid was confirmed in 25% of dolphins tested (2/8) at low concentrations in the stomach contents (10–13 ng g^{-1}) and while indicative of toxin exposure, was not necessarily confirmatory of cause of death (Fire et al. 2011). Phytoplankton samples collected during the mortality event from the central and southeastern Louisiana coast were found to contain *Pseudo-nitzschia pseudodelicatissima*, a known producer of domoic acid and a common member of the phytoplankton community along this coastline in winter and spring. No toxin analyses on the phytoplankton samples were conducted (W. Morrison et al. unpubl. data). Tissues (primarily lung samples) were submitted from 7 dolphins for morbillivirus testing and all were negative by PCR. Because of the large proportion of perinates stranded in this event, the investigative team suspected that an infectious agent that could lead to late-term abortions or early neonatal death, such as the bacterium *Brucella*, may have been involved. However, that could not be confirmed due to the advanced decomposition of the carcasses and limitations of the available tests at the time. The cause of this event remains undetermined (NMFS OPR 2013a).

2008 Texas UME

The 2008 Texas UME began on 1 February 2008, lasted through 31 March 2008, and involved 113 cetaceans (111 bottlenose dolphins, 1 unidentified dolphin, and 1 melon-headed whale). The majority of strandings occurred in Galveston and Jefferson Counties. A drift analysis indicated that the dolphins that stranded around Galveston in early March likely came from offshore within about 50 km of the coast between Galveston, Texas, and Grande Lake, Louisiana (M. Kinsey pers. comm.). Similarities between the 2007 and 2008 Texas events include the location, time of year, high proportion of perinate strandings (44%), and high proportion of carcasses that were moderately to severely decomposed. Of 75 bottlenose dolphins examined using mitochondrial DNA sequence data, all were of the coastal ecotype. Thirty-eight percent (3/8) of dolphins tested were positive for domoic acid and okadaic acid in their digestive tract (up to 39 and 10 ng g^{-1}, respectively; Fire et al. 2011). Seventeen percent (1/6) of dolphins tested for brevetoxins were positive, though at low concentrations, and this may have been indicative of background or subacute exposure (Fire et al. 2011). Similar to the 2007 event, the investigative team suspected that an infectious agent that could lead to late-term abortions or early neonatal death, such as the bacterium *Brucella*, may have been involved due to the high proportion of perinates stranded. Unfortunately, samples were not tested for morbillivirus or *Brucella* due to loss of these samples during Hurricane Ike in September of 2008 (H. Whitehead pers. comm.). This UME overlapped spatially and temporally with a *Dinophysis* bloom and *Porocentrum* bloom (both known to produce the toxin okadaic acid) and a *Pseudo-nitzschia* bloom (known to produce domoic acid). No *K. brevis* bloom was detected during this event (Fire et al. 2011). Southern Texas bays were closed on 7 March 2008 for shellfish harvesting due to the *Dinophysis* threat (Swanson et al. 2010). While the concentration of each individual toxin in the dolphins was considered to be low, the impacts of chronic low-dose exposure or multiple toxin exposure are unknown. This was the first time exposure to 3 distinct classes of harmful algal bloom toxins was documented during a marine mammal mortality event (Fire et al. 2011). The cause of this event remains undetermined (NMFS OPR 2013a).

2010–2014 NGUME

The NGUME as currently defined includes over 1000 cetacean mortalities and is ongoing as of September 2014 (NMFS OPR 2013b). In recent years, the NGUME area (Franklin County, Florida, through Louisiana) experienced an average of 75 strandings per year (2002 to 2009, previous UMEs excluded; Table 2). The NGUME has exceeded the annual mean plus 2 standard deviations for each of the first 4 yr of the event (2010 to 2013; Table 2). Similar to previous GoM events, the majority of strandings were bottlenose dolphins (87%), and 8% could not be identified to genus. The remaining 5% represented 10 genera: *Balaenoptera, Feresa, Globicephala,*

Table 2. Northern Gulf of Mexico cetacean strandings by year and state (February 2010 to 2013) for the designated northern Gulf of Mexico unusual mortality event (NGUME) area. Data include any strandings with a strand date from 1 February 2010 through 2013 reported before September 2014. 2SD: 2 times the standard deviation; see Table 1 for state abbreviations

Location	Historical average (2SD)	2010 (Feb–Dec)	2011	2012	2013	Grand total
FL Panhandle	21 (27)[a]	33	33	29	26	121
AL	14 (14)	25	59	30	26	140
MS	20 (21)	64	111	49	50	274
LA	20 (21)	137	163	94	145	539
Grand total	75 (46)	259	366	202	247	1074

[a]Historical data from 2002 to 2009 exclude 2 previous UMEs in the Panhandle of Florida (March to April 2004 and September 2005 to April 2006)

Grampus, *Kogia*, *Mesoplodon*, *Peponocephala*, *Physeter*, *Stenella*, and *Steno*. The 3 most commonly stranded non-*Tursiops* species were *Stenella longirostris* (n = 13), *Stenella frontalis* (n = 13), and *Peponocephala electra* (n = 8).

A breakdown of stranding numbers by state and year for 2010 through 2013 is shown in Table 2. Twenty-five dead bottlenose dolphins were reported in and around Lake Pontchartrain, Louisiana, between 12 March and 16 April 2010 following a single stranding reported there on 15 February 2010. A group of dolphins had been sighted in the lake since 2007, and historically, it had been rare to have reports of any cetacean strandings in Lake Pontchartrain (Barry et al. 2008). The historical stranding rate (mean plus 2 SD, 2002–2009 with previous UMEs excluded) for March and April in the NGUME area was 55 animals, compared to 103 reported strandings in March and April 2010. Following April 2010, Louisiana continued to have higher strandings than the other states both per year and for the first 4 yr combined. NGUME strandings were higher in the second year of the event (2011, n = 366) than the other years (range 202–259 yr^{-1}; Table 2). In the spring of 2011, there was a large increase in strandings, particularly of perinates in Mississippi and Alabama. Conversely, the spring 2011 peak in Louisiana was not driven by perinate strandings (Fig. 2). Overall, perinates made up a higher proportion of the bottlenose dolphin strandings in January to April 2011 and 2014 compared to 2010, 2012, and 2013 (Table 1, Fig. 2).

To date, 618 dolphins from the NGUME have been tested, and 616 have been confirmed as the coastal ecotype and 2 as the offshore ecotype using mitochondrial DNA sequence data. A subset of bottlenose dolphins from the Florida Panhandle through Louisiana that stranded during February 2010 through April 2012 were screened for marine biotoxins (brevetoxin, domoic acid, okadaic acid, and saxitoxin). Primarily fecal and gastric samples were submitted, but a few other tissue types were also submitted, including liver (n = 9) and urine (n = 2). A total of 12% (6/50) of dolphins tested through early 2012 for brevetoxin were positive at low levels by ELISA but were below the detection limit or trace by LC-MS (range: 1.9–16 ng g^{-1} in fecal or gastric samples), and 8.6% (5/58) of dolphins tested for domoic acid were positive at low levels (range: trace to 43 ng g^{-1}). All dolphins tested for okadaic acid (n = 10) and saxitoxin (n = 12) were negative. For brevetoxin and domoic acid, the low level concentration cases were spread out geographically (from Louisiana, Mississippi, and Alabama) and temporally (2010 and 2012). None of the dolphin samples were positive for both brevetoxin and domoic acid.

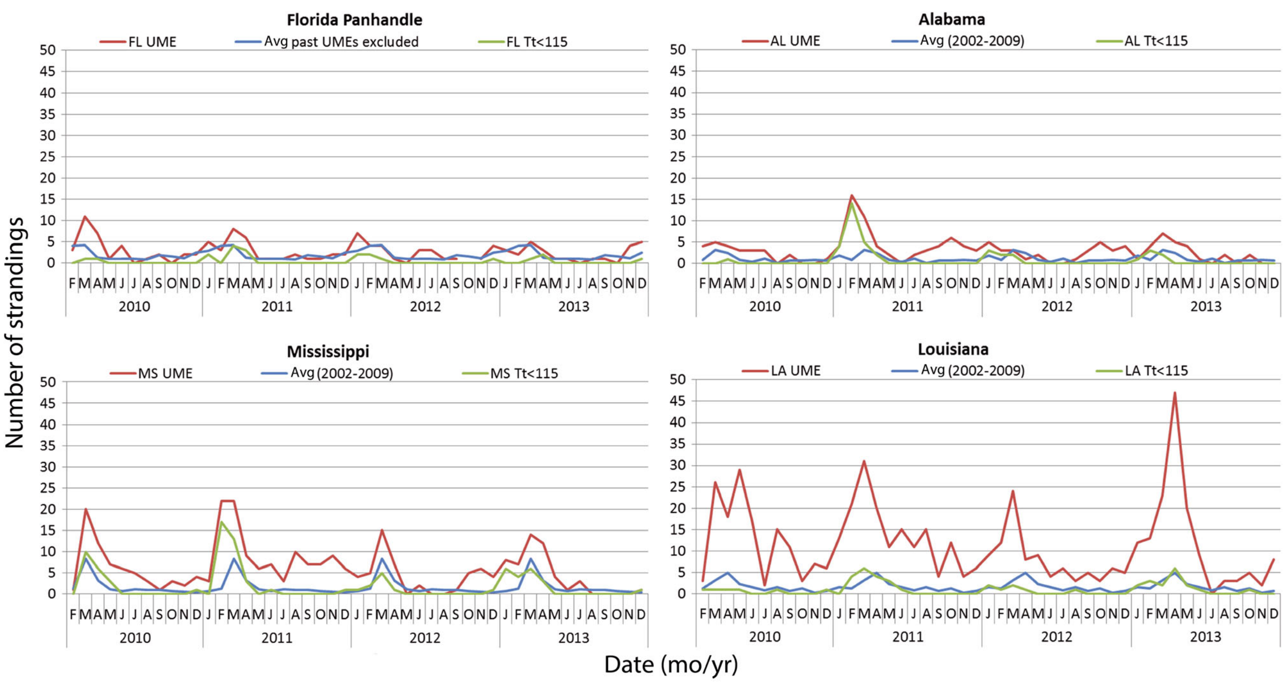

Fig. 2. Strandings per month from February 2010 through 2013, showing all cetaceans (red) compared to the 2000 to 2009 average monthly cetacean strandings (blue) and the unusual mortality event (UME) bottlenose dolphins *Tursiops truncatus* (Tt) <115 cm (green) per state (FL: Florida; AL: Alabama; MS: Mississippi; LA: Louisiana)

Initial screening for morbillivirus was performed using an RT-PCR assay on frozen tissues from 41 fresh dead or moderately decomposed bottlenose dolphins stranded from Alabama through Louisiana during 2010 and 2011. Following the initial screening, an additional 5 animals were selected for testing based on histopathological findings where morbillivirus was suspected. Lung was the most common tissue tested; however, some spleen, brain, and lymph node samples were also submitted, and there was at least 1 positive result for each of the tissue types submitted. In total, samples from 46 dolphins were evaluated for morbillivirus, and 15% (7/46) were positive on PCR and histopathology. Histopathological lesions in all 7 PCR positive cases (6 *Tursiops*, 1 *S. longirostris*) were consistent with those described in previous dolphin morbillivirus cases (Lipscomb et al. 1996, Schulman et al. 1997). Initial genetic sequencing of the cetacean morbillivirus detected in all 7 dolphins yielded sequences most closely related (99.6–100% similarity) to dolphin morbillivirus based on a 300 bp segment of the phosphoprotein gene.

DISCUSSION

Prior to 2010, the only cetacean mortality event to span the entire northern GoM region was the Gulf-wide 1990 event (Fig. 1), which also had the most dolphin mortalities recorded of any previous GoM event (n = 344; Table 1). The average duration of past GoM UMEs was 6 mo. The longest-lasting GoM UME prior to 2010 lasted 17 mo and was due to brevetoxicosis (2005–2006 CWF UME; Table 1). When all events were plotted by their duration and the number of mortalities recorded, the current event is clearly unique in terms of both its duration and the number of reported strandings (Fig. 3). There is an expected relationship between the duration of an event and the number of strandings; however, this correlation for UMEs prior to 2010 is weak (Pearson r = 0.32, p > 0.3) due to the high variability of these metrics among events. The historically highest event in terms of the number of stranded animals (1990 Gulf) lasted only 5 mo, while the historically longest event (2005–2006 CWF) had fewer stranded dolphins (n = 190) but lasted 17 mo. The considerable variation in the intensity and timing of UMEs may reflect differences in the intensity and duration of underlying causes. Under the currently defined boundaries, the NGUME lies well outside of the level of variability in duration and intensity that has been observed in previous GoM UMEs.

Based on comparisons with previous GoM cetacean UMEs and a current morbillivirus epizootic in the Mid-Atlantic (NMFS OPR 2014), initial diagnostic tests for the first 2 yr of the NGUME do not indicate a morbillivirus epizootic as a cause of this UME. Three of 11 past events in the GoM were confirmed or suspected to have morbillivirus as a causal factor. While no cause for the 1990 or 1992 events were officially declared, retrospectively, there was strong suspicion that morbillivirus played a role in the high stranding numbers throughout the early 1990s (Duignan et al. 1996). The only confirmed morbillivirus epizootic (1994 Texas UME) in the GoM involved dolphins with a high prevalence (62%, 29/47) of PCR-positive morbillivirus cases, and dolphins from neighboring states stranding prior to the event had histological evidence of viral infection in addition to positive PCR tests (Lipscomb et al. 1996). These findings are consistent with those from the 1987–1988 east coast die-off in which 97% of the cases tested (35/36) were PCR positive, 52% (49/95) were positive by IHC, and histological evidence of morbillivirus infection was seen (Schulman et al. 1997). Similarly, 96% (215/225) of cases from the ongoing 2013–2014 morbillivirus-

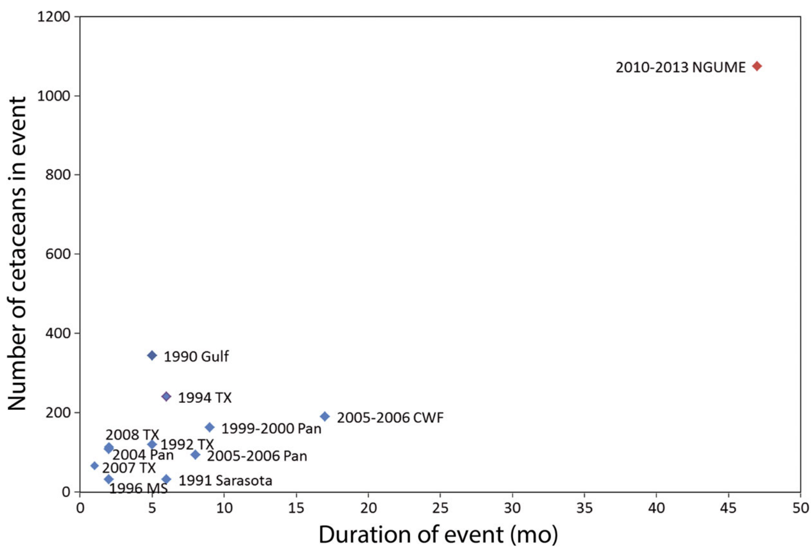

Fig. 3. Mortality events plotted by event duration in months and the number of cetaceans in the event. The northern Gulf of Mexico unusual mortality event (NGUME) is ongoing, and data in this chart include the total cetaceans stranded through 2013 (February 2010 to December 2013). Pan: Florida Panhandle; CWF: central west Florida; Gulf: throughout the Gulf of Mexico; TX: Texas; MS: Mississippi

associated east coast die-off are positive for morbillivirus by either PCR and/or IHC methods (NMFS OPR 2014). The percentage of PCR positive morbillivirus cases for the first 2 yr of the NGUME is only 15% (7/46). Current evidence does not indicate an active morbillivirus epizootic as a cause of the first 2 yr of the NGUME.

Similarly, brevetoxicosis is unlikely a primary cause of the first 2 yr of the NGUME. Brevetoxicosis was the confirmed cause of 4 previous cetacean UMEs in the GoM, all in Florida between 1999 and 2006 (Table 1; Twiner et al. 2012, NMFS OPR 2013a). Brevetoxicosis events in the past typically involved deaths of dolphins across all age classes, were often concurrent with algal blooms and/or mortalities of other species, and had high toxin levels in dolphin samples or exposure routes (prey or water) (Flewelling et al. 2005, Twiner et al. 2012). Brevetoxin UMEs demonstrated a high prevalence (range of 52 to 100%) of tested dolphins positive for brevetoxin (Table 2). Only 12% of dolphins screened for biotoxins from the first 2 yr of the NGUME were positive for brevetoxin and 8.5% for domoic acid. Levels of both were low and in fact similar to those measured in live, free-swimming dolphins in Sarasota Bay (Twiner et al. 2011). These levels are considerably lower than levels detected during previous brevetoxin-related UMEs (Twiner et al. 2012) and may be indicative of background exposure (Fire et al. 2011). In addition, the positive cases were spread out spatially and temporally over the 2 yr tested. This differs from the past events where the positive cases tend to be more clustered in both time and space. The low prevalence of positive cases and low concentrations of brevetoxins measured do not support brevetoxicosis as a major contributor to the mortalities of the first 2 yr of the NGUME.

Two previous events, the 2007 and 2008 Texas UMEs, had a high proportion of stranded perinates, similar to the spring of 2011 during the NGUME. However, unlike the NGUME, both of these prior UMEs were short in duration. While high levels of domoic acid have been associated with premature parturition in pinnipeds, the levels found in dolphins from these events have been low compared to those found with acute toxicosis (Scholin et al. 2000, Goldstein et al. 2009). Evidence does not suggest that domoic acid has played a role in perinatal loss in these GoM cetacean events. *Brucella* or other infectious disease causing low neonatal survival and late-term abortions were suspected to be involved in the 2007 and 2008 events. The involvement of *Brucella*, however, could not be confirmed because of the decomposition of the carcasses and lack of appropriate and validated diagnostic tests at that time. *Brucella* is a known cause of late-term abortions in dolphins and has been shown to cause skin, joint, and brain lesions in non-perinatal cetaceans (Miller et al. 1999, Guzmán-Verri et al. 2012). While *Brucella* has been confirmed in many marine mammal species globally, to date it has not been confirmed as causing epizootics or die-offs in marine mammals, including dolphins. *Brucella* testing for the NGUME is ongoing (NMFS OPR 2013b) as of September 2014. Additional analyses will be needed to further understand the potential role of *Brucella* in the NGUME.

Among previous cetacean GoM UMEs, 2 included environmental influences as possible contributing factors. Low salinity due to heavy rainfall and associated runoff of land-based pesticides were identified as potential contributing factors to the 1992 Texas UME, in addition to possible morbillivirus exposure (Duignan et al. 1996, Colbert et al. 1999). Early in the 1990 Gulf-wide mortality event, a mass stranding of 26 dolphins occurred in East Matagorda Bay, where the bay had frozen over for 2.5 d during the previous month (Miller 1992). In addition, an analysis of Texas stranding data from 1986 to 1990 found an inverse relationship between spring stranding numbers and winter sea surface temperature, with the coldest winter (1989–1990) preceding the highest spring strandings (1990) (Hansen 1992). While cold weather and morbillivirus are indicated as possible contributing factors to the 1990 mortalities, the overall cause of this event was undetermined (Hansen 1992). Low air and water temperatures occurred in the spring of 2010 throughout the GoM prior to and during the start of the NGUME. Fish kills associated with cold water were reported in some bays, estuaries, and shallow water areas throughout the Gulf during early 2010 (Carmichael et al. 2012, FWCC 2013, E. Cotton pers. comm.). In January 2010, there was a severe sea turtle cold stunning event in Florida, including the Florida Panhandle (Avens et al. 2012). In addition, from February through April 2010, a portion of the increase in dolphin strandings was due to the atypical strandings in Lake Pontchartrain, Louisiana, concurrent with lower than average salinity in the lake (USGS 2013). Thus, a large part of the increased dolphin strandings in the early spring of 2010 may have been due to a distinct and temporary event involving a combination of cold temperatures and low salinity. In addition to the spring of 2010, Carmichael et al. (2012) suggested that a cold and freshwater event centered on Mobile Bay, Alabama, may have played a role in mortalities observed during the spring of 2011. While portions of the NGUME may be correlated with cold weather and/or low-salinity events, the long duration of the event and the continued high levels of mortalities beyond winter and spring of each year indicate that freshwater and cold water exposure alone were unlikely primary causes for the ongoing NGUME.

The number of strandings recorded can be influenced by the level of stranding network activity as well as public awareness and reporting. Increased reporting by oil spill responders following the DWH oil spill has been raised as a factor that may have increased the detection of strandings following April 2010. If the increase in reported strandings during the NGUME was primarily driven by better detection during the oil spill response and/or increased public awareness, the number of reported strandings should have been highest immediately following the spill in the summer and fall of 2010, when in fact they were higher throughout 2011. While increased effort can be difficult to quantify, future analyses as part of the ongoing UME investigation will continue to take effort into consideration.

The cause of the NGUME remains undetermined, and the investigation was ongoing as of September 2014. As the UME investigation continues, it is possible that the regional extent, duration, and the number of animals associated with the event will be re-evaluated and spatial and temporal boundaries may be redefined based on new information and analyses. For example, the Florida Panhandle region was initially included in the NGUME because of elevated stranding rates in March and April 2010; however, the stranding rates in the Panhandle did not remain elevated throughout the event (Table 2, Fig. 2). There are also demographic differences within this event, with strandings in Mississippi and Alabama largely driven by perinates, particularly in the spring of 2011, whereas strandings in Louisiana are not primarily driven by perinates (Fig. 2). For these reasons, a statistical analysis of the bottlenose dolphin strandings to identify distinct demographic, temporal, and spatial clusters within the event has been undertaken and will be reported separately (Venn-Watson et al. in press). This event is complex and comparisons to historical events indicate that the characteristics of the NGUME are unique. In addition to being longer in duration, the 2 most common causes of past GoM events, morbillivirus and brevetoxin, do not appear to be significant factors during at least the first 2 yr of the NGUME. The potential effects of multiple or cumulative factors, including environmental influences, *Brucella*, and the DWH oil spill, will continue to be investigated.

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