Harmful Algae 8 (2009) 598–607

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Review

Karenia brevis red tides, brevetoxins in the food web, and impacts on natural resources: Decadal advancements

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ARTICLE INFO

Keywords:
Animal mortalities
Brevetoxins
Decadal advancements
Effects
Food webs
Harmful algal blooms
Karenia brevis
Red tide
Toxin transfer

ABSTRACT

As recently as a decade ago, *Karenia brevis* red tides and their effects on animal resources in the Gulf of Mexico were principally perceived as acute blooms that caused massive fish kills. Although occasional mortalities of higher vertebrates were documented, it has only been in the past decade that conclusive evidence has unequivocally demonstrated that red tides and their brevetoxins are lethal to these organisms. Brevetoxins can be transferred through the food chain and are accumulated in or transferred by biota at many trophic levels. The trophic transfer of brevetoxins in the food web is a complex phenomenon, one that is far more complicated than originally conceived. Unexplained fish kills and other animal mortalities in areas where red tide is endemic are being increasingly linked with post-bloom exposures of biota to brevetoxins. Mass mortality events of endangered Florida manatees (*Trichechus manatus latirostris*) follow a consistent spatial and temporal pattern, occurring primarily in the spring in southwestern Florida. Persistent blooms can also cause a cascade of environmental changes, affecting the ecosystem and causing widespread die-offs of benthic communities. Ongoing fish kills from sustained blooms can lead to short-term declines in local populations. Although animal populations in areas where red tide is endemic are unquestionably at risk, it remains to be determined to what extent populations can continue to recover from these sustained effects.

Contents

1. Introduction

As recently as a decade ago, red tides and their effects on animal resources in the Gulf of Mexico, were principally perceived as acute blooms that caused massive fish kills and occasional mortalities of higher vertebrates (Steidinger et al., 1973; Landsberg, 2002). Although in earlier decades, mass mortality events involving, for example, turtles and a few bottlenose dolphins (*Tursiops truncatus*) (1946–1947), manatees (*Trichechus manatus latirostris*) (1982), and bottlenose dolphins (1987), co-occurred with red tides, definitive proof that brevetoxins were lethal to marine mammals was still lacking (Gunter et al., 1948; Geraci, 1989; O'Shea et al., 1991).

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doi:10.1016/j.hal.2008.11.010

Information on the fate and effects of brevetoxins in the environment was minimal. There was no real clear understanding about the stability of brevetoxins outside of *Karenia brevis* cells or recognition that toxins could be transferred through the food chain and accumulated or transferred by biota at many trophic levels.

Annual kills caused by *K. brevis* red tides involve hundreds of thousands of fish and other animal species. However, data describing declines in fisheries, and the evident rebound of selected fish species every year have provided no indication that frequent red tides could lead to an unsustainable reduction in fish populations or even threaten the long-term survival of local populations of imperiled species.

During the past decade, the following have enabled us to address critical research questions about red tide: the improved availability of funding for research through federal initiatives such as the Ecology and Oceanography of Harmful Algal Blooms and from Florida's state legislature; the increased support from local grass-roots movements such as Solutions To Avoid Red Tide; and the enhancements in technological and analytical capabilities. Such initiatives have provided the impetus to resolve many outstanding questions about the transfer of brevetoxins in the food web and to assess their widespread effects on natural resources.

This paper summarizes key research findings and breakthroughs of the past decade that have provided opportunities for revising management strategies and for making further strides in protecting critical fisheries, endangered species, and key habitats from the sustained effects from Florida red tides.

2. Verification that red tides are lethal to higher vertebrates

Brevetoxins acutely affect humans from neurotoxic shellfish poisoning (NSP) (Steidinger et al., 1998; Poli et al., 2000) or respiratory irritation as well as other chronic sequelae (Backer et al., 2003; Fleming et al., 2005, 2007). However, no fatalities have been documented, probably because humans are not typically exposed to lethal doses.

Since they were first reported in the Gulf of Mexico in 1648 (Magaña et al., 2003), *K. brevis* red tides have been documented to have killed hundreds of animals at higher trophic levels, such as marine birds, turtles, and mammals (Gunter et al., 1948; Quick and Henderson, 1974; Forrester et al., 1977; Kreuder et al., 2002; Landsberg, 2002). Despite the overwhelming evidence for the co-occurrence of red tides with wide-scale animal die-offs, definitive proof that brevetoxins can kill a wide diversity of aquatic organisms as well as terrestrial species connected via the food chain has been hard to obtain.

Brevetoxins were proposed to be a primary causative agent in an unprecedented die-off of more than 740 bottlenose dolphins from June 1987 to February 1988 throughout the southeastern USA (Geraci, 1989). The event coincided in part with an ongoing red tide bloom that was transported from Florida to North Carolina during 1987, a rare occurrence (Tester et al., 1991). The evidence for brevetoxin involvement remained equivocal because the analytical methods available at the time were inadequate to confirm its identity (Van Dolah et al., 2003). Other etiologies, particularly for a viral pathogenesis, were considered as primary contributing factors (Lipscomb et al., 1994).

We have yet to determine via controlled experiments the level of brevetoxins that would be lethal to specific species. The ability to determine toxin concentrations in tissues, fluids, and stomach contents in carcasses found during red tide-mass mortality events has improved considerably because of rapid response, refinement of field protocols, and accurate analysis using reliable technologies. Presumptively acute lethal brevetoxin concentrations can be evaluated against tissue concentrations from "control" animals that died from other causes in endemic red tide areas.

During the past few years, several mortality events involving higher vertebrate have provided more epizootiological field data. Extensive efforts have been undertaken to obtain fresh tissues for toxin analyses, to make accurate assessments of the distribution of carcasses, to compare the spatial and temporal distributions of *K. brevis* cell concentrations with brevetoxin levels in water and tissues, to obtain relevant environmental data, and to identify toxin vectors in the food chain.

In Florida, wide-scale mass mortalities caused by *K. brevis* blooms that were associated with brevetoxicosis occurred for manatees in 1996, 2002, 2003, and 2005 (Bossart et al., 1998, 2002; Landsberg and Steidinger, 1998; Flewelling et al., 2005; Florida Fish and Wildlife Conservation Commission [FWC], unpublished) and for bottlenose dolphins in 1999–2000, 2004, and 2005–2006 (Mase, Leighfield, and Baran, National Oceanic and Atmospheric Administration [NOAA], personal communications; Flewelling et al., 2005) (Table 1).

In some cases, the manatee die-offs occurred concurrently with a *K. brevis* bloom, but in others, lag effects resulted in a delayed toxin exposure (see below). In years when the manatee mortality coincided with a red tide, it was postulated that animals were exposed to brevetoxins through inhalation as well as by ingestion (Landsberg and Steidinger, 1998), although the effects induced by each of these routes of exposure are not easily separated. In 1996, the presence of very low concentrations of brevetoxins in the nasal and lung tissue demonstrated that aerosol could cause consistent inflammatory lesions of the upper mammalian respiratory tract (Bossart et al., 1998). Thus far, there is no indication that inhalation of aerosolized brevetoxins alone can produce lethal doses, but this route of exposure can potentially debilitate manatees, cause pathologies, and lead to fatal secondary effects.

During August 1999–February 2000, more than 120 bottlenose dolphins stranded along the Florida Panhandle, coincident with a

Table 1
Summary of major mass mortality events of marine mammals associated with red tides.

Species	Year	Period	Number	Location	Reference
Tursiops truncatus	1946–1947	Nov–Aug	<5	SWFL	Gunter et al. (1948)
Trichechus m. latirostris	1963	March–April	7	SWFL	Layne (1965)
Trichechus m. latirostris	1982	Feb–April	39	SWFL	O'Shea et al. (1991)
Tursiops truncatus	1987–1988	June–Feb	>740*	Eastern U.S.	Geraci (1989)
Trichechus m. latirostris	1996	March–May	149	SWFL	Landsberg and Steidinger (1998); FWC, unpublished
Tursiops truncatus	1999–2000	Aug–Feb	>120	NWFL	NOAA, unpublished
Trichechus m. latirostris	2002	March–April	30	SWFL	Flewelling et al. (2005); FWC, unpublished
Trichechus m. latirostris	2003	March–April	69	SWFL	FWC, unpublished
Tursiops truncatus	2004	March–April	107	NWFL	NOAA, unpublished
Trichechus m. latirostris	2005	March-April	45	SWFL	FWC, unpublished
Tursiops truncatus	2005–2006	July–June	136**	SWFL	NOAA, unpublished
Tursiops truncatus	2005–2006	Sept–April	93**	NWFL	NOAA, unpublished

* Brevetoxin suspected but not definitively proven (Geraci, 1989).
** Preliminary data from NOAA.

persistent *K. brevis* bloom. Brevetoxin (as PbTx-3) was confirmed by LC–MS/MS in about a third of the animals tested, with the highest concentrations in stomach contents. Analysis for morbillivirus was negative, and no other obvious etiological factors were suspected. These data provided more conclusive evidence that brevetoxin played a role in the dolphin mortalities (Van Dolah et al., 2003), but definitive proof was lacking because of the absence of lethal dose values and because brevetoxins were not detected in two thirds of the animals tested.

Thus far, in mass mortality events of marine mammals associated with red tides, the most likely route of lethal toxin exposure has been by ingestion. For bottlenose dolphins in 2004, for example, exposure occurred principally from their ingestion of live fish prey vectoring brevetoxin (see below). Manatees are exposed to lethal doses of brevetoxin through ingestion of seagrass (Flewelling et al., 2005) – their primary food source (McDonald and Flamm, 2006). These findings demonstrate the importance of the trophic transfer of toxins through the diet to higher level consumers (see below).

Despite the earlier controversy, the sustained co-occurrence of red tides and marine mammal die-offs eventually leads to an overriding weight of evidence for a direct link between red tides and higher vertebrate mortalities (Table 1; Bossart et al., 1998, 2002; Landsberg and Steidinger, 1998; Flewelling et al., 2005).

Birds also succumb to brevetoxicosis, especially those that consume toxic shellfish or fish. During the Florida red tide from October 1973 to May 1974, large numbers of birds were found moribund or dead, particularly double-crested cormorants (*Phalacrocorax auritus*), red-breasted mergansers (*Mergus merganser*), and lesser scaup (*Aythya affinis*). During an eight-week period, the deaths of several thousand lesser scaup were attributed to brevetoxicosis via ingestion of toxic shellfish (Forrester et al., 1977). Substantial numbers of sick and dying double-crested cormorants have co-occurred with red tide outbreaks along the west Florida coast since 1995 (Kreuder et al., 2002), and in spring 2002, a mortality of more than 20 lesser scaup was also attributed to brevetoxicosis in southwestern Florida (FWC, unpublished). Directed investigations have provided evidence for the role of brevetoxins in bird die-offs in the last few years (Vargo et al., 2006; Landsberg et al., 2007; FWC, unpublished).

Sea turtle strandings in southwestern Florida increase (FWC, unpublished) during red tides. During the extended 2005 red tide event that was responsible for wide-scale persistent mortalities of many animal species, the average number of stranded sea turtles also increased, particularly during the summer in the Tampa Bay region, when a wide-scale benthic mortality co-occurred with the red tide (see below). The number of stranded turtles increased significantly when compared to the average number of turtles stranded during the last decade (Fig. 1). Turtle species principally affected by the 2005 red tide in southwestern Florida were loggerheads (*Caretta caretta*), green turtles (*Chelonia mydas*), and Kemp's ridley (*Lepidochelys kempii*) (FWC, Sea Turtle Standing and Salvage Network Data Base; Foley, FWC, personal communication).

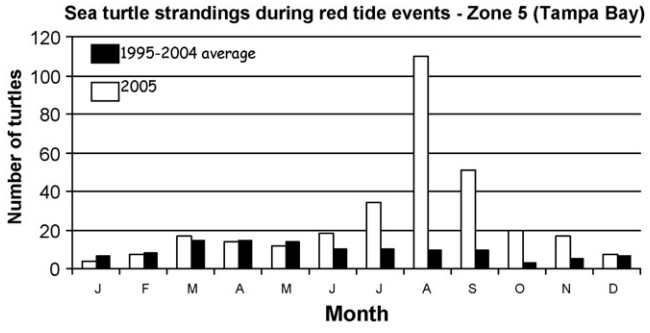

Fig. 1. Sea turtle strandings during red tide events in the Tampa Bay region, southwestern Florida (FWC, unpublished).

Although red tides are responsible for wide-scale mortalities, it has also been recognized that animals exposed to sublethal doses of brevetoxins can survive if removed from the wild and provided with remedial treatment. Local rehabilitation centers have successfully recovered multiple species of birds (Landsberg et al., 2007), turtles, and manatees (FWC, 2007) affected by red tide.

3. Trophic transfer of brevetoxins is a significant process – toxins that have accumulated in biota and are then vectored through the food web can cause mass mortalities of aquatic organisms

Although data suggest that shellfish can be killed by *K. brevis* red tides (Summerson and Peterson, 1990; Leverone et al., 2006), it has long been recognized that filter-feeding organisms, such as bivalves, usually survive red tides and accumulate lipophilic brevetoxins that cause NSP if consumed by humans (McFarren et al., 1965; Roberts et al., 1979; Ishida et al., 1996, 2004; Steidinger et al., 1998; Dickey et al., 1999; Poli et al., 2000; Plakas et al., 2002, 2004; Naar et al., 2004; Pierce et al., 2004; Wang et al., 2004). Depending on the species, shellfish are able to accumulate varying concentrations of brevetoxins (Landsberg, 2002) and thus are significant sources for the potential transfer of toxins up the food chain.

Although these data may not extrapolate to field conditions, when Pacific oysters, *Crassostrea gigas*, were experimentally exposed to 10–25 $\times$ 10^6 *K. brevis* cells oyster^{-1} over a 24 h period, brevetoxin levels were 25–100 mouse units (MU) 100 g^{-1} of drained oyster meat. After three days of depuration, brevetoxin concentrations in oysters were considerably reduced and almost at acceptable regulatory limits (20 MU 100 g^{-1}) for human consumption (Fletcher et al., 1998). More typically, naturally exposed hard clams (*Mercenaria* sp.) and eastern oysters (*Crassostrea virginica*) remain toxic at levels dangerous to humans for two to eight weeks after a red tide (Morton and Burklew, 1969; Pierce et al., 2004; FWC, unpublished). Mollusc species naturally exposed to brevetoxins can accumulate up to hundreds of thousands of ng g^{-1} (FWC, unpublished), with documented reports up to hundreds of MU 100 g^{-1} (Table 2).

Brevetoxins are potent ichthyotoxins that are commonly responsible for fish kills involving millions of fish and affecting hundreds of species (Steidinger et al., 1973; Baden, 1989; Landsberg, 2002). Fish kills usually originate with the lysis of *K. brevis* cells and the subsequent release of brevetoxins, which are absorbed directly across the gill membranes (Abbott et al., 1975; Baden, 1988). Fish may also be killed by ingesting *K. brevis* cells or toxins in the water, or by consuming toxic biota (Landsberg, 2002).

Because the prevailing assumption has been that most blooms are ichthyotoxic, little attention has been directed towards the possibility that toxins might be cycled via fish in the food web. The overall presumption was that because the red tide-affected fish were dead, typical predators of live prey (e.g., marine mammals, non-scavenging birds) would not be feeding on the carcasses and so the decomposing fish would become "recycled" and incorporated into the benthic food web, and brevetoxins would not be transferred up the food chain. However, some trophic components of the food web do survive red tide blooms (Naar et al., 2007).

Brevetoxins from *K. brevis* have been traced through experimental food chains from dinoflagellates through zooplankton grazers to juvenile fish (Tester et al., 2000; Prince et al., 2006). The generality of this food chain transfer was demonstrated by using three different combinations of copepods (*Temora turbinata*, *Labidocera aestiva*, *Acartia tonsa*) and various juvenile fish (spotted mojarra, *Eucinostomus argenteus*; striped killifish, *Fundulus majalis*;

Table 2
Maximum brevetoxin concentrations reported from various mollusc species (MU = mouse units).

Species	Common name	Maximum brevetoxin concentration (MU 100 g^{-1})	Reference
Donax variabilis	Coquinas	550	Cummins et al. (1971)
Mercenaria campechiensis	Hard clams	270	Cummins et al. (1971)
Crassostrea virginica	Eastern oyster	180	Tester and Fowler (1990)
Crassostrea gigas	Pacific oyster	146	Pierce et al. (2004)
Macrocallista nimbosa	Sunray Venus clams	140	Cummins et al. (1971)
Chione cancellata	Cross-barred Venus	95	Steidinger et al. (1998)
Mercenaria mercenaria	Hard clams	69	Pierce et al. (2004)
Busycon sp.	Whelk	22	Pierce et al. (2004)

pinfish, *Lagodon rhomboides*; spot, *Leiostomus xanthurus*) during different seasons. Juvenile spot were fed toxin-laden copepods so that vectorial intoxication could be examined. Toxins were shown to move from fish viscera to muscle tissue after 2–6 h to 25 h (Tester et al., 2000).

Additional mechanisms of trophic toxin transfer to fish have been demonstrated. When fed healthy *K. brevis* at bloom densities, planktivorous fish (juvenile striped mullet, *Mugil cephalus*) accumulated brevetoxins to levels commensurate with *K. brevis* densities in the aquarium and with exposure duration (6–24 h). At high densities, fish exposed to intact *K. brevis* cells survived, whereas those exposed to lysed cells died quickly without accumulating brevetoxins. Similarly, omnivorous pinfish and Atlantic croakers (*Micropogonias undulatus*) fed brevetoxin-contaminated shellfish over a two-week period also accumulated brevetoxins in both muscle and viscera (Naar et al., 2007).

These studies demonstrate that fish can tolerate exposure to brevetoxins and can accumulate brevetoxins in both muscle and (at higher levels) in the viscera. Although the lethal limit of brevetoxins ingested by live fish remains unknown, contaminated shellfish may have been responsible for a monospecific fish kill. In March 2003, hundreds of dead striped burrfish (*Chilomycterus schoepfi*) washed up along southwestern Florida beaches following a red tide bloom. Analyses of freshly dead fish ($N = 4$) revealed high concentrations of brevetoxins in both the muscle and internal tissues. Stomach contents of the burrfish principally contained the remains of small bivalves, whose brevetoxin concentrations ranged from 3780 to 6494 ng g^{-1}. HPLC–MS analyses identified brevetoxin metabolites in both fish tissues and stomach contents (FWC and University of North Carolina at Wilmington [UNCW], unpublished). We postulate that following exposure to a red tide bloom, small filter-feeding bivalves, a primary food source of striped burrfish (Motta et al., 1995), contained sufficient quantities of brevetoxin to be lethal.

Therefore, fish and shellfish can survive red tides and act as significant vectors for the transfer of brevetoxins to higher trophic levels – a process that could result in mortalities of fish- or shellfish-consuming birds, marine mammals, and sea turtles. The vectoring of brevetoxins by fish (Naar et al., 2007) was demonstrated in conjunction with a bottlenose dolphin mortality event in 2004 (Flewelling et al., 2005). In response to this mortality, an extensive epidemiological, pathological, and environmental investigation was conducted that failed to identify any consistent mortality factors other than brevetoxin (NOAA and FWC, 2004). Although no *K. brevis* was observed in the area of the strandings, bottlenose dolphins had acutely toxic stomach contents that were comprised mostly of planktivorous menhaden (*Brevoortia* sp.). Analyses of several undigested fish revealed extremely high levels of brevetoxins that were also found to a lesser extent in all eight fish species collected live from the area two weeks after the onset of the dolphin mortalities. This confirmed that fish can be vectors of lethal doses of brevetoxin to marine mammals that consume live prey (Flewelling et al., 2005)

Other novel mechanisms for the exposure of brevetoxins to higher vertebrates have recently been demonstrated. In spring 2002, three weeks after a *K. brevis* bloom had almost dissipated (see below), 30 manatees died in southwestern Florida. Elevated brevetoxin concentrations were found in the visceral tissues, with only low concentrations present in the lungs. High brevetoxin concentrations (maximum of 1132 ng g^{-1}) were found in stomach contents containing only seagrass, confirming recent ingestion exposure as opposed to inhalation in all animals tested ($N = 27$). Minimal levels of brevetoxins in the seawater (<1.3 ng ml^{-1}) were in contrast to the high toxicity levels of seagrass (PbTx-3, up to 1263 ng g^{-1}) at four mortality sites. Although the mechanism of toxin accumulation (active uptake versus the passive adsorption of toxin) in seagrass is unknown, fractionation analysis showed brevetoxin in all seagrass components (epiphytes [83%], blades [7%], and rhizomes [10%] of total brevetoxin concentration measured) (Flewelling et al., 2005; FWC, 2007). Although filter-feeding tunicates attached to seagrass were suspected brevetoxin vectors in the 1982 manatee mortality event (O'Shea et al., 1991), Flewelling et al. (2005) demonstrated that not only are seagrass and its associated epiphytes significant sources of brevetoxin but such substrates can provide lethal toxin doses to manatees even in the absence of red tide (see below).

Because many lower-trophic-level animals survive red tides and store brevetoxins, toxin transfer up the food chain is possible in many different ways. Food-web transfer of brevetoxins is now a recognized route by which higher level organisms can become exposed to these toxins (Tester et al., 2000; Flewelling et al., 2005). In cases such as those described above, brevetoxins persist in the environment well after a red tide bloom has dissipated, toxic biota remain a latent source of toxicity throughout the food web, and lethal thresholds at higher levels are evidently met.

4. Demonstration of lethal lag effects from post-bloom exposures

Unexplained fish kills and other animal mortalities in red tide endemic areas are increasingly linked with post-bloom exposures of biota to brevetoxins. Following several recent mortality events, we were able to collect animal tissues and environmental samples for brevetoxin analyses. These analyses showed that persistence of high concentrations of brevetoxins in various biotic reservoirs can remain a stable source of toxicity, even in the apparent absence of *K. brevis* cells.

A persistent fish kill in northwestern Florida from mid-January to May 2006 demonstrated the potential for lag effects from red tides. All of the reported fish mortalities occurred in Choctawhatchee Bay near Fort Walton Beach, with juvenile spot (*L. xanthurus*) being the most affected. A few dead and dying fish, including Gulf sturgeon (*Acipenser oxyrinchus desotoi*), longnose gar (*Lepisosteus osseus*), bay anchovies (*Anchoa mitchilli*), skipjack shad (*Alosa chrysochloris*), and invertebrates such as blue crabs (*Callinectes sapidus*) and lion's mane jellyfish (*Cyanea capillata*) were also reported (FWC and UNCW, unpublished). Dying spot and longnose gar displayed behavioral symptoms typical of red tide-affected fish (Landsberg, 2002). High concentrations of brevetoxins

found in the muscle and internal organs of these fish (sturgeon, gar, and multiple samples of juvenile spot) confirmed exposure. Brevetoxin concentrations in the muscle of some dying juvenile spot exceeded 1000 ng g^{-1}, and intestinal contents were as high as 6438 ng g^{-1}. Intestinal contents (e.g., sand grains, foraminifera, and benthic invertebrates) of examined spot indicated that they had been feeding on the benthos. Following a red tide in the area that diminished in December 2005, weekly monitoring detected no *K. brevis*. We hypothesized that the fish kills resulted from post-bloom exposure to brevetoxin reservoirs in the environment, likely the benthos. The possibilities that exposure occurred outside the area of the fish kills (i.e., a spatial lag) or from unknown brevetoxin sources inside the bay have not been ruled out (FWC and UNCW, unpublished).

In some years, such as 1996 and 2003, the duration of the manatee mortality event was coincident with the duration of a red tide bloom that occurred in the same inshore area where manatees were exposed to brevetoxins. There was no lag between the end of 1996 manatee mortality event and the dissipation of the red tide (Landsberg and Steidinger, 1998). High concentrations of red tide cells appeared inshore during the winter–spring period, and manatees started dying within a few days. In 1996, after several months, the mortalities essentially stopped towards the end of April, when *K. brevis* cell concentrations dropped to below 10^5 cells l^{-1} (FWC, unpublished). However, in other years, such as in 1982 and 2002, manatee mortalities continued after the red tide bloom had diminished.

We now recognize that manatees can be exposed to brevetoxins after a red tide bloom has dissipated, particularly if they consume toxic filter-feeding organisms attached to seagrasses (principally turtle grass, *Thalassia testudinum*) or are exposed to toxins directly associated with the seagrass (Flewelling et al., 2005). In 1982, three weeks elapsed between the time when the red tide cell counts decreased to below 10^6 cells l^{-1} (FWC, unpublished) and when the last manatee died. Sick and dying manatees were reported during the red tide event and following the bloom (O'Shea et al., 1991). It was suspected that filter-feeding tunicates were still a toxic source to manatees some three weeks after the bloom had ended. During the 2002 red tide in southwestern Florida, a die-off of 30 manatees also followed the dissipation of the bloom (see above). Parallels between this die-off (Flewelling et al., 2005) and that of 1982 were apparent, but in 2002 no tunicates or any other filter-feeders were found on seagrass or in the gastrointestinal tracts of any of the recovered manatee carcasses.

The bottlenose dolphin mortality event of 2004 (see above) also illustrates a lag between red tide events and animal die-offs, although it is not clear whether the lag was a separation both in time and space. In this instance, bottlenose dolphins were exposed to lethal levels of brevetoxins by feeding on toxic fish prey, but no *K. brevis* bloom was located. Possible explanations for the decoupling are (1) a red tide event affected the area before the onset of mortality and fish remained toxic, or (2) the *K. brevis* bloom was located elsewhere and toxins were actively transported by the fish. In either case, this unusual mortality event illustrated that toxin-vectoring by fish can result in delayed or remote animal exposure and that toxins should always be considered as etiological factors during unusual aquatic animal mortalities even in the apparent absence of toxigenic blooms.

5. Recognition that mass mortality events of manatees tend to follow a consistent spatial and temporal pattern

Karenia brevis red tides co-occurred with, and were likely responsible for, the large-scale mortalities of the endangered

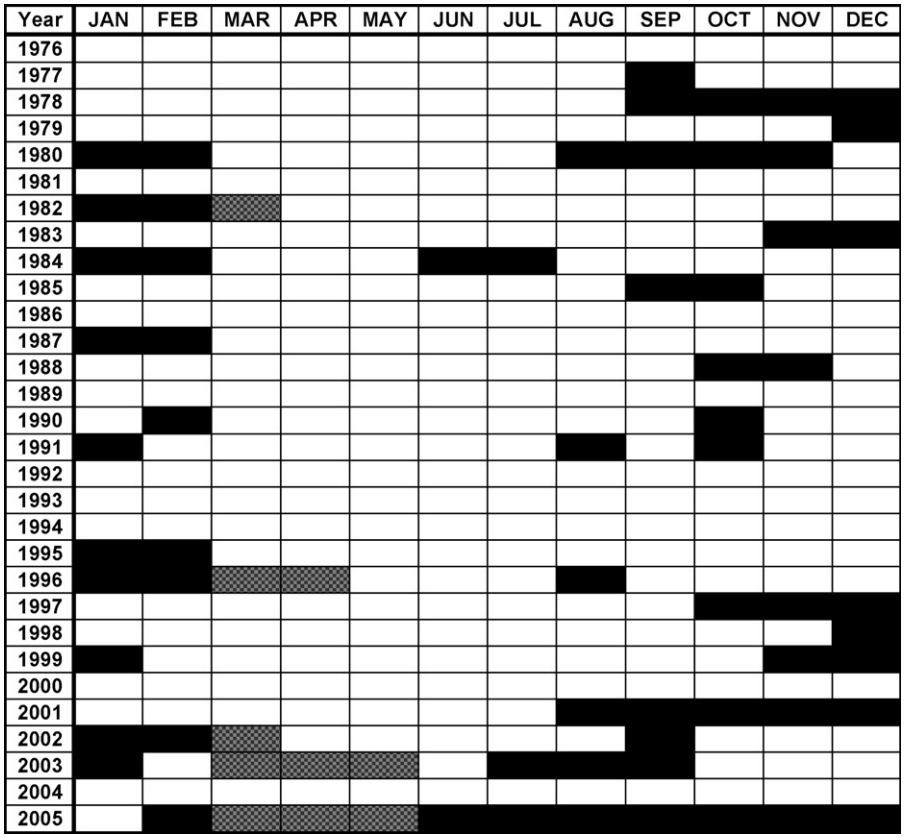

Fig. 2. Comparison of the temporal distribution of red tide with manatee mortality events in southwestern Florida (black box = counts above 1×10^5 cells l^{-1}; striped box = month indicates mass mortality of manatees during red tide). Modified from Landsberg and Steidinger (1998).

Florida manatee in 1963 (Layne, 1965), 1982 (O'Shea et al., 1991), 1996 (Bossart et al., 1998, 2002; Landsberg and Steidinger, 1998; Trainer and Baden, 1999), 2002, 2003, and 2005 (FWC, unpublished) (Table 1) during March–April in southwestern Florida.

Prior to the 1996 red tide manatee mortality event, there was no indication that large-scale die-offs were anything but random. In retrospect, it is possible to discern some temporal and spatial patterns now that several mass mortalities have occurred. Time of year is a key factor contributing to the mass mortalities. Since 1982, when the red tide came inshore during March in southwestern Florida and persisted for several weeks in high salinities, a wide-scale mortality event has occurred (Fig. 2). Following the 1996 manatee mortality, Landsberg and Steidinger (1998) reviewed the epidemiological factors that contributed to the event and determined that "a unique combination of environmental, geographical, and biological factors must co-occur to cause these mortalities." After over-wintering in the southwestern region, manatees are particularly at high risk of exposure to red tide and brevetoxins:

(1) If red tide has come inshore in the winter–spring,
(2) If salinities are high (>24 ppt), and
(3) If red tide or brevetoxins persist in the area for several weeks to months.

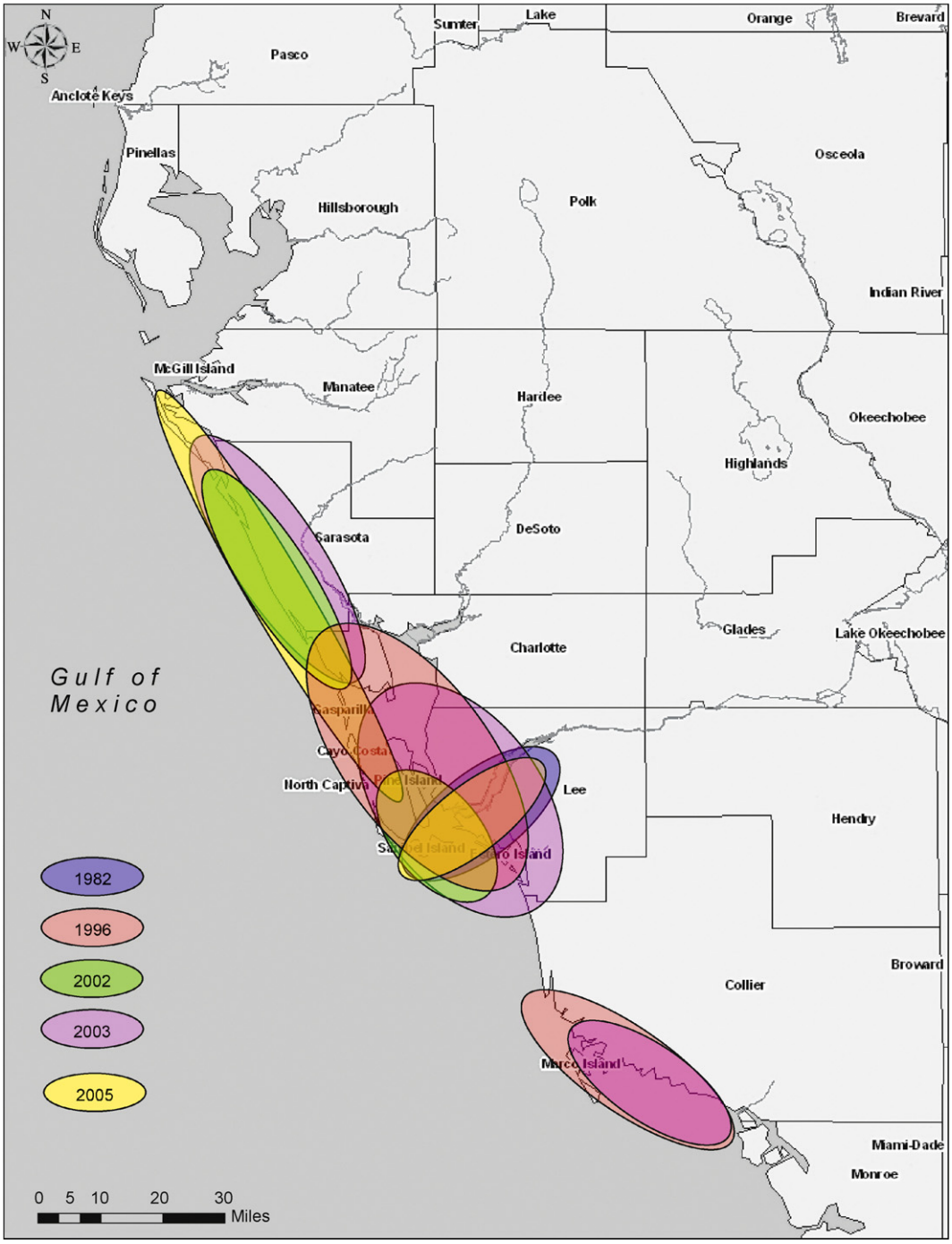

Fig. 3. Geographical locations of the five manatee mass mortality incidents in southwestern Florida since 1996.

The same situation applied to the mortality events occurring in 2002, 2003, and 2005.

Several aspects of the large-scale manatee mortality events during the last five time periods were different in terms of longevity, mortality rate, and the numbers of animals affected. In 1982 and 2002, there was a lag between the end of the bloom and the cessation of the mortalities (see above), whereas in 1996 and 2003, the majority of the mortalities coincided with the red tide. The number of debilitated animals also varies – in part related to proximity of the animals to red tide and the concentration of toxin in the exposure area.

Geographical hotspots for manatee exposure to red tide exist in and around the mouth of the Caloosahatchee River, the Sanibel area, Sarasota and Lemon bays, and the Marco Island area (Fig. 3). Mapping the distribution of manatee carcasses in GIS demonstrates some spatial pattern to the mortalities, even though the location of a carcass is not necessarily the exact location where animals were exposed to brevetoxins. In part, the likelihood that manatees are exposed to red tide depends upon their winter location (usually in warm, freshwater areas) (McDonald and Flamm, 2006) and their proximity to the bloom during their post-winter migrations to higher-salinity areas.

Additional factors that likely contribute to an increased susceptibility of manatees to brevetoxicosis include their general health status and immune function (Bossart et al., 2002; Walsh et al., 2005), extent of the cold temperatures during the preceding winter (McDonald and Flamm, 2006), manatee fitness and nutritional condition, their post-winter movements, and the location of seagrass-bed feeding grounds in relation to salinity, red tide distribution, and brevetoxins (Landsberg and Steidinger, 1998).

6. Cascading effects of *K. brevis* blooms cause significant mortalities

Despite the almost annual occurrence of red tide, persistent blooms that linger for months and cause extended ecosystem impacts occur, or are documented, less frequently. Even though an extensive red tide lasted for many months in southwestern Florida during 1947–1948, effects on benthic communities were not reported (Gunter et al., 1948).

The first report of a large-scale mass mortality involving hundreds of fish and invertebrate species with marked benthic community effects was recorded in 1971 (Simon and Dauer, 1972; Steidinger and Ingle, 1972; Steidinger et al., 1973; Smith, 1975a, 1975b, 1976). Red tide was present off southwestern Florida from April to August. Mass mortalities of benthic communities, including invertebrates and reef fish on patch reefs between Charlotte Harbor and Tampa Bay were documented over 1500 km^2 of shelf. At inshore reefs (12–18 m depths), 45–58 of the resident fish species were reported absent. The persistent red tide bloom was directly, or indirectly, responsible for almost completely extirpating the patch reef biota, including fish, alcyonarian and scleractinian corals, molluscs, decapod crustaceans, polychaetes, tunicates, porifera, echinoderms, and benthic algae (Smith, 1975a). Although the mortalities were partly attributed to direct toxicity, environmental sequelae of the lingering bloom included anoxia, hypoxia, and hydrogen-sulfide poisoning. Decreased water transparency inhibited photosynthesis of hermatypic corals and benthic macroalgae and contributed to their mortalities (Smith, 1975a). Bottom-fish kills were not only limited to smaller benthic fish species but included groupers (Serranidae), snappers (Lutjanidae), triggerfishes and filefishes (Balistidae), porgies (Sparidae), and grunts (Pomadasyidae). Decomposing fish also contributed to oxygen depletion.

A similar mass mortality event of benthic communities and associated fauna of Tampa Bay was documented during a prolonged red tide (more than nine months) in August 2005. An estimated 5600 km^2 was affected, involving fish, loggerhead sea turtles, crustaceans, echinoderms, corals, sponges, and polychaetes (FWC, unpublished). As in 1971, cascading effects included hypoxia or anoxia, hydrogen-sulfide poisoning, a high biological oxygen demand due to decomposition, and a thermocline. Interestingly, higher water temperatures occurred in 1971 (maximum temperature recorded was 33 °C – Steidinger and Ingle, 1972) and 2005 than in any of the intervening years in which red tides occurred (FWC, unpublished). High concentrations of brevetoxins were found in some species of crustaceans, molluscs, and other invertebrates in the "dead benthic zone" (FWC, unpublished). It is unclear how to interpret these data because we lack comparative controls on these species, and it is impossible to determine if toxicity was the cause of death.

Along Boca Chica Beach, Texas, in early September 2005, an initial *K. brevis*-associated fish kill that affected approximately 31,000 Gulf menhaden (*Brevoortia patronus*), 26,000 mullet (*Mugil* sp.), and 26,000 scaled sardines (*Harengula jaguana*) progressed to include crevalle jack (*Caranx hippos*), whiting, hardhead catfish (*Ariopsis felis*), common snook (*Centropomus undecimalis*), and mangrove snapper (*Lutjanus griseus*). The fish kill preceded by two weeks a benthic mortality of thousands of crustaceans (principally penaeid shrimp and mole crabs), various molluscs, and polychaetes, all of which stranded on the beaches. The maximum *K. brevis* count exceeded 5×10^6 cells l^{-1}. Direct correlation between the red tide, toxicosis, and indicators of poor water quality (anoxia, hypoxia, or hydrogen-sulfide poisoning) was suspected but not confirmed, However, the bloom and the wide-scale mortality appear to be more than coincidental (Buzan, Texas Parks and Wildlife Service, personal communication; *The Monitor*, McAllen, Texas, 2 October, 2005).

7. Short-term effects on fisheries can be documented

Since 1844, when the first fish kill was reported in southwestern Florida, red tides must have killed billions of fish throughout the Gulf of Mexico (Ingersoll, 1882; Gunter et al., 1948; Steidinger et al., 1973; Quick and Henderson, 1975; Landsberg, 2002). Despite ongoing incidents, fish kills caused by red tide cannot be reliably quantified because of their magnitude and the spatial and temporal scale over which they occur. Additionally, there is no accountability for the number of eggs or larval stages killed (Kimm-Brinson and Ramsdell, 2001; Colman and Ramsdell, 2003), the effect on juvenile recruitment (Riley et al., 1989; Warlen et al., 1998), or the extent of post-bloom mortalities.

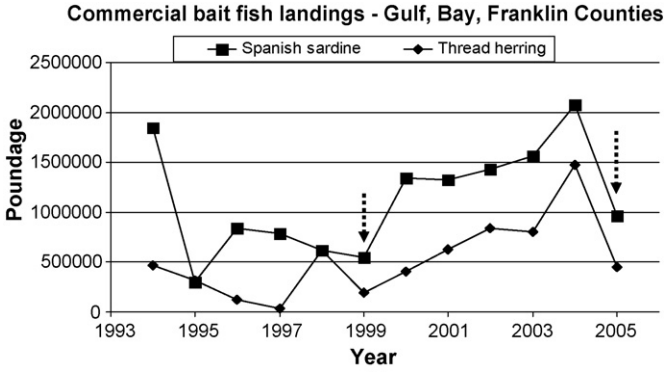

Fig. 4. FWC fishery landings data in pounds for the Florida Panhandle (Gulf, Bay, and Franklin counties). Note the general drop in landings during the red tide years, 1999 and 2005.

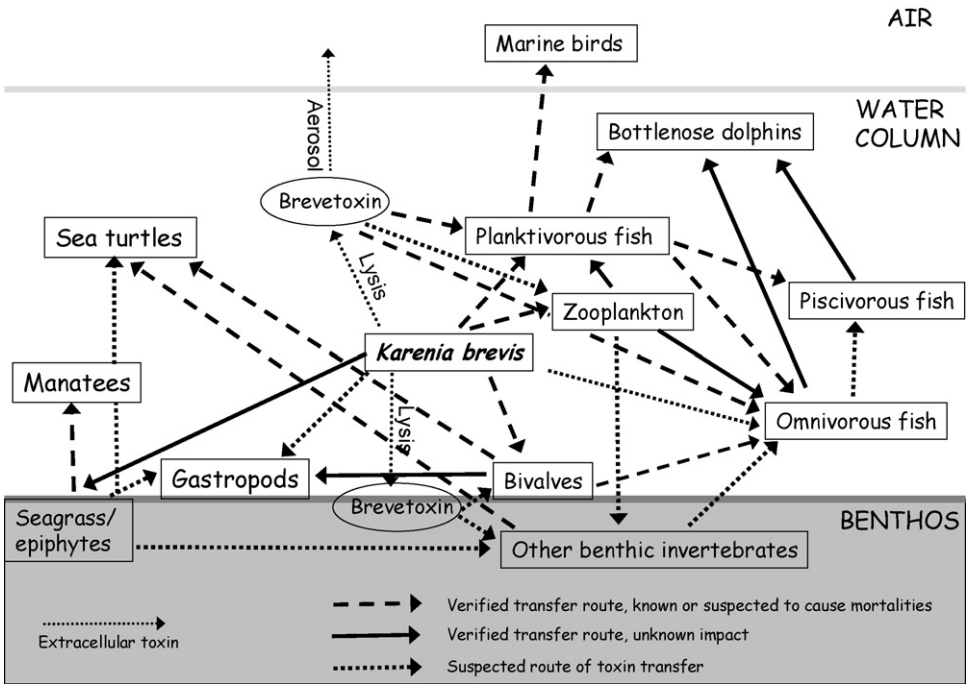

Fig. 5. Schematic of the transfer and impact of brevetoxins in the marine environment.

Estimates of the number of fish killed by red tides have been attempted in Texas (Rubec, 1999) and Florida (FWC, unpublished). Counts of the numbers of dead fish stranding along a specific beach area are likely to be underestimates. One possible method to evaluate short-term effects is to review the commercial fisheries-landings data (FWC, unpublished; Brown, personal communication) or to conduct independent assessments. Such assessments when compared to non-red tide years or regions may help to determine if short-term declines during or following red tides appear to influence fishery numbers in areas where red tides are endemic.

Northwestern Florida provides one example of a short-term effect on fishery populations. The baitfish landings and independent survey data indicate a short-term decline during and immediately following a red tide. For example, in 1999 and 2005 in the Florida Panhandle, there were extensive red tides. FWC unpublished data for commercial bait fish landings of Spanish sardine (*Sardinella aurita*) and thread herring (*Opisthonema oglinum*) in three main counties (Gulf, Bay, and Franklin) showed coincident general declines (Fig. 4). Independent assessments by the FWC's Fisheries-Independent Monitoring group in Apalachicola Bay also revealed a similar short-term decline (FWC, unpublished). Although there are likely to be a number of variables contributing to these short-term trends, there is no evidence that red tides have had sustained effects on any particular species in the Gulf of Mexico. Further research is needed to determine the potential effect of other environmental factors (such as salinity) that may also contribute to short-term declines.

8. Conclusions

Data gathered over the past decade have shown that trophic transfer of brevetoxins through the food web is a complex phenomenon and one far more complicated than originally conceived. During a red tide bloom, brevetoxins are initially intracellular in *K. brevis*, but as the cells lyse or die, the toxins become more widely available in the environment (Pierce et al., 2001). Brevetoxins can be present in water and sediments, adsorbed to particles, aerosolized, or stored in the biota and available for transfer through the food web (Pierce et al., 1990; Tester et al., 2000; Landsberg, 2002). During persistent blooms, brevetoxin concentrations in the environment can increase and affect numerous animal species in the food web via different routes of exposure, including vectoring or accumulation of toxins at different trophic levels. A generalized scheme for the transfer of brevetoxins in the environment is shown in Fig. 5.

The persistence of brevetoxins in the environment results in low-level chronic or sustained exposures that can affect the health of many species within diverse habitats and demonstrates that there are less acute mechanisms by which mortalities can occur. Recent evidence suggests that brevetoxins are stable in the environment and are transferred through the biota in a complex form – either as PbTx-3 or as newly identified toxin metabolites (Plakas et al., 2002) with unknown potencies. Although some metabolites are being assessed for their potential public-health risk, it is unclear to what extent they pose a threat to living resources in areas where red tides are endemic.

The controversy continues as to whether *K. brevis* red tides are occurring more frequently in the Gulf of Mexico as does the debate about whether or not animal mortalities from red tides are also increasing. Since 1996, definitive evidence that brevetoxins kill animals has emerged, and an increasing number of large-scale mortalities have been documented, particularly of marine mammals, to have been caused by these toxins. Regardless of primary assignation of cause, it is still necessary to determine if interacting natural factors other than brevetoxins or anthropogenic factors also contribute to these events. It is important to evaluate whether the perceived increase in red tides and animal mortalities reflect difficulties in managing or controlling red tides, increases in the protection of commercially significant species (as the populations increase, so will the number of deaths), enhanced reporting and response to animal die-offs, or more definitive diagnoses as to the cause of death. Identifying the causes of animal mortalities will not lessen the ever-increasing environmental pressures on animal populations, but it can help us to recognize that red tide events should not necessarily be viewed as isolated

incidents and that they are not always the single, conclusive cause of death. Animal populations in areas where red tides are endemic are unquestionably at risk from multiple stressors, and as these stressors continue to increase, we may find that fewer populations will be able to recover from the sustained effects induced by exposure to red tides.

Acknowledgments

The authors wish to acknowledge the following staff at FWRI for discussions on the manuscript: Judy Leiby, Sara McDonald, Merrie Beth Neely, Jim Quinn, Karen Steidinger, and Jennifer Wheaton. We thank staff from the Fish and Wildlife Health, Harmful Algal Bloom, Habitat, Fisheries-Independent Monitoring, Fisheries-Dependent Monitoring, Marine Fisheries Biology, Marine Fisheries Stock Assessment, Marine Turtle Research, and Marine Mammal Research subsections at FWRI who provided unpublished data or their technical support. We acknowledge Dave Buzan, Texas Parks and Wildlife Department, for providing unpublished data. We dedicate this paper to the memory of our late colleague Dr. Carl Beaver, FWRI-FWC, who contributed to the benthic-community assessment surveys following the persistent 2005 red tide in southwestern Florida.[SS]

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