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T. K. Rowles
National Marine Fisheries Service

L. S. Schwacke
NOAA's Ocean Service

R. S. Wells
Mote Marine Laboratory

J. T. Saliki
University of Georgia

L. Hansen
National Marine Fisheries Service

See next page for additional authors

Authors
T. K. Rowles, L. S. Schwacke, R. S. Wells, J. T. Saliki, L. Hansen, A. Hohn, F. Townsend, R. A. Sayre, and A. J. Hall

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T. K. Rowles
National Marine Fisheries Service,
Marine Mammal Health and Stranding Response Program,
1315 East-West Highway,
Silver Spring, Maryland 20910, U.S.A.

L. S. Schwacke
NOAA's Ocean Service,
Center for Human Health Risks,
331 Fort Johnson Road,
Charleston, South Carolina 29412, U.S.A.

R. S. Wells
Chicago Zoological Society,
c/o Mote Marine Laboratory,
1600 Ken Thompson Parkway,
Sarasota, Florida 34236, U.S.A.

J. T. Saliki
Athens Veterinary Diagnostic Laboratory,
University of Georgia,
Athens, Georgia 30602, U.S.A.

L. Hansen
A. Hohn
National Marine Fisheries Service,
Southeast Fisheries Science,
NOAA Beaufort Laboratory,
101 Pivers Island Road,
Beaufort, North Carolina 28516, U.S.A.

F. Townsend
Bayside Hospital for Animals,
251 N.E. Racetrack Road,
Fort Walton Beach, Florida 32547, USA

R. A. Sayre
NOAA's Ocean Service,
Center for Human Health Risks,
331 Fort Johnson Road,
Charleston, South Carolina 29412, U.S.A.

A. J. HALL[1]
Sea Mammal Research Unit,
Scottish Oceans Institute,
University of St Andrews,
Fife, KY16 8LB, United Kingdom
E-mail: ajh7@st-andrews.ac.uk

ABSTRACT

Cetacean morbilliviruses (CeMV) are viruses that can cause mass mortalities among various odontocete species. In this study levels of "herd" immunity in cetaceans from the U.S. coast are described from the distribution and prevalence of antibodies against morbilliviruses. Neutralizing antibody titers against dolphin morbillivirus (DMV), porpoise morbillivirus (PMV), phocine distemper (PDV), and canine distemper viruses (CDV) were measured. Positive samples had higher titers against the CeMV than against the other morbilliviruses tested, indicating that although PDV or CDV can be used to investigate exposure their use may result in a higher false negative rate. The results suggest that morbillivirus did not persist in coastal populations of bottlenose dolphins (*Tursiops truncatus*) after the major outbreaks that occurred in the 1980s and 1990s. Bottlenose dolphins from Beaufort, North Carolina; St. Joseph Bay, Florida; and Cape May, New Jersey had anti-DMV seroprevalences ranging from between 15% and 33% but those from Charleston, South Carolina and Sarasota Bay, Florida, sampled in recent years were largely negative. These latter groups are therefore now vulnerable to infection and could experience high mortality if exposed to CeMV. Sero-surveys of this kind are therefore vital for assessing the risk of new and recurring viral outbreaks in coastal cetaceans.

Key words: infectious disease, bottlenose dolphin, *Tursiops truncatus*, serology, Atlantic Ocean.

The emergence and history of morbillivirus infection in cetacean populations worldwide has been well documented (Domingo *et al.* 1990; Van Bressem *et al.* 1991, 1998, 2001; Welsh *et al.* 1992; Aguilar and Raga 1993; Barrett *et al.* 1993; Lipscomb *et al.* 1994*a*, *b*; Barrett *et al.* 1995; Duignan *et al.* 1995*a*, 1996; Hall 1995; Saliki *et al*. 2002). Two antigenically and genetically very similar cetacean morbillivirus strains have been described, namely dolphin morbillivirus (DMV, first isolated in striped dolphins (*Stenella coeruleoalba*) from the Mediterranean sea in 1990) and porpoise morbillivirus (PMV, first isolated in harbor porpoises (*Phocoena phocoena*) from the NE Atlantic in 1988) (Kennedy *et al.* 1988, McCullough *et al.* 1991, Visser *et al.* 1993). These viruses, together with phocine distemper virus (PDV) and canine distemper virus (CDV), have impacted various marine mammal populations worldwide since they were first identified in the late 1980s (Hall 1995, Kennedy 1998). These relatively recent additions to the *Paramyxoviridae* family have affected a variety of small odontocete cetaceans in Europe and North America. A major outbreak of the disease occurred in 1987–1988 among common bottlenose dolphins (*Tursiops truncatus*) in the United States, which spanned stocks from New Jersey to Florida

[1]Author to whom correspondence should be addressed.

(Geraci 1989, Lipscomb *et al.* 1994*b*). In addition, analysis of tissues from dolphins that stranded in the Gulf of Mexico in 1992–1993 during an unusual mortality event also found morbilliviral RNA (Lipscomb *et al.* 1994*a*, *b*). Further sequence analyses of the isolates from these outbreaks found DMV to be more prevalent in the north Atlantic whereas PMV was more common farther south (Taubenberger *et al*. 1996). Indeed, only PMV was detected in samples from dolphins that died during the 1993 Gulf of Mexico epidemic whereas both viruses were found in victims of the 1987–1988 outbreak. However, the first documented dolphin morbillivirus epidemic in the United States may have occurred as early as 1982 in the Indian/Banana River Lagoon of Florida. Duignan *et al.* (1996) concluded retrospectively that the marked increase in strandings in the region and the presence of seropositive individuals in a previously naïve population was indicative of an outbreak which did not persist.

However, probably the largest outbreak of morbillivirus among cetaceans occurred between 1990 and 1992 among striped dolphins in the Mediterranean Sea (Domingo *et al*. 1990). Thousands of animals died during this outbreak and subsequent genetic studies of the isolated virus indicated that it was distinctly different from PDV (Barrett *et al*. 1993), which had emerged in the European harbor seal (*Phoca vitulina*) in 1988 (Osterhaus and Vedder 1988). It was subsequently named dolphin morbillivirus (Visser *et al.* 1993). In 1994 common dolphins (*Delphinus delphis*) in the Black Sea were also affected by a morbillivirus (Birkun *et al*. 1999). In the summer of 2007 there was then a second outbreak of dolphin morbillivirus among the Mediterranean striped dolphins (Raga *et al*. 2008) when >100 animals stranded due to infection. Other species affected include the long-finned pilot whale (*Globicephala melas*) that stranded along the southern Spanish Mediterranean coast in 2006–2007 (Fernandez *et al*. 2008).

In addition, during the PDV outbreak among seals, a morbillivirus was isolated from two harbor porpoises on the coast of Northern Ireland. At necropsy these animals displayed all the pathological signs consistent with morbillivirus infection including severe pneumonia, syncytia formation, and necrosis of the bronchial and bronchiolar epithelium. Acidophilic intracytoplasmic inclusion bodies were seen in the bronchial and bronchiolar epithelium that is highly specific for morbillivirus infection (Kennedy *et al*. 1988). Subsequently a morbillivirus was isolated from two harbor porpoises that died in the Dutch Wadden Sea (Visser *et al*. 1993), distinctly different from DMV when tested against a panel of monoclonal antibodies and in cell culture. This virus was therefore named porpoise morbillivirus.

These outbreaks prompted a number of serological surveys to determine previous morbillivirus exposure, particularly among the odontocete cetaceans, which appear to be especially vulnerable to infection (Visser *et al*. 1993; Duignan *et al.* 1995*a*, 1996; Van Bressem *et al.* 1998, 2001; Nielsen *et al.* 2000). Serological surveys remain the predominant method for determining exposure to disease as well as identifying current immune status, particularly for marine mammals and other wildlife, where samples for viral antigen studies are difficult to obtain (Haydon *et al.* 2002, Thompson *et al*. 2002). The prevalence of pathogen-specific antibodies (*i.e.*, the proportion of seropositive animals with antibody titers above an established threshold) is determined from serum samples. In the case of viruses inducing a long-lasting immunity, such as morbilliviruses, past exposure can be determined using serology. Disease endemicity has been inferred for morbilliviruses in marine mammal populations from studies where very high sero-prevalence proportions were found (Dietz *et al.* 1989; Markussen and Have 1992; Duignan *et al*. 1995*a*, *b*; Van Bressem *et al*. 1998, 2001) but this can only be definitively concluded when it can

be demonstrated that both young and older age-classes have antibodies. However, the distribution of antibody titers can be of value even without age-specific details. Because antibody levels generally increase shortly after exposure to an infection and then decline over time (Lloyd-Smith *et al*. 2007), the titer values can provide information on time since exposure or infection. Therefore, if a pathogen was present but it has not persisted in a population a few individuals might have high titers but most are likely to have lower titers (Cornwell *et al*. 1992), whereas if the pathogen is still present, the mean titers within the population are likely to be higher, as was seen during the PDV outbreaks in Europe in 1988 and 2002 (Pomeroy *et al*. 2005).

Here we investigate the distribution and prevalence of virus neutralizing antibodies to morbilliviruses in cetaceans from around the United States. We used two sources of serum samples, (1) sera collected during live capture-release programs for bottlenose dolphins and (2) additional sera obtained from freshly stranded cetaceans (various species) sampled as part of the U.S. Marine Mammal Health and Stranding Response Program. By comparing titers to different morbilliviruses, the primary antigen to which the dolphins were exposed can be inferred.

Methods

Bottlenose dolphins were sampled from five locations along the U.S. Atlantic and Gulf of Mexico coasts during capture-release operations between 1989 and 2006. In the stranded animals, a variety of different cetacean species were sampled between 1999 and 2004. Neutralizing antibody titers against the two cetacean viruses, DMV and PMV, were measured, together with titers against the pinniped morbillivirus, PDV and the terrestrial carnivore morbillivirus, CDV.

Live Capture-Release

Serum samples were collected during live capture release, health assessment studies being carried out in U.S. coastal waters (Schwacke *et al.* 2004). Figure 1 shows the five locations sampled: Beaufort, North Carolina; Cape May, New Jersey; Charleston, South Carolina; Sarasota Bay, Florida; and St. Joseph Bay, Florida. The capture and release techniques are fully described in Wells *et al.* (2004, 2005) and are summarized here. Small groups of dolphins were encircled using a seine net and each individual was placed in a stretcher and lifted onto a veterinary examination vessel, where it was weighed and measured. Blood samples were drawn from a vessel in the fluke into serum separator tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The serum was removed after centrifugation and stored at -20°C or below for serology and other clinical studies. A tooth was removed for age determination only when an animal's age was unknown (Hohn *et al.* 1989).

The number of serum samples collected by location and year (1999–2006) is shown in Table 1. This ranged from between 12 and 14 serum samples obtained from Cape May and Charleston, up to 70 samples obtained from the more frequent sampling effort for the Sarasota Bay population. In addition 27 serum samples (from 8 females and 5 males) from Sarasota Bay dolphins captured, released and subsequently recaptured and resampled between 1989 and 2001 were screened for the presence of anti-DMV antibodies. To avoid duplication of the results the recaptured animals were excluded from the first group. This was the case for only one animal (FB63, Table 5) which was captured and released four times between 1989 and 2001.

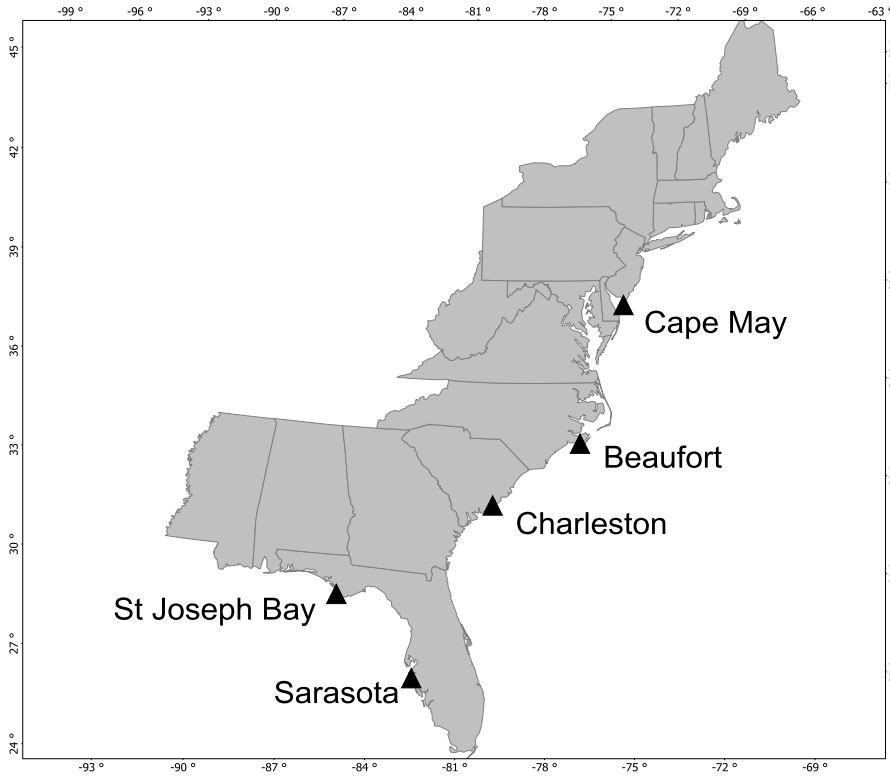

Figure 1. Locations of the bottlenose dolphin cross-sectional live capture study sites.

Stranded Cetaceans

Blood samples collected from animals that stranded live (stranding condition code 1) were obtained from the tail fluke using the same method as for the live captured animals. Those obtained from freshly dead or euthanized individuals were collected from the aorta into a plain Vacutainer tube. Samples were centrifuged and the serum stored at −20°C until testing. The number of animals sampled by species and year is shown in Table 2. Most samples were collected from single stranded animals except for two groups of cetaceans that mass-stranded during the study

Table 1. Number of bottlenose dolphin serum samples tested by year.

Location	1999	2000	2001	2002	2003	2004	2005	2006	Total
Beaufort	2	11						18	31
Cape May				7	5				12
Charleston	14								14
Sarasota Bay			5	8	16	33	8		70
St. Joseph Bay							9	18	27
Total	16	11	5	15	21	33	17	36	154

Table 2. Number of stranded cetaceans tested by year.

Common name	Year					Total
	1999	2001	2002	2003	2004	
Atlantic white-sided dolphin				1		1
Bottlenose dolphin		1	1	3	4	9
Clymene dolphin					2	2
Common dolphin (short-beaked)				1	3	4
Fin whale					1	1
Fraser's dolphin				10		10
Harbor porpoise	1				5	6
Humpback whale			1			1
Killer whale			1			1
Long-finned pilot whale				1		1
Melon headed whale					1	1
Pantropical spotted dolphin					1	1
Pygmy sperm whale					2	2
Risso's dolphin				1	3	4
Rough-toothed dolphin					8	8
Sei whale			1			1
Spinner dolphin				1	1	2
Unspecified dolphin					1	1
Total	1	1	4	18	32	56

period. These comprised 10 Fraser's dolphins (*Lagenodelphis hosei*) that stranded alive in Charlotte Harbor, Florida, in April 2003 and 7 rough-toothed dolphins (*Steno bredanensis*) that stranded alive on Hutchinson Island, St. Lucie, Florida, in August 2004 (an additional rough-toothed dolphin was also sampled in 2004).

Serological Testing

Antibody titers against four morbilliviruses were measured using the microtiter virus neutralization test (Rossiter *et al*. 1985) as described in Saliki and Lehenbauer (2001). The Rockborn strain of CDV, PDV strain 1-2-6A and the Belfast strains of DMV and PMV (kindly donated by Dr. S. Kennedy, Department of Agriculture and Rural Development, Belfast, Northern Ireland) were used in the assays. Viruses were grown in African green monkey kidney (*Cercopithecus aethiops*, Vero) cells (a common cell line for morbillivirus isolation and propagation) using the alpha modification of Eagle's minimum essential medium supplemented with Earle's salts, L-glutamine, 10% fetal bovine serum (FBS), and antibiotics (100 U of penicillin and 100 μg of streptomycin per mL). Twofold dilutions of sera (25 μL) were made in triplicate in 96-well microtiter plates with minimum essential medium with Earle's salts (EMEM) containing 5% fetal bovine serum. An equal volume of virus (25 μL) containing approximately 100 $TCID_{50}$ was added to two wells of each triplicate with the third well containing EMEM as a control. Plates were incubated at 37°C for 1 h. Vero cells (1.5×10^4 cells in 150 μL) were added and the plates incubated at 37°C in 5% CO_2. The plates were read after 4 d by examining cell monolayers for virus-specific cytopathic effects (CPE). Titers were expressed as the reciprocal of the

highest dilution of serum that completely neutralized CPE in both duplicate wells. Titers were also $\log_2$ transformed to linearize the scale.

RESULTS

Cross Sectional Live Capture Samples

The sera of 137 bottlenose dolphins were titrated against DMV. The sera of 114 of these were also tested for the presence of antibodies against PMV, PDV, and CDV. The relationship among the titers ($\log_2$ transformed) is shown in Figure 2A, B. The DMV titers were generally higher than for the other morbilliviruses, especially when compared to the CDV and PDV titers (Fig. 2B shows that among the seropositive titers all were higher against DMV than CDV, above the line of equivalence shown). This indicates the animals were most likely to have been exposed to DMV, since the titers against the homologous virus will always be the highest (Visser *et al.* 1990, Duignan *et al.* 1994). If the other morbillivirus antigens (PDV or CDV) had been used as a surrogate this would have produced a very high proportion of false negative results (11%).

Table 3 shows the frequency of serum DMV titers by location, combining all years (1999–2006) as there was insufficient data to consider temporal as well as spatial trends. Titers $\geq$1:16 ($\log_2 = 4$) were taken as seropositive. The distribution of titers against PMV in the Beaufort and St. Joseph Bay animals are also shown. In the animals from Beaufort sampled in 2006, the two seropositive individuals had higher titers against PMV than against DMV and all the seropositive animals sampled in St. Joseph Bay in 2006 ($n = 4$) had titers that were higher against PMV. Overall, in Beaufort in 2006 and St. Joseph Bay in both 2005 and 2006, 16% (7/45) of the sampled individuals had seropositive titers that were higher against PMV than DMV which may indicate that the virus now circulating along the east coast of the United States and into the Gulf of Mexico is more likely to be the PMV strain than the DMV strain. However, all the PMV seropositive animals were also seropositive against DMV. Thus, in the further analyses of the prevalence of morbillivirus antibodies, the DMV titers will be used for all sites, since even though the PMV titers were higher in some animals from these two regions, the prevalence proportions do not change.

Table 3 also shows the prevalence (%) of seropositive animals at each location and the 95% confidence intervals around these prevalences. The confidence intervals indicate the likely range and therefore the reliability of the prevalence estimates, given the sample sizes in each group. A Fisher's exact test (two-sided) for differences among proportions found the prevalences to be significantly different among the locations ($P < 0.0001$). Charleston and Sarasota Bay had no seropositive samples, in contrast to the longitudinal samples from Sarasota Bay (see section further). Beaufort and Cape May had similar seropositive proportions of about 20%–30%. These two sites also included the animals with the highest titers ($\geq$1:256). The prevalence of seropositive dolphins from St. Joseph Bay was 18% (Table 3).

We found that increasing the seropositive threshold from $\geq$1:16 to $\geq$1:32 did not significantly affect the results. The prevalences in samples from Beaufort and Cape May at $\geq$1:32 reduced to 13% and 25%, respectively. This did not substantially affect any conclusions regarding exposure and susceptibility, thus we retained a seropositive threshold of $\geq$1:16.

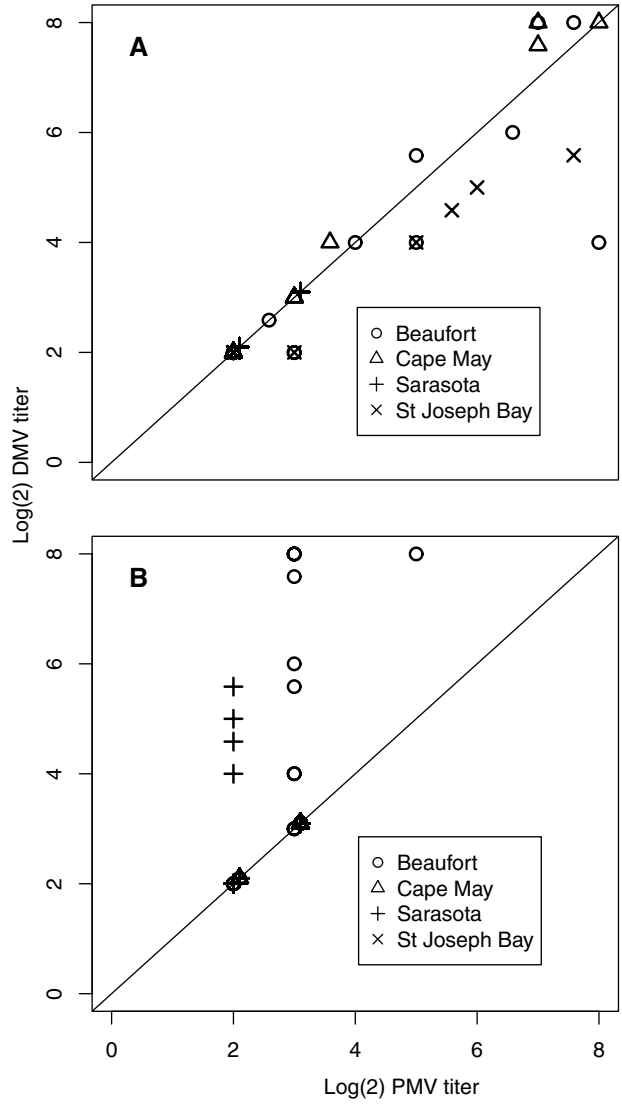

Figure 2. Comparison of $\log_2$(DMV) against (A) $\log_2$(PMV) titers and (B) $\log_2$(CDV) titers.

The percent positive (prevalence) and the number of seronegative or seropositive by region and year of birth (where this was available, $n = 125$) are shown inTable 4. The majority of seropositive samples ($\geq$1:16, $\log_2 \geq 4$) were from individuals born prior to a documented morbillivirus outbreak within their region of capture. For samples from the eastern U.S. Atlantic coast (Cape May, Beaufort, and Charleston), only one seropositive individual was born subsequent to the 1987–1988 morbillivirus outbreak. This individual's titer was relatively low (1:16) and his estimated year of birth was 1989 (11 yr old at time of capture), suggesting that the virus circulated

Table 3. Frequency of DMV and PMV serum titers in live captured bottlenose dolphins by location (<1:16 = seronegative, ≥1:16 = seropositive).

DMV titer	<4	6	8	16	24	32	48	64	96	128	192	256	Total	Negative	Positive	Seropositive prevalence (%)	95% CI
Beaufort	15	1	8	3			1	1				2	31	24	7	23	10–42
Cape May	5		3	1							1	2	12	8	4	33	10–65
Charleston			14										14	14	0	0	0–2
Sarasota	19		51										70	70	0	0	0–5
St. Joseph Bay	18			1	1	1	1						22	18	4	18	5–35
Total	57	1	76	5	1	1	2	1	0	0	1	4	149	134	15	10	6–16
PMV titer	<4	6	8	16	24	32	48	64	96	128	192	256					
Beaufort	13	1	2	1		2			1	1	1	1	23	16	7	30	16–51
St. Joseph Bay	21		1			1	1	2			1		27	22	5	19	8–37
Total	34	1	3	1		3	1	2	1	1	2	1	50	38	12	24	14–37

Table 4. Number animals with positive ($P \geq 1{:}16$) and negative ($N < 1{:}16$) titers to DMV by year of birth (age) and region.

Region / Year of birth	Beaufort			Cape May			Charleston			Sarasota			St. Joseph Bay		
	N	*P*	Prevalence (%)	*N*	*P*	Prevalence (%)	*N*	*P*	Prevalence (%)	*N*	*P*	Prevalence (%)	*N*	*P*	Prevalence (%)
1970–1975	0	0	0	0	1	100	2	0	0	6	0	0	2	0	0
1976–1980	0	0	0	0	2	100	4	0	0	2	0	0	0	1	100
1981–1985	0	1	100	0	0	0	2	0	0	11	0	0	3	1	25
1986–1990	3	1	25	1	1	50	2	0	0	10	0	0	2	0	0
1991–1995	4	0	0	4	0	0	1	0	0	13	0	0	4	0	0
1996–2000	1	0	0	2	0	0	3	0	0	16	0	0	3	1	25
2001–2005	0	0	0	1	0	0	0	0	0	9	0	0	4	1	20

for some months past the end of the observed die-off. Similarly, two relatively young (~2 yr and 8 yr old) dolphins sampled from St. Joseph Bay with estimated birth years subsequent to the 1992–1993 Gulf of Mexico morbillivirus outbreak also had low titers (1:16 and 1:32). There is a small possibility that the observed titer, at least in the younger animal, was remnant maternal antibody. There was no difference in the distribution of titers between the sexes (Mann Whitney *U*-test, $P = 0.596$).

Longitudinal Samples from Sarasota Bay

The results from serum samples collected longitudinally from the Sarasota Bay dolphins between 1989 and 2001 are shown in Table 5. Between 1989 and 1994 almost half of the animals sampled (6/13, 46%) had a high DMV titer ≥1:64 up to a maximum of 1:256 (Table 5). The four individuals that were followed until 2000–2001 showed a subsequent decline or essentially no change in titers between around 1993 and 2001 (a fourfold change in titer is generally considered clinically significant, Bonilla *et al*. 2005). Interestingly, one animal (FB63), captured four times over an 11-yr period had a consistently relatively high titer ranging from 1:64 to 1:192, These results are in contrast to the recent results reported above, in which none of the additionally captured Sarasota Bay dolphins between 1999 and 2006 had seropositive morbillivirus titers.

Stranded Cetaceans

The stranded cetacean samples were obtained from live and freshly dead animals and they comprised two categories, single stranded animals and groups. The geographical locations of single stranded animals that provided samples are plotted in Figure 3. The most common species were bottlenose dolphins ($n = 9$) and harbor porpoises ($n = 6$, Table 2). Thirty-three animals were seronegative and six were seropositive. Since genetic studies were not carried out on the bottlenose dolphins, it is not possible to determine whether they were coastal or offshore animals. The titers against the four morbilliviruses for the seropositive animals are listed in Table 6. In four species titers were higher against DMV than the other morbilliviruses, but in two bottlenose dolphins, titers were again higher against PMV than DMV. One rough-toothed dolphin stranded in the Gulf of Mexico had a very high titer against DMV but all serum samples from the two mass-strandings (Fraser's dolphins and rough-toothed dolphins, $n = 17$) were negative.

Discussion

This study describes the distribution of neutralizing antibody titers against various morbilliviruses in cetaceans from U.S. waters. Three different sample sources were used; serum collected during live capture-release studies, from live-stranded animals, and from freshly dead animals. Here we used a titer threshold of ≥1:16 to classify animals as having positive, protective antibody levels. Other studies of neutralizing antibodies to morbilliviruses in marine mammals have used different seropositive thresholds, ranging between ≥1:16 and ≥1:64 (Duignan *et al.* 1996, Thompson *et al.* 1992, Nielsen *et al.* 2000, Thompson *et al.* 2002). Because of this variation, we investigated the effect of changing the threshold. We found that increasing the cut-off from ≥1:16 to ≥1:32 did not substantially change the prevalence results

Table 5. Longitudinal morbillivirus titers in adult bottlenose dolphins from Sarasota Bay, Florida.

Dolphin	Age in 1990	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
Females														
FB29	27	64		<16			<16							
FB17	29			512			<16							
FB13	31					>16							12	
FB35	34			>16										
FB63	36		128				64				64			192
FB57	38		256		256	256								
FB15	40			>16									8	
FB19	46			128		64								
Males														
FB46	12			32		<16								
FB14	17	128				<16							<6	
FB98	37			>16										
FB74	39	>16												
FB112	39	>16												

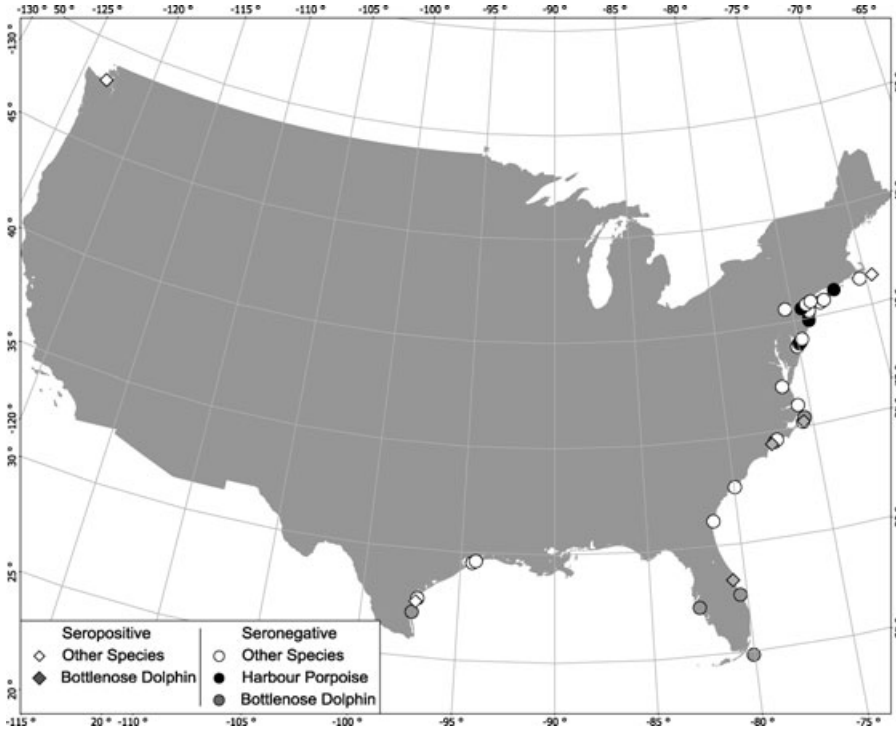

Figure 3. Geographical locations of single stranded cetaceans tested for morbillivirus antibodies.

or conclusions from the study, indicating the robustness of using ≥1:16 as the seropositive threshold titer. While other studies might continue to use a higher threshold (Thompson *et al.* 2002), a slightly more cautionary approach is warranted in studies of vulnerable species, particularly when they are not sampled during an epidemic. However, a higher threshold is also recommended when using hemolyzed sera that may be toxic to the virus.

Table 6. Differential morbillivirus neutralization titers in single stranded cetaceans.

Latitude (°N)	Longitude (°W)	Date	Species	DMV	PMV	CDV	PDV
28.35139	80.65060	12 July 2001	Bottlenose dolphin	16	16	4	8
48.17629	123.13700	2 January 2002	Killer whale	64	32	16	64
41.37820	69.57470	18 October 2002	Humpback whale	32	32	<8	<8
34.43000	77.54330	30 July 2004	Bottlenose dolphin	16	32	<8	<8
35.25469	75.52030	6 August 2004	Bottlenose dolphin	64	128	4	<4
27.56667	97.21670	26 August 2004	Rough-toothed dolphin	≥256	192	<4	<4

The largest sample size for this serological survey was provided by the capture-release studies of bottlenose dolphins that have been carried out on the east coast of the United States and in the Gulf of Mexico since 1989 (although in some locations the time series of samplings through capture-release has been much longer (Wells and Scott 1990)). The results suggest that, although morbilliviruses did not persist in coastal stocks after the 1980s outbreaks, PMV in particular may still be circulating in the southerly regions. This is in line with the findings of Taubenberger *et al*. (1996) who only found PMV and not DMV in a sample of the bottlenose dolphins that died during the Gulf of Mexico epidemic in 1993. In the study presented here, animals sampled in St. Joseph Bay had higher titers against PMV than against DMV. Although PMV was first isolated in a harbor porpoise (Kennedy *et al.* 1988), its natural host range has not been fully established. By contrast, in the Atlantic regions both viruses may be circulating, with DMV being more prevalent in the north, as was also reported in the late 1980s (Taubenberger *et al*. 1996). Clearly isolation and characterization of the viruses responsible for the serological test results would be advantageous.

Heterologous antigen has been widely used in neutralization assays to detect antibodies to emerging viruses from the same family, particularly for viruses that are difficult to isolate and culture (OIE 2008). While it is desirable to use homologous antigen, this is not always possible for both practical and economic reasons. However, as has been shown in this study, this compromise may produce biased results. A panel of four morbilliviruses was used to screen 114 bottlenose dolphin serum samples. When the correlation among the titers was examined (Fig. 2A, B) it indicated that the CeMV titers were largely greater than those for PDV or CDV. Thus, if CDV or PDV antigen alone had been used this would have resulted in a very high number of false negatives, illustrating the importance of using or developing a specific assay wherever possible.

In addition to the difference in the response to different morbillivirus antigens in the St. Joseph Bay population, two of the younger animals captured and sampled had seropositive titers, which could suggest recent exposure, since the animals would not have been present in the location at the time of the previous outbreaks. Future serological surveys in conjunction with viral RNA detection and phylogenetic analyses are needed to establish the likelihood of this conclusion *vs.* the possibility that the observed titers were remnant maternal antibody. The lactation period for bottlenose dolphins is ~2–3 yr (Wells and Scott 1999), with the possibility of some continued passive immunity occurring throughout lactation (Corthésy-Theulaz *et al*. 2003). However, although calves as old as 7 yr have been observed with lactating mothers in Sarasota Bay (Wells and Scott 1999) it seems unlikely that a juvenile as old as 8 yr is continuing to receive maternal antibodies.

Serological surveys are, therefore, also important for establishing potential past exposure scenarios, particularly where age-prevalence data are available (Marschner 1996). It seems likely that bottlenose dolphins from estuaries in the Beaufort area were exposed in the past, possibly during the 1987–1988 morbillivirus outbreak. It had been thought that the estuary animals were not involved in this die-off (Geraci 1989) but either morbillivirus still is, and has been, circulating in the stock for over 15 yr without establishing itself sufficiently to cause an epidemic (or indeed to become endemic), or that animals over 15 yr of age were exposed in the 1980s and have life-long immunity. Dolphins monitored with electronic tags in Beaufort during 1999 and 2000 were found to primarily inhabit the estuaries near Beaufort but many of them spent some time in nearshore waters (Hohn and Hansen, unpublished

data). It is reasonable to assume that they would have come into contact with coastal migratory dolphins infected with morbillivirus during the 1987–1988 epizootic.

The most important conservation result from this study is that the bottlenose dolphins in Sarasota Bay, Florida, and in Charleston, South Carolina, which are relatively isolated, estuarine resident communities (Scott *et al*. 1990, Zolman 2002), have not been exposed to morbilliviruses in recent years, have essentially no protective antibody titers and are therefore highly vulnerable to infection if morbillivirus were to be reintroduced. Of some note is the finding that a morbillivirus was circulating in the long-term Sarasota Bay residents in the late 1980s and although one animal still had high antibody levels in 2001 animals sampled since then have been seronegative.[2] In addition the animal with a high titer in 2001 (FB63) had been consistently highly seropositive since its first capture in 1990, perhaps indicating the individual variation in response and maintenance of titers, as is seen in human measles (Itoh *et al*. 2002). It appears that the virus did not persist perhaps because there was insufficient contact between infective and susceptible animals for the virus to spread or cause an epidemic and the disease then faded out. From the recent data we conclude that this population, spanning five generations, is now once again naïve. That dolphins inhabiting estuaries near Charleston were seronegative while those in Beaufort were seropositive might reflect differences between these locations in movements of dolphins from estuarine to coastal waters, with dolphins in Charleston being more insular. We know from the outbreak of DMV in striped dolphins in the Mediterranean (Aguilar and Raga 1993) and its re-emergence in 2007 (Raga *et al*. 2008) that initial mortality rates are likely to be very high if the virus were to be reintroduced in such naïve populations without immunity.

Obtaining samples from different cetacean species for morbillivirus serology through strandings is very difficult, as animals are often too decomposed for blood sample collection (Gulland 1999). Most samples in this study were obtained from animals that stranded alive and then died or were euthanized. Here we investigated morbillivirus titers in samples collected from these sources and found that three bottlenose dolphins from the east and Gulf of Mexico coasts of the United States were seropositive. This is in line with the findings from the capture-release studies. Perhaps an important finding was the very high titer in a stranded rough-toothed dolphin from the Gulf of Mexico, sampled in August 2004. Although an isolated case, it could indicate the circulation of the virus in these offshore species. However, it is only a single animal and is in contrast to the results from a group of animals that live stranded the same year and region and that were all seronegative. This illustrates the problem of making inferences about exposure among particular species in specific regions from single stranded animals and the need for continued long-term surveillance studies that will allow sample sizes to increase with time and opportunity.

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[2]It should be noted that no dolphins >37 yr of age have been sampled since 2001, in spite of the presence of residents in their 40s and 50s in the population.

was conducted under NMFS Marine Mammal Health and Stranding Response Program Permit No. Permit No. 932-1489. Field sampling in Sarasota, Florida, was conducted under NMFS Scientific Research Permit Nos. 522-1569 and 522-1785, approved by the Mote Marine Laboratory Institutional Animal Care and Use Committee, and supported by NMFS, Dolphin Quest, and the Chicago Zoological Society. We also extend our thanks to the dedicated volunteers of the U.S. Southeast and Northeast Marine Mammal Stranding Networks.

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