3-28-2008

Brevetoxin Body Burdens in Seabirds of Southwest Florida

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by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
College of Marine Science
University of South Florida

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Date of Approval:
March 28, 2008

Keywords: *Karenia brevis,* harmful algal blooms, HABs, red tide, neurotoxic shellfish poisoning

DEDICATION

This study is dedicated to Skipper, a lively, sweet double crested cormorant who stole my heart but later died from brevetoxicosis at the Save Our Seabirds rehabilitation center in 2001

ACKNOWLEDGEMENTS

I would like to thank my family for their support and Michelle van Deventer for all of her hard work in the laboratory. I would also like to thank my committee and my fellow employees at the Florida Fish and Wildlife Research Institute for all of their encouragement through this arduous process. I would especially like to thank Lee Fox, Executive Director of Save Our Seabirds, who showed me that one person can make a difference and to Leanne Flewelling who encouraged me every step of the way.

I would also like to thank all of the groups who provided bird collections including SEANET Beached Bird Survey of Shell Key, the St. Petersburg Audubon/Eckerd College Least Tern Nesting Study, Peace River Wildlife Center, The Pelican Man Sanctuary, Save Our Seabirds (SOS), The Center for Rehabilitation of Wildlife (CROW), The Wildlife Center of Venice (WCV) and Suncoast Seabird Sanctuary (SSS). Profuse gratitude goes to funding institutions including the Tampa Bay Parrot Heads, the Florida Department of Environmental Protection (Award # DO157160), and the Florida Department for Health and Centers for Disease Control.

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ABSTRACT

Harmful algal blooms (HABs, or “red tides”) of the brevetoxin-producing dinoflagellate *Karenia brevis* occur periodically along Florida’s Gulf coast. Mass mortalities of marine birds have long been associated with these blooms, yet there are few data documenting the accumulation of brevetoxins (PbTx) in the tissues of birds.

Post-mortem evaluations were performed on 185 birds representing 22 species collected from October 2001 through May 2006 during red tide and non-red tide events to quantify their body burdens of brevetoxins. A variety of tissues and organs were selected for brevetoxin analysis including blood, brain, heart, fat, stomach or gut contents, intestinal contents or digestive tract, muscle, lung, liver or viscera, kidney, gonads, gallbladder and spleen. Brevetoxin levels in avian tissues ranged from <LD (below level of detection) to 9989 ng/g PbTx, with the highest levels generally found in liver, gall bladder, stomach and intestinal contents of affected birds. These results indicate that marine birds are exposed to a range of levels of brevetoxin in their diet during blooms of *K. brevis* which

may amass in various tissues of the body. As a consequence, the birds may exhibit acute brevetoxicosis during red tide events or show chronic accumulation effects during non-red tide events.

INTRODUCTION

Microalgae or phytoplankton are single-celled photosynthetic organisms that make up the lowest trophic level of aquatic ecosystems. Of the thousands of species of marine algae found in marine bodies throughout the world, a small number are known to produce chemicals that are toxic to other organisms including fish, shellfish, birds, marine mammals and humans (Creekmore, 2001). When these toxic microscopic algae in seawater proliferate to higher than normal concentrations, they are called Red Tide or Harmful Algae Blooms (HABs) due to the water discoloration they often cause, including colors of red, brown, green or yellow (Anderson, 1994).

There are many types of HABs found throughout the world in various locations, which can range from recurrent in some areas to episodic or persistent in others (Shumway et al., 2003). The most common toxins involved in these events include domoic acid, saxitoxin, brevetoxin, okadaic acid and ciguatoxins (Table 1).

Table 1. Illnesses associated with HABs in humans. (Morris, 1999; Anderson et al., 2001; Shumway et al., 2003)

Illness	Causative organism	Associated toxin	Clinical symptoms
Paralytic shellfish poisoning (PSP)	*Alexandrium* spp., *Gymnodinium catentatum, Pyrodinium bahamese*	Saxitoxin & derivatives	Neurological manifestations, respiratory distress, muscular paralysis & death
Neurotoxic shellfish poisoning (NSP)	*Karenia (Gymnodinium) brevis*	Brevetoxins	Gastrointestinal & neurological symptoms, respiratory & eye irritation
Diarrhetic shellfish poisoning (DSP)	*Dinophysis* spp., *Prorocentrum* spp.	Okadaic acid & dinophysis toxins	Acute gastroenteritis
Amnesic shellfish poisoning (ASP)	*Pseudonitzschia* spp.	Domoic acid & isomers	Gastroenteritis, neurological symptoms leading to severe amnesia & permanent short-term memory loss, coma & death
Ciguatera fish poisoning	*Gambierdiscus toxicus*	Ciguatoxins	Gastrointestinal & neurological symptoms

Paralytic Shellfish Poisoning (PSP) is caused by the consumption of shellfish that have been contaminated with the saxitoxins which are produced by several species of dinoflagellate organisms. The effects are neurological and are very fast acting, and can include numbness, loss of coordination, difficulty in breathing, nausea, vomiting, dizziness, loss of sight and headaches. PSP has been found in shellfish on the west and east coasts of North America and most reports refer to incidents involving human illness and sometimes human death.

During the first recorded outbreak of a toxic dinoflagellate in Massachusetts in 1972, one dead and several sick gulls were noted and a kill of about 100 birds including black ducks and gulls were reported (Shumway et al., 2003). Autopsies showed hemorrhaging as has been seen in other episodes of PSP poisoning, the causative organisms were identified as *Gonyaulax tamarensis*, and toxic shellfish was found in some gut contents of dead birds (Bicknell & Collins, 1972). In November 1987, fourteen dead humpback whales (*Megaptera novaeangliae)* washed ashore in Massachusetts along Cape Cod Bay and Nantucket Sound. The whales had eaten atlantic mackerel *(Scomber scombrus)*, which tested positively for the PSP toxin (Geraci et al., 1989; Anderson & White, 1992).

Amnesic Shellfish Poisoning (ASP) is caused by ingestion of clams, mussels or crabs contaminated with Domoic acid, a neurotoxic produced by several species of pinnate diatoms. Effects include memory loss, brain damage and even death, as in the case of over 400 sea lions off of the Central Californian coast in 1998. Birds have also been affected as can be seen from the deaths of large numbers of brown pelicans (*Pelecanus occidentalis*) and double crested cormorants (*Phalocrocorax aurelius)* in Monterey Bay, CA, in 1991 (Wright & Quilliam, 1995).

Diarrhetic Shellfish Poisoning (DSP) is caused by eating shellfish that has been contaminated with Okadaic acid or other related toxins. Effects include gastrointestinal distress including diarrhea, nausea, vomiting, chills and abdominal cramps. No human deaths have been reportedly caused by DSP and most symptoms pass within three days. Large and unexplained die-offs of loons in Long Island waters, as well as bird deaths in Europe in the summer of 2002, have been attributed to DSP (Shumway et al, 2003).

Although there are over 40 toxic species of algae which live in the Gulf of Mexico, the most common in the Tampa Bay Florida region is the unarmored toxic dinoflagellate *Karenia brevis* (Figure 1). *Karenia brevis* produce brevetoxins, which can cause fish kills and other marine animal mortalities including birds, manatees and dolphins. *Karenia brevis* can also cause filter-feeding animals such as oysters or clams to become toxic to humans (i.e., NSP) and cause an air-borne toxin (Steidinger et al., 1998). In addition to the Tampa Bay area, *K.brevis* has been found throughout the Gulf of Mexico, including along the coasts of Mexico, Texas and Louisiana, along the east coast of Florida and as far north as North Carolina (Steidinger et al., 1998). *Karenia brevis* is found year round throughout the Gulf of Mexico at concentrations of about 1,000 cells per liter or less and usually blooms in the late summer or early fall. Kim and Martin (1974) found that *Karenia brevis* thrives in salinity ranges of 30-34 PSU, but can tolerate a wide salinity range (22-39 PSU). *Karenia brevis* also survives most water temperatures common in the Gulf of Mexico (Kamykowski, 1981). The primary means of reproduction of these organisms is by simple asexual fission, which can increase these blooms to very high concentrations (Anderson, 1994).

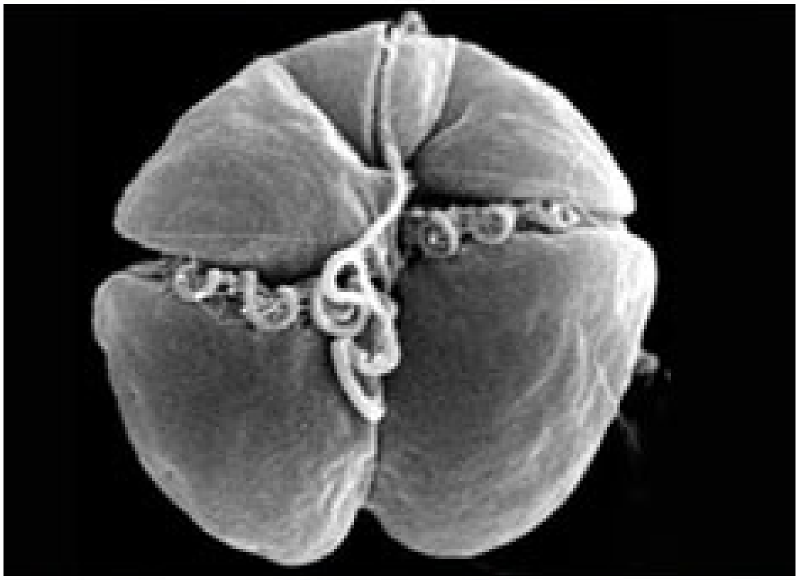

Figure 1. ***Karenia brevis*** **SEM photograph.** ***K. brevis*** **cells usually average 20 microns in width. Courtesy of the HAB lab at FWRI.**

Florida red tide events have been known to cause marine animal deaths since the 1500's (Appendix 1), including episodes of high fish and bird mortality as well as human respiratory illnesses. High mortality rates have also been seen in bottlenose dolphins, turtles and manatees (Fairey et al., 2001). In humans, the effects of NSP include gastrointestinal and neurological symptoms comprising of dizziness and seizures as well as headaches, diarrhea and muscle or joint pain. Symptoms can include difficulty in breathing, altered perceptions of hot and cold and double vision. When the toxin becomes airborne in sea spray, asthma-like symptoms in humans have also been documented. Aerosol effects have also been recorded in marine wildlife, including manatees (Bossart et al., 1998) and double crested cormorants (Kreuder, et al., 2002).

Brevetoxins act by binding to a specific site near voltage-gated sodium channels and then allow an unchecked flow of Na+ ions into and out of the cells. This disruption of ion flow is responsible for the neurological effects that have been associated with NSP.

There seem to be several pathways in which brevetoxins can accumulate in marine wildlife:

1. Aquatic organisms can become contaminated through the direct ingestion of cells, such as the case for filter feeders like sponges, mollusks and crustaceans. In fact, shellfish are often used as an indicator of HAB occurrence in an area and are studied for toxin absorption and retention (Shumway, 1990).
2. Marine life can also be exposed to waterborne toxins after cell lysis caused from wave action. This can happen to birds such as double crested cormorants (*Phalocrocorax auritus)*, common loons (*Gavia immer*) and red breasted mergansers (*Mergus merganser*) that swim underwater to catch their prey.
3. Marine animals may also be contaminated through aerosolized toxins that can cause respiratory irritation in mammals (e.g., manatees and birds) (Bossart et al., 1998; Kreuder et al, 2002). This can happen when an organism comes up for air in the middle of a bloom or when birds fly over a bloom. Kreuder et al. (2002) reported that between 1995 and 1999, 360 birds showing signs of toxin contamination were admitted to a rehabilitation center off of the southwest coast of Florida at the same time that high levels of *K. brevis* were reported in the area. Brevetoxins were found in the spleens and lungs of all four double crested cormorants tested, which could indicate inhalation as an exposure route.

4. Marine life can also be exposed to toxins through the ingestion of other organisms in which the toxin has bioaccumulated (e.g., bivalves such as crustaceans and gastropods) or through bioaccumulation in the organism's own body.

The majority of sea birds reportedly sick from brevetoxins are admitted during times in which large HAB blooms have been reported in the area. Save Our Seabirds, Inc. treats about 350-400 birds annually and sees anywhere from 12-25 birds exhibiting brevetoxicosis. The criteria used to diagnose patients with brevetoxicosis at local avian rehabilitation centers include states of seizures, shaking, inability to stand, weakness, slumping of the head, nasal discharge, dehydration, reduced body mass or atrophied musculature in comparison to a healthy individual (personal communication, Lee Fox). The birds diagnosed with brevetoxin poisoning are treated using a protocol which has a reported 90% success rate (pers. Comm., Lee Fox). Suncoast Seabird Sanctuary (SSS), a large sea bird rehabilitation center in the Redington Beach area of Tampa Bay, treats about 10,000 birds annually. SSS provided data on double crested cormorants admitted to the facility since 1982 (Table 2). Peaks in the numbers of admitted birds tend to occur in years when major red tides were documented (Appendix 1, Figure 2 and Table 2).

Table 2. The number of cormorants admitted per year at the Suncoast Seabird Sanctuary. Data received from Barbara Suto, head Wildlife Biologist of the sanctuary. See Figure 2 for a plotted graph of same data. Compare to Appendix 1 which shows a pattern similar to the interannual differences in red tide events.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1982	19	36	22	31	21	24	8	18	10	17	24	32	262
1983	14	17	27	8	7	3	5	4	8	10	53	27	183
1984	23	19	68	18	19	14	14	7	8	4	6	13	213
1985	7	13	10	15	7	10	20	7	6	11	37	12	155
1986	9	15	22	12	7	1	7	4	5	35	33	8	158
1987	13	19	25	7	13	41	33	16	12	13	16	10	218
1988	7	2	10	17	12	17	20	19	15	19	28	15	181
1989	58	23	114	68	14	18	13	5	8	9	11	1	342
1990	7	45	89	30	17	8	18	14	11	4	18	5	266
1991	15	8	25	32	12	9	7	18	39	53	28	18	264
1992	7	16	26	20	16	8	14	11	10	17	11	3	159
1993	23	6	7	15	7	6	6	18	16	13	18	2	137
1994	3	7	9	9	13	12	14	15	11	41	75	20	229
1995	8	2	23	14	15	52	18	27	12	35	12	15	233
1996	53	76	129	114	15	6	5	6	7	15	13	4	443
1997	8	19	58	21	7	10	10	7	7	14	9	5	175
1998	19	13	10	12	5	6	8	8	7	11	24	14	137
1999	6	8	15	12	11	12	7	6	8	21	25	19	150
2000	10	2	31	31	9	15	21	10	23	29	34	7	222
2001	1	19	37	28	18	17	15	11	13	55	73	116	403

For example, in January through June, as well as August and October through December, 1982, 17 or more double crested cormorants per month were admitted at Suncoast Seabird Sanctuary, which corresponded with red tide events reported in the area in January through April and July through October; red tide events were suspected but not confirmed in May and June of 1982. This trend is seen again in the following periods: November and December 1983, January through May 1984, November 1985, October and November 1986, January through July 1987. Sporadic coinciding months are seen in 1988, 1989, 1990 and 1991, almost all of 1995, the first half of 1996 and early 1997, as well as towards the end of 2001. High yearly averages of affected double crested cormorants also coincide with years of high blooms (Figure 2).

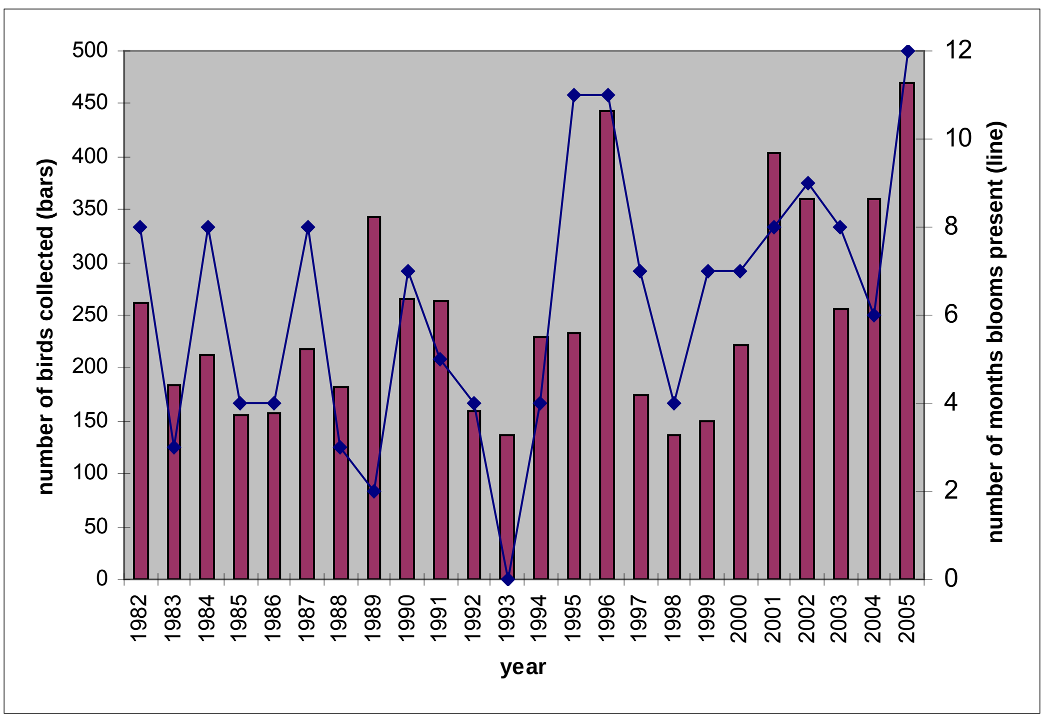

Figure 2. Annual total of double crested cormorants admitted to the Suncoast Seabird Sanctuary plotted per year from 1982-2005 and *Karenia brevis* bloom duration. Double crested cormorant data received from Barbara Suto, head Wildlife Biologist of the sanctuary. Bloom duration courtesy of FWRI.

Reports are not available for the total numbers of double crested cormorants admitted during red tide events from The Pelican Man Sanctuary in Sarasota, FL, a rehabilitation center for wild birds which treats about 6-7000 birds annually. However, workers at the facility have given anecdotal reports that many birds, mostly double crested cormorants, have been seen with breveitoxicosis symptoms during red tide events in the area.

Brevetoxins have also been implicated in high mortality rates among many other species of marine life. Mortalities of frigate birds, terns, gulls, ducks and vultures from Tampa Bay to Key West were reported by Glazier (1882), Moore (1882) and Walker (1884). Dead double crested cormorants (*Phalocrocorax auritus)*, ducks, frigate birds (*Frigata magnificens*), gulls, terns and vultures due to *K. brevis* were reported in 1973 off of the coast of Florida (Steidenger et al., 1973). Large number of lesser scaup (12,000-20,000) and some double crested cormorants and red breasted mergansers died during red tides in the Tampa, Florida area in 1975 (Quick & Henderson, 1975).

Even though HABs have historically been natural, the frequency of occurrence and intensity of some blooms throughout the world seems to have increased in recent decades (Shumway, 1990; Smayda, 1990; Hallagraeff, 1993; Burkholder, 1998; Shumway et al., 2003). Natural events like hurricanes can dilute or terminate toxic algae blooms. Algae can also be transported in ship ballast waters. Hallagraef (1993) postulated that agricultural runoff into the oceans and other pollutants dispersed into the environment by human activities (including human sewage) has resulted in increased nutrient loading of phosphorus and nitrogen, which can provide conditions favorable to the growth of HABs.

Clearly, HABs are adversely affecting marine life in many areas around the world and in particular, the Tampa Bay area of Florida. Research in this field of study is direly needed and plainly an opportunity presents itself to study birds coming into the rehabilitation centers in the Tampa Bay area. It is surprising, considering the impacts these toxins have on seabirds, that these types of studies have not been undertaken previously, since sea birds are among the most valuable indicators of environmental problems due to their sensitivity to environmental pollutants (Swennen, 1997; Boersma 1978, 1986).

The objectives of my study were:

1. Determine the levels of brevetoxins present in the blood and tissues of various species of sea birds during bloom periods.

2. Compare the capture location and brevetoxin levels in birds with the timing and location of *K. brevis* blooms to assess any potential inter-relationships.

METHODS

Necropsies

Bird carcasses and blood samples are obtained from local rehabilitation centers, including Suncoast Seabird Sanctuary in Indian Rocks Beach, The Center for Rehabilitation of Wildlife in Sanibel, The Wildlife Center of Venice and Save Our Seabirds in Wimauma. Birds are stored at -20 º C until necropsied. Necropsies are performed following procedures outlined in The Avian Necropsy Manual by Work (2000). Subsamples of tissues are taken after homogenization and then refrozen until extraction.

Toxin Analysis

Extraction Methods for Blood Samples. Blood samples are taken by the staff at the rehabilitation centers and are placed in either heparanized or non-heparinized glass tubes. Heparinized tubes are kept at room temperature and processed as soon as possible (within a few hours). Non-heparinzed samples are refrigerated until processed. The samples are centrifuged at 3000 rpm for 15 minutes. The resulting serum is then collected and analyzed directly using the ELISA method developed by Naar et al. (2002).

Extraction Methods for Tissue Samples From 2001 to 2005. Three grams of each tissue type were weighed and added to 10 ml of acetone in a 50 ml Falcon tube, then placed on a plate shaker for 30 minutes. The sample was centrifuged at 3000 rpm for 15 minutes and the acetone phase was separated from the tissue. This process was repeated in the same condition and acetone phase were combined in a separate Falcon tube. The combined acetone extract was the dried and resuspended in 8 ml of MeOH plus 1.5 ml of nanopure water. The aqueous methanol extract was defated using 5 ml of hexane. After hexane addition, the falcon tube was shaken and vented 3 times, then allowed to settle. The resulting separated hexane was removed and the sample was dried. After drying, 3 ml of MeOH was added and the sample was refrigerated until analysis using the ELISA method.

Extraction Methods for Tissue Samples From 2006 to Present. In 2006, a new extraction method was developed based on modifications suggested by Paul McNabb (personal communication). In this method, tissue samples are homogenized and a sub-sample of 2 grams is weighed out. Nine ml of 80% methanol is added and the sample is then heated at 60 $^{\circ}$C for 20 minutes. It is then centrifuged at 3000 rpm for 10 minutes and the resulting liquid poured into a new tube. The MeOH extraction is then repeated. Finally, 5ml of hexane is added to the sample, which is shaken and centrifuged at 3000 rpm for 10 minutes. The resulting bottom layer is removed and analyzed in the ELISA method.

ELISA Brevetoxin Methods. ELISAs (enzyme-linked immunosorbant assays) are widely used in both clinical and research fields to help rapid, simple, accurate and specific quantitation of many biological small molecules. For brevetoxins, we are using a brevetoxin competitive ELISA developed by Naar et al. (2002). The original protocol was shortened for use in the FWRI laboratory by Naar (personal communication). For this assay, samples and controls compete with plate-bound brevetoxin for goat anti-brevetoxin antibodies. The antibodies bound to the plate are then visualized using an HRP-conjugated secondary antibody (rabbit anti-goat antibodies), and the HRP substrate TMB (3,3′,5,5′-Tetramethylbenzidine). Absorbance of the wells is read at 450 nm. The color intensity is inversely proportional to the concentration of brevetoxins in the sample. This assay recognizes all congeners and metabolites of brevetoxin that have a PbTx-2-type backbone. Results are reported as nanograms brevetoxin per gram sample (ng/g PbTX) for tissues and nanograms brevetoxin per microliter (ng/ml PbTX) for blood serum samples.

Karenia brevis Cell Counts

Karenia brevis cell count data used in this study was obtained from the FWRI Harmful Algae Bloom Historical Database, which is updated twice weekly with data collected as part of FWRI's Routine Red Tide Monitoring Program. Samples collected as part of the Routine Red Tide Monitoring Program are processed following the method reported in Naar et al., 2007. Identifications of *Karenia brevis* were made using Haywood et al., 2004 and Steidinger et al., 2008.

RESULTS

A total of 185 birds representing 22 species were tested for the presence of brevetoxin (Table 3). To simplify analysis, the 22 species were split into 8 groups (Table 3). The groups were chosen on the animals' feeding habitat and diet (Table 4). Data for each bird, including identification number, collection date, collection location, tissues tested, specific test results, and history, are provided in Appendices 3 and 4.

A variety of tissues and organs were selected for brevetoxin analysis and included blood, brain, heart, fat, stomach, or gut contents, intestinal contents or digestive tract, muscle, lung, liver or viscera, kidney, gonads, gallbladder and spleen. Not all of these tissues were tested for each bird and some types of tissues were tested more often than others, depending on availability. Of the 185 birds tested, 144 tested positive for at least one sample type (Figure 3). A total of 820 tissue and organ samples were analyzed. Of these, 391 were negative and 429 were positive. Gallbladders (20 out of 21) showed the highest percentage of positive results at 95% while lungs (19 of 72) showed the lowest percentage of positive results at 26% (Figure 4). Since there was generally considerable variation of brevetoxin content in tissues and organs, I have examined each broad group (Table 3) to determine if certain types of birds contain more toxin than others or show a wider distribution in their tissues and organs.

Table 3: List of all species of birds used for toxin assays by group and family and the total number of each species tested.

Group	Family	Common name	Species name	Number tested
Cormorants	Phalacrocoracidae	double crested cormorant	Phalacrocorax auritus	101
Gulls	Laridae	herring gull	Larus arentatus	1
		laughing gull	Larus atricilla	8
Herons & Egrets	Ardeida	great blue heron	Ardea herodias	6
		great white heron	Ardea herodias (white morph)	3
		green heron	Butorides virescens	6
		yellow crowned-night heron	Nycticorax mauritianus	3
		great egret	Ardea alba	2
Loons & Gannets	Gaviidae	common loon	Gavia immer	4
	Sulidae	northern gannet	Morus bassanus	5
Terns	Sternidae	least tern	Sternula antillarum	11
		royal tern	Thalasseus maximus	6
		sandwich tern	Thalasseus sandvicensis	1
Pelicans	Pelicanidae	brown pelican	Pelecanus occidentalis	12
		white pelican	Elecanus erythrorhynchos	1
Shorebirds	Laridae	black skimmer	Rynchops niger	1
	Scolopacidae	ruddy turnstone	Arenaria interpres	1
		sanderling	Calidris alba	8
	Rallidae	sora rail	Porzana carolina	1
Other	Pandionidae	osprey	Pandion haliaetus	2
	Gruidae	whooping crane	Grus americana	1
	Ciconiidae	wood stork	Mycteria americana	1
			TOTAL	**185**

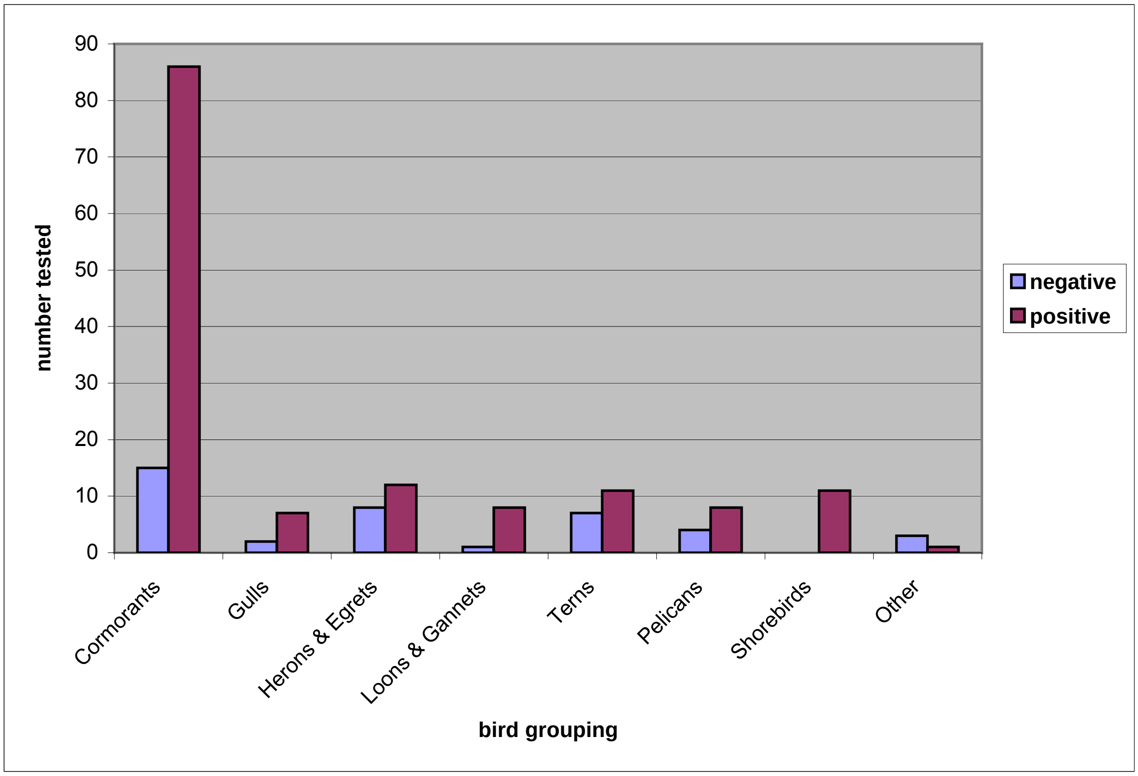

Figure 3. The number of birds which tested positive or negative for brevetoxin content.

Table 4 . Species of birds tested showing general habitat and diet type. (adapted from Kaufman, 1996)

Species	**Feeding habitat**	**Diet**
Black skimmer	Ocean beaches, inlets, tidewaters & estuaries along the coast	Mostly small fish & crustaceans
Brown pelican	Coastal marine & estuarine environments	Mostly fish, small marine invertebrates
Common loon	Coastal marine near shore areas & large freshwater lakes & ponds	Mostly small fish, aquatic vertebrates & invertebrates
Double crested cormorant	Ponds, lakes, rivers, lagoons, estuaries & open coastline	Fish, other aquatic animals, insects & amphibians
Egret	Wetlands, marshlands, swamps, streams, rivers, ponds, lakes, tidal flats, canals & flooded fields	Fish, invertebrates, reptiles, birds & small mammals
Northern gannet	Offshore islands & marine coastlines, often well offshore	Mainly fish & some squid

Table 4 (Continued)

Great blue & white heron	Calm, shallow freshwater & seacoasts	Fish, invertebrates, amphibians, reptiles, birds & small mammals
Green heron	Swamps, creeks, streams, marshes, ponds, lake edges, salt marshes, ponds & pastures, winters in coastal areas & mangrove swamps	Mostly small fish, invertebrates, frogs & other small animals
Herring gull	Along beaches, mudflats & dumps	Fish, marine invertebrates, insects, birds, eggs, carrion, garbage
Laughing gull	Along oceans, on rivers, at landfills & urban parks	Aquatic & terrestrial invertebrates, fish, squid, garbage, flying insects & berries
Least tern	Seacoasts, beaches, bays, estuaries, lagoons, lakes & rivers	Small fish & some invertebrates
Osprey	Large bodies of water containing fish including boreal forest ponds, desert salt-flat lagoons, temperate lakes & tropical coasts	Almost entirely fish
Royal tern	Along marine coastlines, sandy beaches & salt bays	Fish & shrimp
Ruddy turnstone	Along rocky shores, sand beaches & mudflats	Aquatic invertebrates & insects, carrion, garbage & birds eggs
Sanderling	Sandy beaches	Aquatic & terrestrial invertebrates
Sandwich tern	Seacoasts, bays, estuaries, mud flats & occasionally ocean far from land	Small fish & some invertebrates
Sora	Shallow wetlands	Seeds & aquatic invertebrates
White pelican	Offshore large bodies of water often far from land	Fish
Whooping crane	Freshwater marshes & prairies, shallow lakes, lagoons & saltwater marshes	Wide variety of insects, fish, frogs & plant & animal matter, including mollusks, crustaceans, waste grain
Wood stork	Shallow wetlands	Fish, amphibians, reptiles

Table 4 (Continued)

Yellow crowned night heron	Exposed tidal flats	Crustaceans, water beetles, leeches, mussels, frogs & small fish

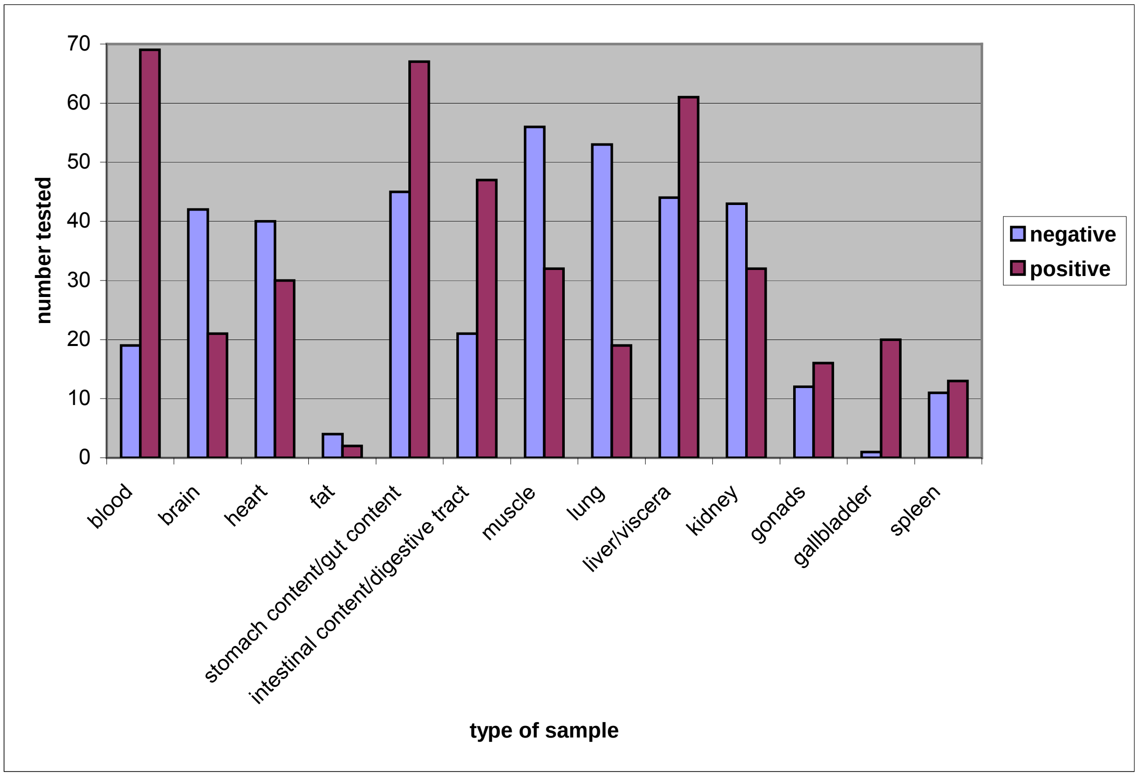

Figure 4. The types of samples which tested positive or negative for brevetoxin content.

Cormorants (Phalacrocoracidae)

Of the 101 double crested cormorants tested, 86 were positive for at least one sample type (Table 5). The fraction testing positive ranged from 54% in lung tissue to 100% of all gallbladders tested (n=12). Brevetoxin concentrations ranged from 0-9,989 ng/g with the highest value reported in a sample of stomach contents. It is interesting to note that the highest level found in any tissue sample in all of the groups tested was this specific stomach content. The four sample types with the highest positive brevetoxin levels were gallbladder, stomach contents, intestinal contents and liver/viscera, whereas the lowest positive brevetoxin levels were found in blood, brain, lung and muscle samples.

Table 5. The fraction of sample types from cormorants that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. Brevetoxin concentrations are given in ng/g or ng/ml.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	86/101	85	0-9,989	98	10
blood	67/87	77	0-12	3	2
brain	16/25	64	0-65	14	12
heart	18/25	72	0-102	33	35
stomach contents	23/30	77	0-9,989	615	40
intestinal contents	20/24	83	0-2,645	172	60
muscle	18/26	69	0-97	40	45
lung	13/24	54	0-196	19	12
liver/viscera	19/27	70	0-198	63	62
kidney	16/25	64	0-105	31	35
gonads	12/14	86	0-87	48	58
gallbladder	12/12	100	20-949	410	446
spleen	6/11	55	0-84	29	12

In April of 2006, 57 double crested cormorants which had been captured and rehabilitated at the Suncoast Seabird Sanctuary for brevetoxicosis were released. Although original blood samples were not taken for these birds upon admission to the rehabilitation center, most blood samples taken upon release showed detectable levels of brevetoxin (Figure 5 and Appendix 2).

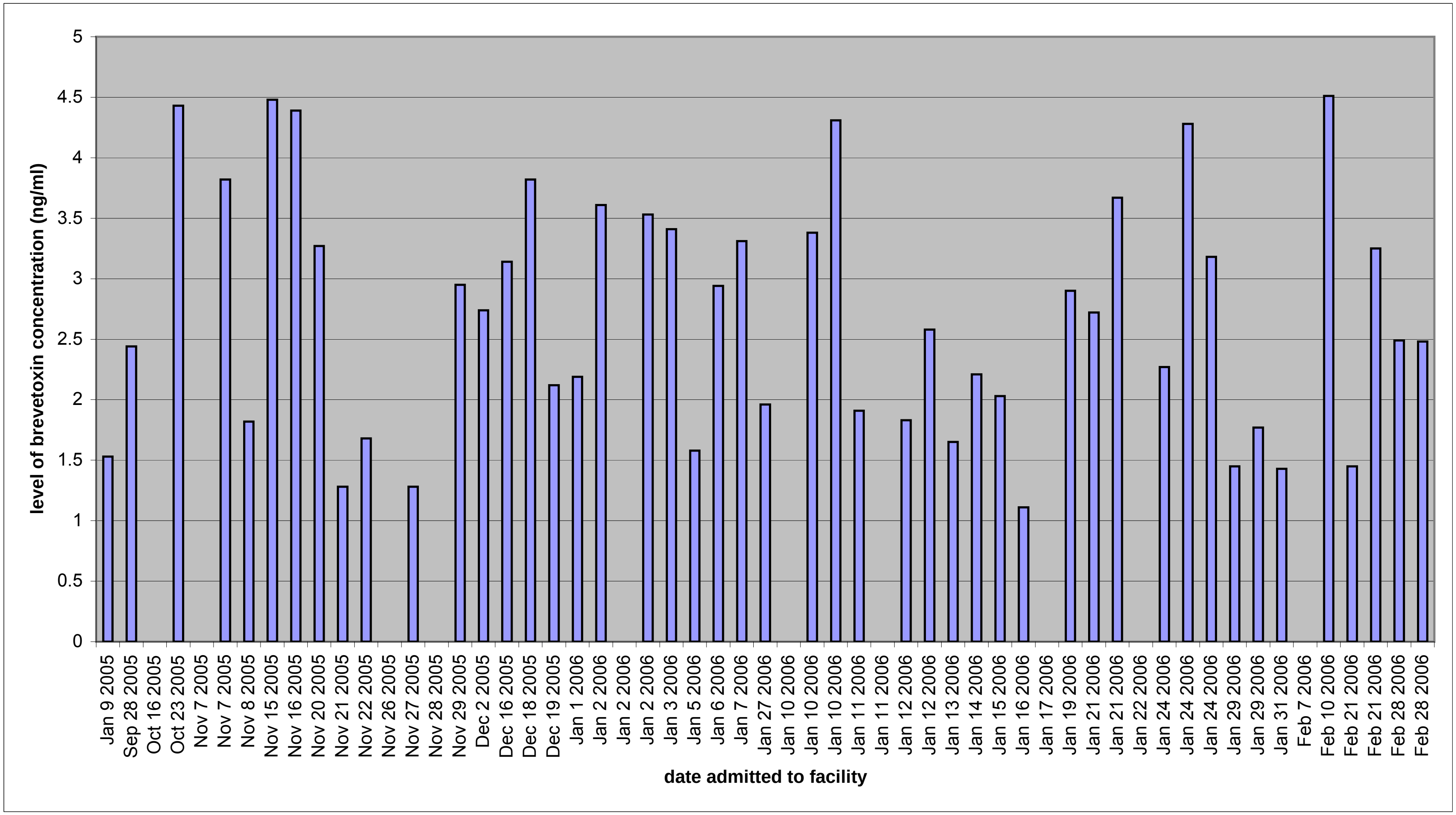

Figure 5. Brevetoxin levels of blood serum taken from 57 double crested cormorants on their release date from a rehabilitation center in April of 2006. The dates shown represent the date the animals were originally brought to the facility for treatment.

Gulls (Laridae)

Of the 9 gulls from two different species tested for this project (Table 3), 7 were positive for at least one sample type (Table 6). There were no positive brevetoxin levels in either fat or brain tissue samples. However, like double crested cormorants, all the gallbladders tested were positive (n=2). No blood samples were tested for this group. Brevetoxin concentrations ranged from 0-2,801 ng/g with the highest value reported in an intestinal content sample. The four sample types with the highest positive brevetoxin levels were gallbladder, intestinal contents, stomach contents and liver/viscera whereas the lowest positive brevetoxin levels were found in gonads, heart, lung and spleen.

Table 6. The fraction of sample types from multiple gull species that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	7/9	78	0-2,801	264	8
brain	0/5	0	0	0	0
heart	1/7	14	0-32	5	0
fat	0/2	0	0	0	*
stomach contents	8/11	73	0-2,216	418	34
intestinal contents	4/9	44	0-2,801	574	0
muscle	3/9	33	0-177	37	0
lung	3/9	33	0-46	12	0
liver/viscera	6/9	67	0-1,355	290	14
kidney	5/8	63	0-398	91	16
gonads	1/5	20	0-10	2	0
gallbladder	2/2	100	897-2,099	1498	*
spleen	1/1	100	35	35	*

Herons and Egrets (Ardeida)

Twenty birds from 5 species were tested for this group (Table 3), 12 of which were positive for brevetoxin (Table 7). Similar to double crested cormorants, no brevetoxin was detected in the single fat tissue sample. Like cormorants and gulls, 100% of the gallbladder tissue samples tested positive for brevetoxin (n=2), although, the highest level was seen in a stomach content sample (811 ng/g). The four sample types with the highest positive brevetoxin levels were gallbladder, stomach content, intestinal content and liver/viscera, whereas the lowest positive brevetoxin levels were found in blood, brain, lung and muscle.

Table 7. The fraction of sample types from multiple heron and egret species that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g or ng/ml.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	12/20	60	0-811	53	0
blood	1/3	33	0-2	1	0
brain	1/10	10	0-15	2	0
heart	6/13	46	0-36	10	0
fat	0/1	0	0	0	*
stomach contents	10/18	56	0-811	154	20
intestinal contents	7/13	54	0-511	117	40
muscle	5/15	33	0-32	7	0
lung	2/10	20	0-9	2	0
liver/viscera	9/17	53	0-296	71	0
kidney	4/11	36	0-108	17	0
gonads	1/3	33	0-39	13	0
gallbladder	2/2	100	8-315	162	*
spleen	2/4	50	0-35	15	13

Loons and Gannets (Gaviidae and Sulidae)

Of the 4 loons and 5 gannets tested, a total of 8 were positive for at least one sample type (Table 8). There were no positive brevetoxin levels found in brain, stomach content and lung tissue samples. The fractions which did test positive ranged from 14% of the muscle tissue samples to 100% of the gallbladders (n=1) and gonads (n=1). Brevetoxin concentrations ranged from 0-60 ng/g with the highest value reported in an intestinal content sample. The four sample types with the highest positive brevetoxin levels were gallbladder, intestinal content, liver/viscera and gonads, whereas the four sample types with the lowest positive brevetoxin levels were muscle, blood, heart and spleen.

Table 8. The fraction of sample types from loons and gannets that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g or ng/ml.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	8/9	89	0-60	6	0
blood	2/3	67	0-4	3	3
brain	0/4	0	0	0	0
heart	1/3	33	0-10	3	0
stomach contents	0/7	0	0	0	0
intestinal contents	1/5	20	0-60	12	0
muscle	1/7	14	0-6	1	0
lung	0/5	0	0	0	0
liver/viscera	6/8	75	0-25	17	17
kidney	2/7	29	0-19	4	0
gonads	1/1	100	14	14	*
gallbladder	1/1	100	58	58	*
spleen	1/2	50	0-8	4	*

Terns (Sternidae)

Of the 18 terns from 3 species tested (Table 3), 11 were positive for at least one sample type (Table 9). There were no positive brevetoxin levels found in gonads and spleen tissue samples. Like all of the previous groups, 100% of the gallbladder samples were positive (n=1). Brevetoxin concentrations ranged from 0.0-4,400 ng/g with the highest value reported in a stomach content sample. No blood samples were tested in this group. The sample types with the highest positive brevetoxin levels other than stomach content were intestinal content, gallbladder and liver/viscera, whereas the sample types with the lowest positive brevetoxin levels were found in brain, muscle, heart and lung tissue samples.

Table 9. The fraction of sample types from multiple tern species that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	11/18	61	0-4,400	83	0
brain	1/7	14	0-8	1	0
heart	2/9	11	0-31	6	0
stomach content	9/20	45	0-4,400	302	0
intestinal content	3/4	75	0-465	131	30
muscle	3/12	25	0-14	3	0
lung	1/4	25	0-33	8	0
liver/viscera	7/20	35	0-141	19	0
kidney	2/8	25	0-90	13	0
gonads	0/3	0	0	0	0
gallbladder	1/1	100	83	83	*
spleen	0/1	0	0	0	*

Pelicans (Pelecanidae)

Of the 13 pelicans tested, 8 birds tested positive for at least one sample type (Table 10). There were no positive brevetoxin levels found in muscle, lung and heart tissue samples and of the fractions that did test positive, 100% of the gallbladders were positive (n=2), much like all the previous groups. Brevetoxin concentrations ranged from 0-2,595 ng/g with the highest level reported in a stomach content sample. The sample types with the highest positive brevetoxin levels other than stomach contents and gallbladder were intestinal content and liver/viscera, whereas the sample types with the lowest positive brevetoxin levels were brain, blood, gonads and kidney.

Table 10. The fraction of sample types from multiple pelican species that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g or ng/ml.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	8/13	62	0-2,595	54	0
blood	4/7	57	0-12	3	2
brain	2/9	22	0-10	2	0
heart	0/5	0	0	0	0
fat	1/2	50	0-12	6	*
stomach content	9/14	64	0-2,595	240	17
intestinal content	3/4	75	0-143	47	21
muscle	0/9	0	0	0	0
lung	0/9	0	0	0	0
liver/viscera	4/9	44	0-31	8	0
kidney	2/9	22	0-42	5	0
gonads	1/2	50	0-10	5	*
gallbladder	2/2	100	17-93	55	*
spleen	2/4	50	0-16	7	7

Shorebirds (Laridae, Scolopacidae and Rallidae)

Of the 11 shorebirds tested from 4 species (Table 3), all were positive for at least one sample type (Table 11). There were no positive brevetoxin levels found in gonad or gallbladder tissue samples, contrary to all of the previous groups. No blood samples were tested for this group. The fractions that did test positive ranged from 14% in lung tissue samples to 100% in liver/viscera (n=11), fat (n=1) and brain tissue samples (n=1). Brevetoxin concentrations ranged from 0-574 ng/g with the highest level reported in a stomach content sample. The tissues with the highest positive brevetoxin levels were liver/viscera, stomach content, intestinal content and kidney, whereas the tissues with the lowest positive brevetoxin levels were lung, muscle, brain and heart.

Table 11. The fraction of sample types from multiple shorebird species that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	11/11	100	0-575	60	16
brain	1/1	100	6	6	*
heart	3/7	43	0-21	7	0
fat	1/1	100	8	8	*
stomach contents	8/9	89	0-575	94	40
intestinal contents	9/11	81	0-372	90	30
muscle	3/8	38	0-20	5	0
lung	1/7	14	0-25	4	0
liver/viscera	11/11	100	8-286	152	196
kidney	2/5	40	0-103	27	0
gonads	0/1	0	0	0	*
gallbladder	0/1	0	0	0	*
spleen	1/1	100	16	16	*

Other (Pandionidae, Gruidae and Ciconiidae)

Of the 4 other birds tested from 3 species (Table 3), only 1 bird was positive, which, surprisingly, was the wood stork (Table 12). There were no positive brevetoxin levels found in the brain, muscle, lung or kidney of that specific bird and although there were no gallbladders or blood samples tested, the tissue with the highest positive brevetoxin level was the liver/viscera (169 ng/g) whereas the lowest positive brevetoxin level was found in the stomach content.

Table 12. The fraction of sample types from other bird species that were positive for brevetoxin and the range of toxin levels found in each sample type. Levels have been rounded to whole numbers. An asterisk denotes insufficient samples available to calculate. Brevetoxin concentrations are given in ng/g.

Sample type	# positive over total	% positive	Range PbTX	Average PbTX	Median PbTX
total	1/4	25	0-169	12	0
brain	0/2	0	0	0	*
stomach content	1/3	33	0-17	16	9
muscle	0/2	0	0	0	*
lung	0/4	0	0	0	0
liver/viscera	1/4	25	0-169	42	0
kidney	0/2	0	0	0	*

Collection Dates, Locations and Brevetoxin Cell Counts

The first bird was collected on 10-29-01. *Karenia brevis* cell counts ranged from not present to medium concentrations from off shore St. Petersburg south to Ft. Meyers for that same time frame (Table 13). One bird, a great white heron, which was collected south of the bloom in the Florida Keys, was negative for brevetoxin (Figure 6). *Karenia brevis* was not present in the Florida Keys during this time frame although low to medium concentrations were detected through the end of December from St. Petersburg south to Ft. Meyers. No birds were collected in that area and time frame for the study.

Table 13. Toxin content and bloom distribution for 2001 through 2004.

Collection date	Species	Collection location	Bloom geographic range	PbTX (+) or (-)
10/29/2001	Great white heron	Monroe county	St. Petersburg to Ft. Meyers	negative
1/10/2002	Common loon	Charlotte county	St. Petersburg to Naples	negative
1/10/2002	Double crested cormorant	Charlotte county	St. Petersburg to Naples	negative
2/5/2002	Brown pelican	Monroe county	St. Petersburg to Naples	negative
2/13/2002	Brown pelican	Monroe county	St. Petersburg to Naples	negative
10/17/2002	Great egret	Monroe county	None	negative
3/10/2003	Brown pelican	Monroe county	Sarasota to Naples	negative
7/30/2003	Great white heron	Monroe county	Tarpon Springs to Naples	negative
7/30/2003	Great white heron	Monroe county	Tarpon Springs to Naples	negative
1/30/2004	Laughing gull	Sarasota	Tarpon Springs to Sarasota	negative
1/30/2004	Sanderling	Sarasota	Tarpon Springs to Sarasota	positive
3/29/2004	Double crested cormorant	Pinellas county	Tarpon Springs	positive
5/12/2004	Brown pelican	Pinellas county	None	negative
5/27/2004	Laughing gull	Pinellas county	None	positive
6/3/2004	Brown pelican	Pinellas county	None	positive
6/13/2004	Least tern	Pinellas county	None	positive
6/13/2004	Least tern	Pinellas county	None	negative
6/20/2004	Least tern	Pinellas county	None	negative
7/9/2004	Least tern	Pinellas county	None	positive
7/9/2004	Least tern	Pinellas county	None	negative
7/11/2004	Royal tern	Pinellas county	None	positive
7/20/2004	Laughing gull	Pinellas county	None	negative
8/22/2004	Royal tern	Pinellas county	None	positive
8/22/2004	Royal tern	Pinellas county	None	positive
10/3/2004	Laughing gull	Pinellas county	Sarasota to Naples	positive
11/14/2004	Double crested cormorant	Lee county	Sarasota to Naples	positive

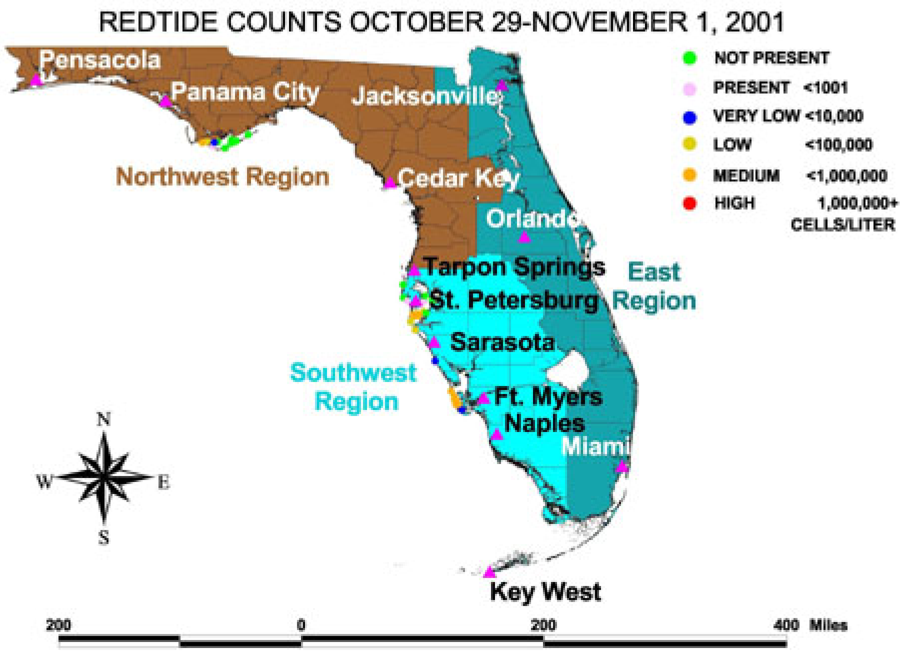

Figure 6. Red tide counts taken by The Florida Wildlife Research Institute from October 29 through November 1, 2001 as represented on the FWRI website.

In 2002 only five birds were collected for use in this study. Two birds were collected in early January from the Peace River Wildlife Sanctuary, which is located in Charlotte County (Figure 7). Both birds, a double crested cormorant and a common loon, were negative for brevetoxin presence and cell counts taken by FWRI from the area showed low *K. brevis* concentrations with ranges of not present to high in various areas from the St. Petersburg coast south to the Naples area (Table 13).

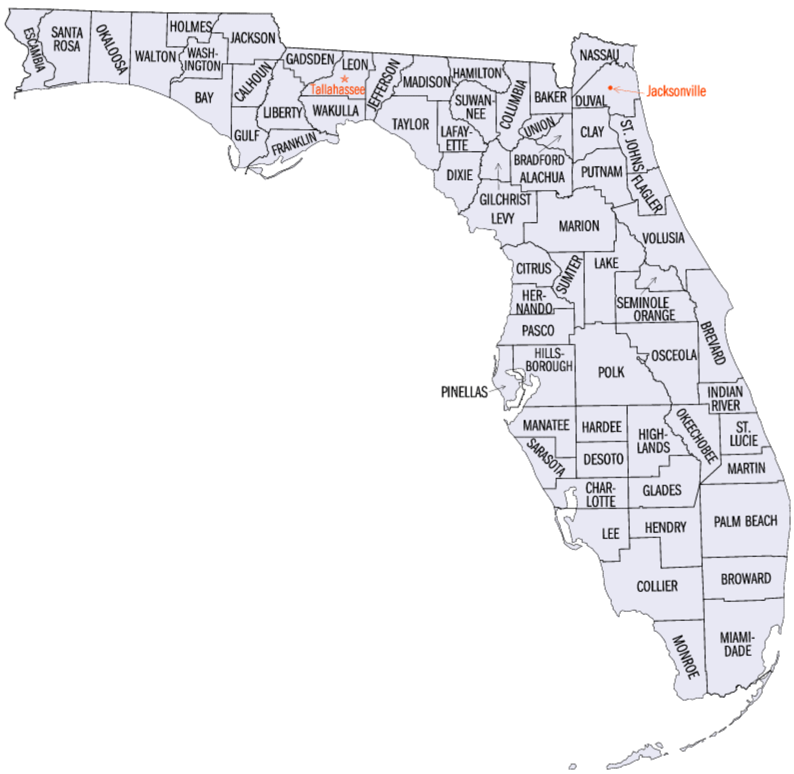

Figure 7. A map of Florida showing the counties.

Two brown pelicans collected in February from the Florida Keys area were also negative for brevetoxin presence. During the time frame the two birds were collected, *K. brevis* cell counts had increased in range from not present to high levels detected from offshore St. Petersburg down to Naples, but were not detected in the Florida Keys (Figure 8).

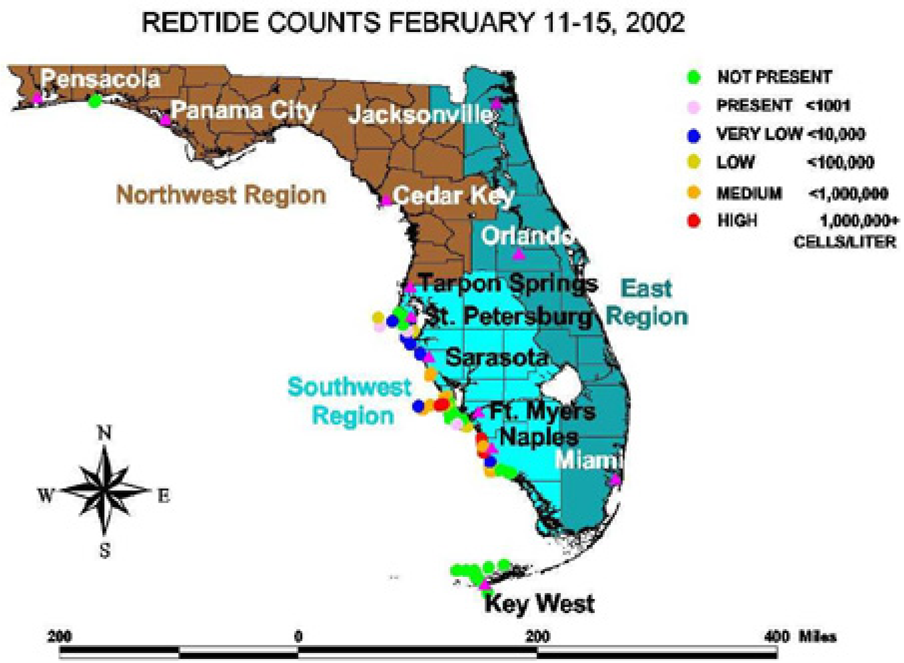

Figure 8. Red tide counts taken by The Florida Wildlife Research Institute from February 11 through 15, 2002 as represented on the FWRI website.

An egret collected in October 2002 in the Florida Keys was also negative for brevetoxin presence and cell counts taken by FWRI showed no presence of *Karenia brevis* in any of the collected areas along the south west coast of Florida.

Three birds were collected in 2003 from various locations in the Florida Keys. All three were negative for brevetoxin presence even though very low to medium *K. brevis* counts were reported up and down the south west coast of Florida throughout the year with a few high patch counts detected in January offshore from the Naples and Ft. Meyers area.

Twenty birds were collected during 2004 and very low to medium *K. brevis* counts seen at the end of 2003 continued along the south west Florida coast from January through April. May to September of that year showed no presence of the red tide organisms but very low to medium counts were reported again through the end of the year offshore from Sarasota south to the Naples area. Although one of the birds collected from the Sarasota area in January was negative for brevetoxin presence, a sanderling collected in the St. Petersburg area in the same month was slightly positive for brevetoxin presence in a liver and intestinal sample. A double crested cormorant collected in St. Petersburg in March was positive for brevetoxin presence in its liver, heart and stomach content, however, a brown pelican collected dead from Shell Key in May was negative for brevetoxin presence. A laughing gull collected on the same island a few weeks later was positive for brevetoxin presence in its stomach contents, although negative for all of its other tissue samples. This type of hit and miss positive or negative resultants continued for several months until November of 2004, when patches of medium to high counts were seen offshore of the Naples and Ft. Meyers areas (Figure 9). No more birds were collected until March 2005, when 3 double crested cormorants were brought into CROW, a rehabilitation center located in Sanibel (near Ft. Meyers), all 3 of which were positive for brevetoxin presence in all of the tissues collected. In 2005, 101 birds were collected that were used in the study and FWRI records show a clear cut event for the entire year with high cell count concentrations detected in the areas of Tarpon Springs south to Sarasota with some patches even further south to Ft. Meyers, Naples and in the Florida Keys. The event appears to have continued into January and February of 2006 with finally no counts being detected in March 2006 along the entire south west coast of Florida. A total of 55 birds were collected in 2006.

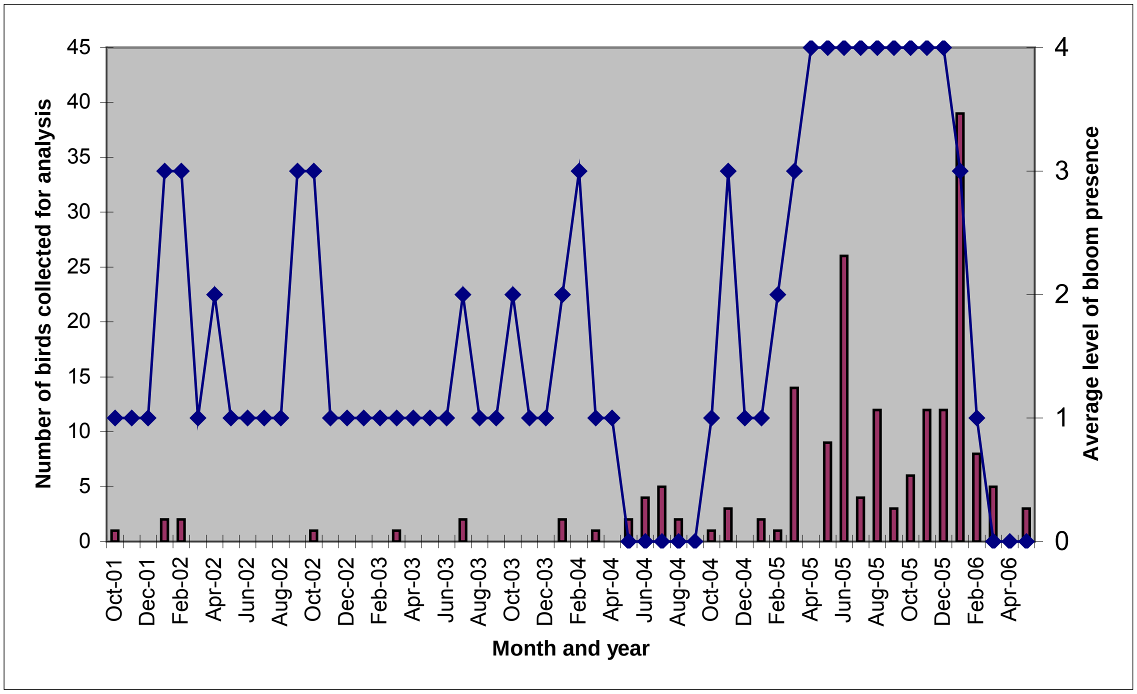

Figure 9. The number of birds collected for analysis by month and year compared to the average level of bloom presence detected through cell counts by FWRI. 0 denotes no presence, 1 is very low counts (<1,000 cells), 2 is low counts (<10,000 cells), 3 is medium counts (<1,000,000 cells) and 4 is high counts (>1,000,000 cells).

Brevetoxin presence in the various tissues tested throughout all 22 species ranged from 0 to 9,989 ng/g. The highest level was found in a stomach content sample from a double crested cormorant that had been collected on August 19, 2005 from Vina del Mar, an area of St. Petersburg Beach in Pinellas County. The bird died en route to the rehabilitation center and all of the tissues tested from the bird were positive. *Karenia brevis* cell counts showed low to high patches in the area where the bird had been collected for several months previously and the bloom was ongoing. In fact, 7 of the 12 highest levels found were from double crested cormorants, with the remaining 5 found in laughing gulls (Figure 10).

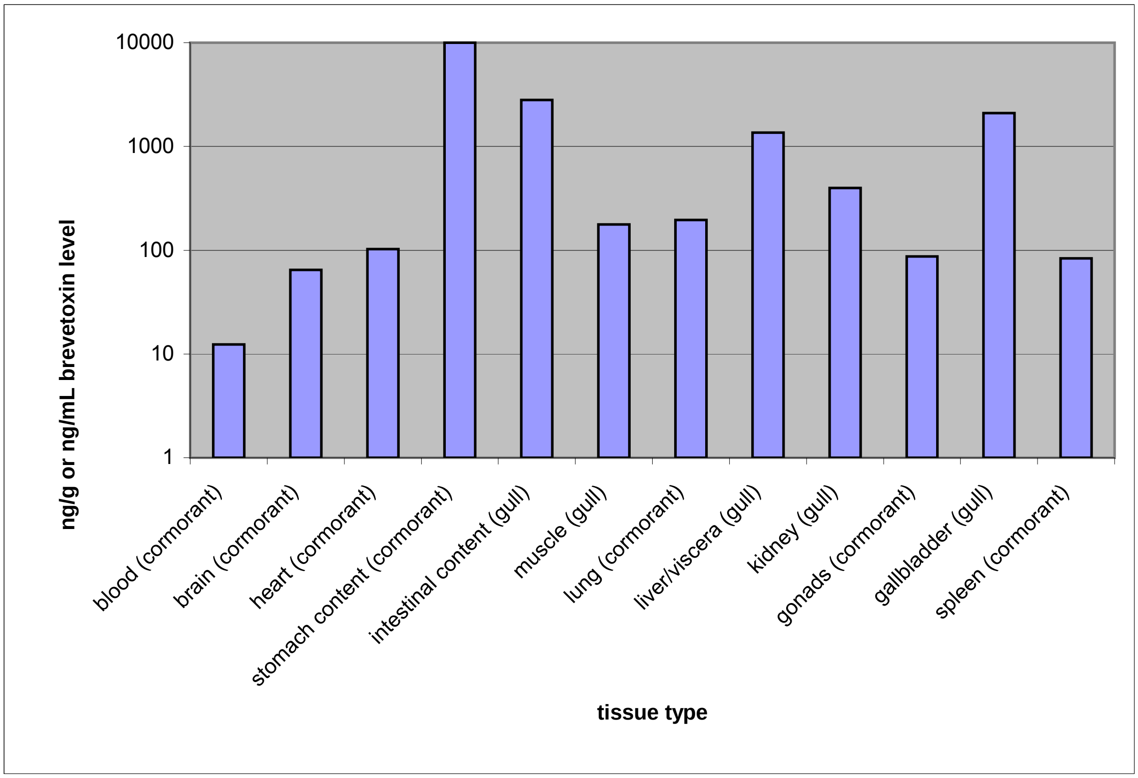

Figure 10. Highest brevetoxin concentrations found by tissue sample type and species in logarithmic scale.

DISCUSSION

The birds involved in the study were collected between 2001 through May 2006, during red-tide event periods and non-red tide event periods. Variability in the collection dates and collection areas are a consequence of an assortment of reasons: firstly, we depended on the staff of the rescue centers to contact us when they had birds that died that could be included in the research study. The rescue centers did not always have time for this when personnel were too busy, especially during event periods. Secondly, we were not in contact with every bird rescue center in Southwest Florida. The centers and groups we used were the SEANET Beached Bird Survey of Shell Key located in Pinellas County, The St. Petersburg Audubon/Eckerd College Least Tern Nesting Study located in Pinellas County, Peace River Wildlife Center located in Charlotte County, The Pelican Man Sanctuary of Sarasota, Save Our Seabirds (SOS) of Tierra Verde located in Pinellas County, The Center for Rehabilitation of Wildlife (CROW) located in Lee County, The Wildlife Center of Venice (WCV) located in Sarasota County, The Suncoast Seabird Sanctuary (SSS) located in Pinellas County, and birds which came from all over Florida into the Fish and Wildlife Research Institute (FWRI) located in St. Petersburg. Thirdly, not all birds that die or get sick in a red tide event come ashore and many that do come ashore may not be found. Therefore, the sample size is minimal and I have no way of estimating the true impact of brevetoxin concentrations in the tissues and organs of birds.

The *Karenia brevis* cell count data used in this study were provided by the Fish and Wildlife Research Institute (FWRI) located in St. Petersburg, Florida, which is a division of the Florida Fish and Wildlife Commission. Most sampling performed by FWRI is response based, i.e., samples are taken after a bloom had begun and reports of dead fish, discolored water, or respiratory irritation had been made. An independent contractor was hired to perform statistical analysis on cell count data collected from 1954 thru 2002 (comprising over 56,000 samples) by FWRI and that contractor determined that data collected from response-oriented monitoring is incomplete and limited, because it is too late to study the initiation and growth phases of the bloom and because it is logistically difficult to mobilize resources quickly enough to document the event adequately. Therefore, FWRI data, which were used to compare dates and locations of bloom detections, as well as the dates and locations of birds collected for this study, are inconsistent and precluded statistical analysis. Comparisons are therefore descriptive and only semi-quantitative.

There were several possible reasons that I observed such high concentrations of brevetoxins in double crested cormorants and species of gulls. More numbers of double crested cormorants were collected than any of the other species, although gull species actually rank towards the lower end of the spectrum for numbers collected (Figure 3). However, both double crested cormorants and gulls inhabit and feed in a wide range of habitats (Table 4), including estuaries and open ocean coastlines, and therefore may be exposed more frequently to areas where red tide is present. Both species also feed on various planktivorous fish in which brevetoxin has been shown to accumulate (Naar, et al., 2007), including baitfish such as threadfin herring and sardine species common to the Tampa Bay area. Gulls also have a tendency to feed on dead organisms that have washed ashore, greatly increasing their chances of being exposed to brevetoxins during events that may cause fish kills (van Deventer, 2007).

In addition to finding that double crested cormorants and gulls had the highest values for brevetoxin presence, the tissues that had the highest and the lowest levels for brevetoxins were also consistent for both groups (Figure 11). This was true not only when looking at the highest concentrations present but at average concentrations of brevetoxin (Figure 12). The highest concentrations were consistently found in stomach contents, intestinal contents, liver/viscera and gallbladder samples not only in double crested cormorants and gulls, but also in herons and egrets, terns and pelicans. Loons and gannets also showed the highest values for intestinal contents, liver/viscera and gallbladder, but differed in that high concentrations were found in gonad samples instead of stomach contents. Concentrations in organs of shorebirds were similar except that high concentrations were found in the kidneys instead of stomach contents or gonads. In the “other” group, the highest values were found in liver/viscera samples.

The lowest concentrations of brevetoxins for tissue samples were most commonly found in the blood, brain, lung and muscle. Such was the case for double crested cormorants and herons and egrets. Gulls showed the lowest values in gonads, heart, lung and spleen tissues. Loons and gannets showed the lowest values in the muscle, blood, heart and spleen samples and terns showed the lowest values in the brain, muscle, heart and lung samples. Pelicans were similar with the lowest values of brevetoxins shown for brain, blood, gonads and kidney samples, while shorebirds showed the lowest values in lung, muscle, brain and heart samples.

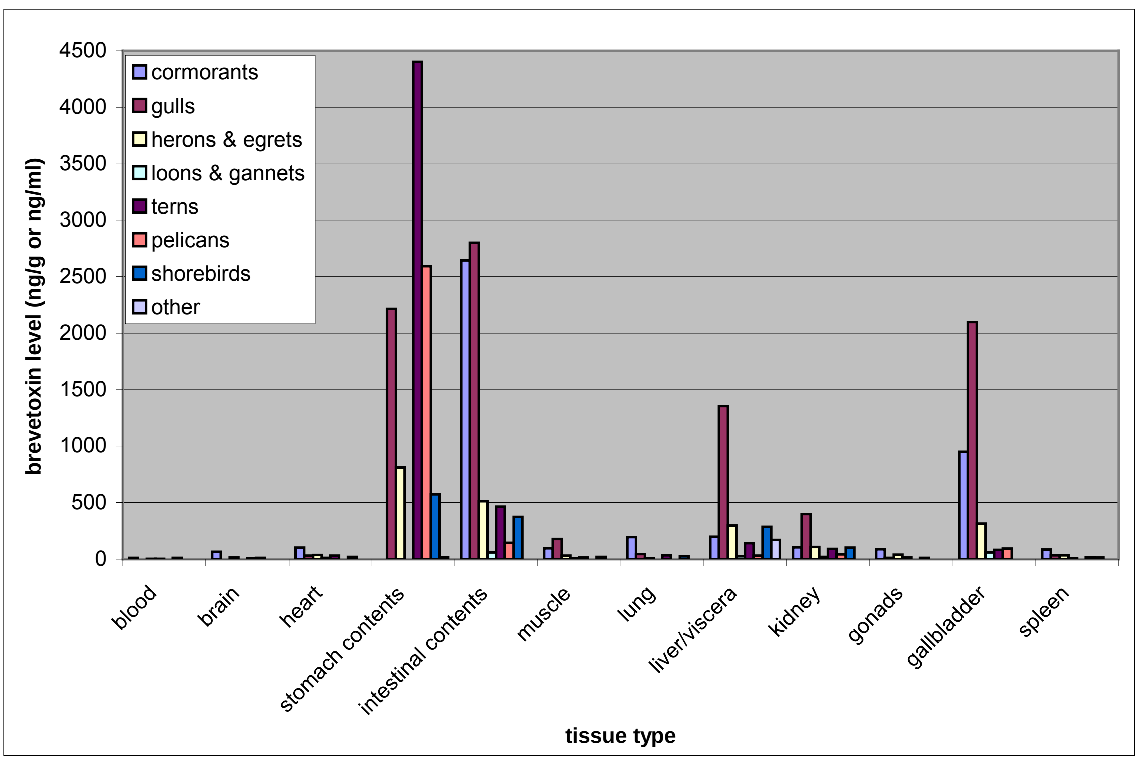

Figure 11. The highest concentration of brevetoxin found in each type of tissue tested in each group of birds.

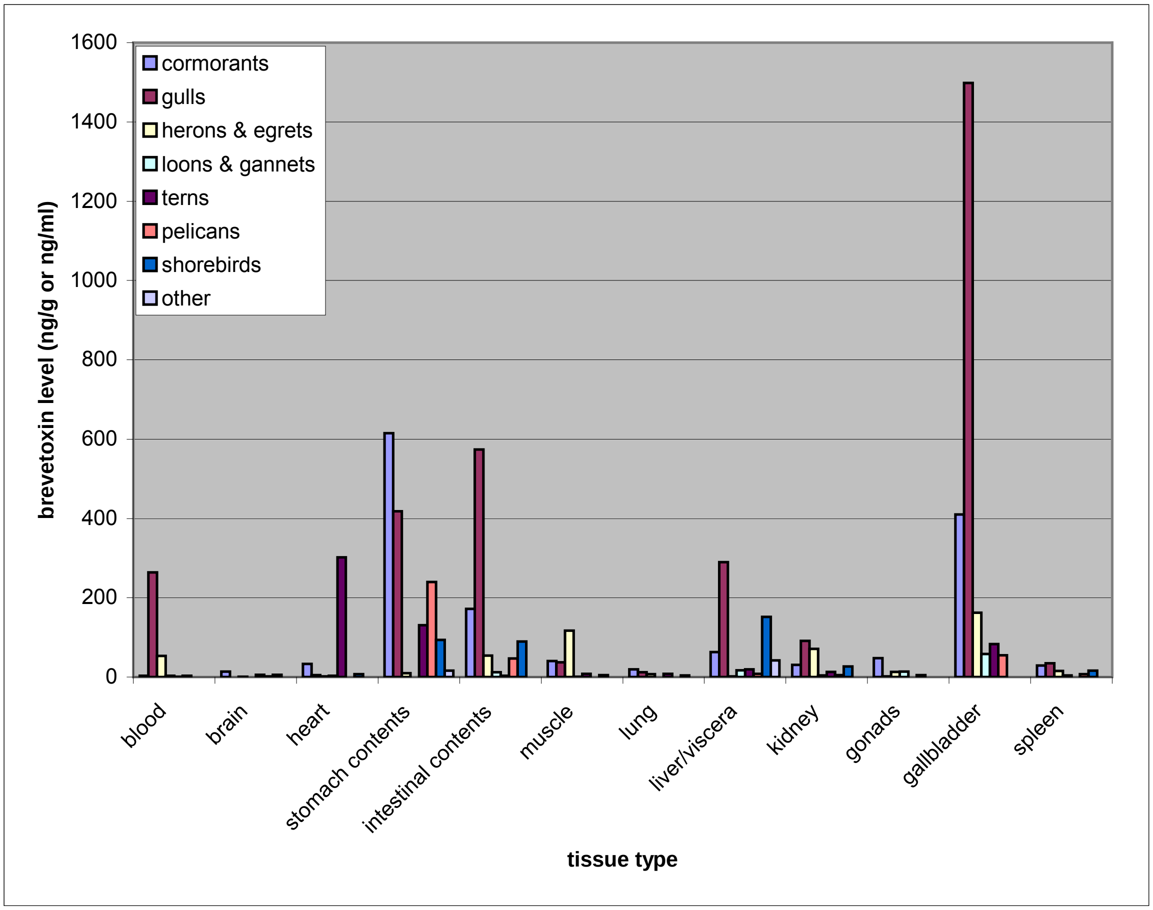

Figure 12. The average concentration of brevetoxin found in each type of tissue tested in each group of birds.

There are currently no other published studies of birds associated with *K. brevis* events with which to compare these values. There is, however, published data concerning manatees and dolphins. During a spring 2002 event, 34 endangered Florida manatees (*Trichechus manatus latirostris*) died in southwest Florida. In the spring of 2004 107 bottlenose dolphins (*Tursiops truncatus*) died in waters off the Florida panhandle (Flewelling, et al., 2005). Of the 63 animals tested (27 manatees, 36 dolphins) all were found to have high concentrations of brevetoxin in their tissues, specifically in the stomach contents (Flewelling et al., 2005), which is comparable to the results found in my study. However, that study excluded the possibility of poisoning through aerosol exposure for these particular animals, consistent with the findings from my study of low concentrations of brevetoxins in the lung tissues. Conversely, in a previous event in 1996 in which 149 manatees died, lung pathology indicated that brevetoxins had been inhaled (Bossart et al., 1998).

Brevetoxin concentrations were generally low in the few specimens placed in the “other” species and the group was unusual in that they showed the lowest positive values in stomach content samples and no positive values in brain, muscle, lung or kidney tissue samples. This observation may result from the small number of individuals (4) available for this group. However, the three species of birds included in this group, the whooping crane, osprey and wood stork, have different feeding habits from birds such as double crested cormorants or gulls. Their habits are more defined and limited to certain areas and certain types of prey. For example, wood storks do eat fish, but are more commonly seen on ponds or ditches where brevetoxin most likely will not be present. However, the wood stork available to my study was the only animal found to have positive brevetoxin levels in tissues from this group. Ospreys also eat fish, and can hunt on ponds and in lakes where brevetoxins will not be found, in addition to tropical coastlines where blooms may occur. Possibly when blooms and fish kills are present, ospreys are may avoid the areas. While working for a rehabilitation center, I also noticed that the resident osprey did not eat fish whole, but picked at its food and usually avoided organs such as the liver, stomach, intestines and kidney where brevetoxins accumulate (Naar et al., 2007), and ate mostly muscle where brevetoxins do not typically concentrate. Whooping cranes, on the other hand, eat a large variety of prey, not only small fish in salt marshes, but insects, frogs and sometimes plant matter, well away from the marine environments where *K. brevis* blooms.

The fact that brevetoxin concentrations in different tissues varies among species seems to be dependant on the animal's physiology. A study by Poli et al. (1990) showed that the liver was the major organ of metabolism and that excretion from bile was an important route of elimination. They showed that within 30 minutes of intravenous administration of brevetoxin to rats, 69.5% of radiolabelled toxin went to the skeletal muscle, 18% to the liver and 8% to the intestinal tract. They deduced that skeletal muscle does not appear to be a site of metabolism but of storage from which toxin is slowly released prior to clearance by the liver with elimination occurring via feces and urine.

Another study by Cattet and Geraci (1993) using ingested brevetoxin, also in rats, showed that although brevetoxin was distributed widely in the body, that it was concentrated in the liver. That study showed that after 6 hours, mean concentrations in organs were highest in the liver with the stomach next, followed by the intestine, heart, kidney, spleen, lung, fat, muscle, plasma, testes and finally brain. The results of this study were similar to those found by Poli et al. (1990) in that the highest concentrations of brevetoxin was found in stomach contents with intestinal contents next, then gallbladder, liver/viscera, kidney, lung, muscle, heart, gonads, spleen, brain and finally blood. Slight differences in the order of highest to lowest can be accounted for in the actual methods of each study. The study by Cattet and Geraci (1993) was controlled in a laboratory setting, whereas the samples obtained for my study were animals from the wild that were all exposed to brevetoxin at different times, at different concentrations and probably by different sources. They were also most likely continuously exposed to brevetoxins due to continued feeding in the environment as opposed to the one time feeding exposure the rats underwent. My study also tested lung tissues of some birds since brevetoxins can become airborne. Gallbladders, an organ which was absent from the other studies, were also tested and consistently high levels were found in positively exposed birds.

I had the lowest brevetoxin concentrations in blood serum samples in contrast to the study by Cattet and Geraci (1993), which found the lowest brevetoxin concentrations in brain samples. This is most likely due to the fact that the majority of blood samples used in this study were from 57 double crested cormorants that were released from the Suncoast Seabird Sanctuary in April of 2006 (Appendix 1), most of which had been at the sanctuary and recovering from brevetoxin exposure for several months. Therefore, the average blood sample levels may be lower than they would have been when the animals were first exposed. Original blood samples were not taken for these birds upon admission to the rehabilitation center because most birds that are exposed to brevetoxins are extremely sick and are usually very dehydrated, making it difficult to get enough of a blood sample to be used for brevetoxin testing. On the other hand, it is also interesting to note that the blood samples of these birds had any levels at all considering they had been in rehabilitation and not exposed to brevetoxin for up to 3 or 4 months or longer. One animal was at the center for over a year and still showed detectable levels of brevetoxin. van Deventer (2007) found generally high levels of brevetoxins in the fish food supplies of at least one rehabilitation center, which could account for this oddity.

Differences can also be seen in the highest concentrations and average concentrations of brevetoxin among the different avian groups (Figure 13 and Figure 14). For example, the highest level seen in the loon and gannet group was only 60 ng/g, compared to the highest double crested cormorant value of 9,989 ng/g and the highest gull value of 2,801 ng/g. The highest values for terns and pelicans were 4,400 ng/g and 2,595 ng/g respectively. The highest values seen in the heron and egret group was 810 ng/g and in shorebirds 574 ng/g, with the last group, other, showing a high value of approximately 168 ng/g.

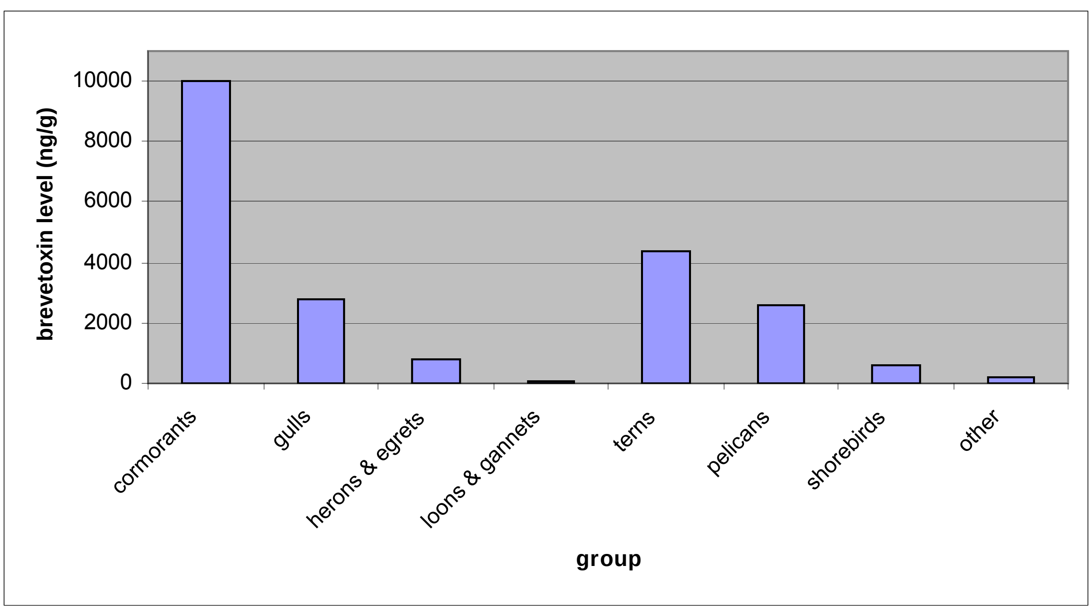

Figure 13. Birds by group compared to the highest concentration of brevetoxin (ng/g) found in a sample from that group.

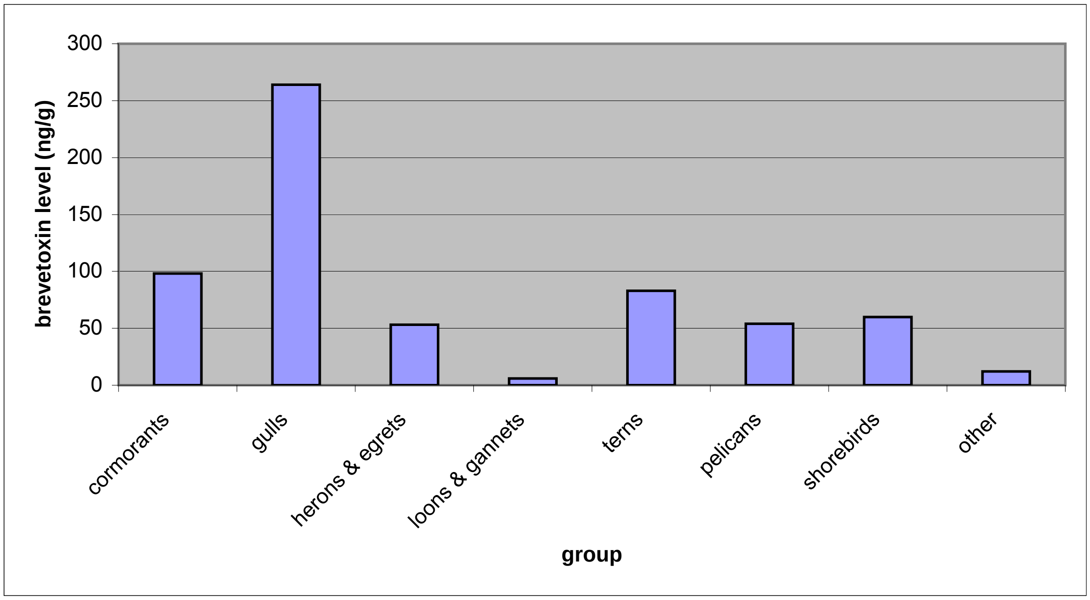

Figure 14. Birds by group compared to the average concentration of brevetoxin (ng/g) found in a sample from that group.

Again, these differences are possibly due to habitat and feeding preferences, although feeding habits do not explain all of the results reported in my study. Double crested cormorants, gulls, terns and pelicans eat marine fish which can accumulate brevetoxins in their tissues (Naar et al., 2007) and the levels of brevetoxins seen in the tissues of those birds support those findings. Herons and egrets have a wide variety of feeding habitats that can include organisms in freshwater areas or from land as well as marine areas. Thus, the brevetoxin levels found in my study tend to be lower than in those species found in strictly marine habitats. Common loons often eat in freshwater areas in the northern parts of the US and Canada, although, in Florida, they seem to be strictly estuarine, so it is surprising to see such low levels of brevetoxins in their tissues. Northern gannets often eat well offshore where brevetoxin blooms may not be as concentrated as they are inshore, although, the individuals occasionally are seen inshore. The shorebird species available for my study perhaps show the most surprising results. It could be argued that these particular species have feeding habits that may keep them from being exposed to areas and foods with high levels of toxins, therefore explaining the low levels seen in the tissues. For example, soras usually eat seeds and aquatic insects and ruddy turnstones, in addition to marine fish, also eat insects, carrion and garbage. Sanderlings eat aquatic and terrestrial invertebrates and sanderlings, ruddy turnstones and black skimmers all feed on crustaceans that have been shown to be highly toxic (Matter, 1994). van Deventer (2007) has shown that these shorebird species also scavenge fish on the beach that showed consistently high levels of brevetoxin in their tissues, therefore raising the question as to why the shorebirds used in my study do not show higher concentrations, similar to double crested cormorants or gulls.

CONCLUSIONS

Through testing of tissues from 185 birds representing 22 species, collected from October 2001 through May 2006, it has been shown that marine birds accumulate brevetoxins in various organs and tissues. Relationships between internal toxin levels and *K. brevis* cell counts within blooms suggest a high coincidence with avian brevetoxicosis. My observations show that levels of brevetoxin in tissues of birds rose significantly during an ongoing red tide event and even rose slightly over several brief red tide event periods (Appendix 5).

Brevetoxins were detected in essentially all internal organs and tissues as well as blood serum. Brevetoxins were detected in 52% of all tissues tested, with 95% of gallbladders positive, 78% of blood serum positive, 69% of intestinal content/digestive tract samples positive, 60% of stomach/gut content samples positive and 58% of liver/viscera samples positive. Values of brevetoxin ranged from 0 to 9,989 ng/g, with the highest level found in a stomach content sample of a double crested cormorant. The highest concentrations of brevetoxins occurred in either double crested cormorants or gulls. Although I have no way of estimating the duration of exposure, the fact that toxin was found throughout the body at high levels suggests that multiple toxic prey items were ingested rather than a single acute exposure.

Results reveal a clear, acute threat to marine birds and therefore to other marine animals, including numerous species of fish as well as mammals such as dolphins and manatees during red tide events. Moreover, little is known about chronic, low level exposure effects to marine animal health. This fact supports the need for further study in this field, not only during obvious red tide events, but during non-event periods as well.

Based on the range of brevetoxin levels I found in the various tissues and organs and, due to the expense and time required to test a wide variety of tissues and organs, I would recommend only using muscle, liver, stomach and/or intestinal contents, and lung tissue for ELISA based tests. Also, given my results for blood serum analyses, a more focused study is in order. Blood should be drawn from all birds upon arrival and release. This will give a data set which will allow for comparisons of blood serum phycotoxin levels in sick animals and allow an assessment of various treatment options.

MANAGEMENT IMPLICATIONS AND AREAS OF FUTURE RESEARCH

Some previous authors note that inshore or coastal birds appear to have developed conditioned aversions to algal toxins. Shumway (1990) suggests, based on a 1983 study by Nisbet, that terns developed an aversion response to toxic fish. Nisbet reported many piles of vomit containing the birds major prey, the sandlance, and estimated that they had been regurgitated within 20-30 minutes after ingestion. He suggested that more birds vomited than were killed and that only birds feeding on fish during the initial bloom period were impacted. Other studies have shown that birds killed in mortality events were naïve (e.g., Fritz et al. 1999, Work et al. 1993, Shumway et al. 2003, and Kreuder et al. 2002). This research points to a possible field of study on the subject of toxic prey aversion in marine birds.

Much research has shown that brevetoxins move through the food web and this bioaccumulation of toxins may affect both ecological communities and individual species, such as sea birds. Seabirds may have the potential to change prey base or feeding location to avoid toxins. Because seabirds may have this ability to move between ecological communities, another area of future research may include studies to see if sea bird mortality events have significant affects on seabird populations, based on their slow rates of reproduction. Coulson et al. (1968) estimated that 80% of the breeding population of shags in Northumberland died during an outbreak of PSP. During the summer of 1989-1990, 150 adult yellow-eyed penguins (out of a population of 240 breeding pairs) were reported dead in New Zealand, apparently due to red tide poisoning (Gill and Darby, 1993). Coulson and Stowger (1999) reported the deaths of over 13,000 black legged kittiwakes in the northeast UK in just two years due to red tide. Many have also noted that the full impact of HABs on marine birds is likely underestimated as many die at sea and never wash ashore, so the need for research obvious (Shumway et al., 2003; Coulson et al., 1968).

Further, a protocol has been established at one bird rehabilitation center that reportedly provides a 90% success rate (pers. Comm., Lee Fox). This claim could be studied and if proven, the protocol could be distributed to other rehabilitation centers located in other areas where brevetoxin events occur.

The combined answers to these questions could bring us a step closer to managing wildlife in a consistent and practical manner and may lead to further research into HABs and its effects on other charismatic marine species, such as manatees, whales and dolphins.

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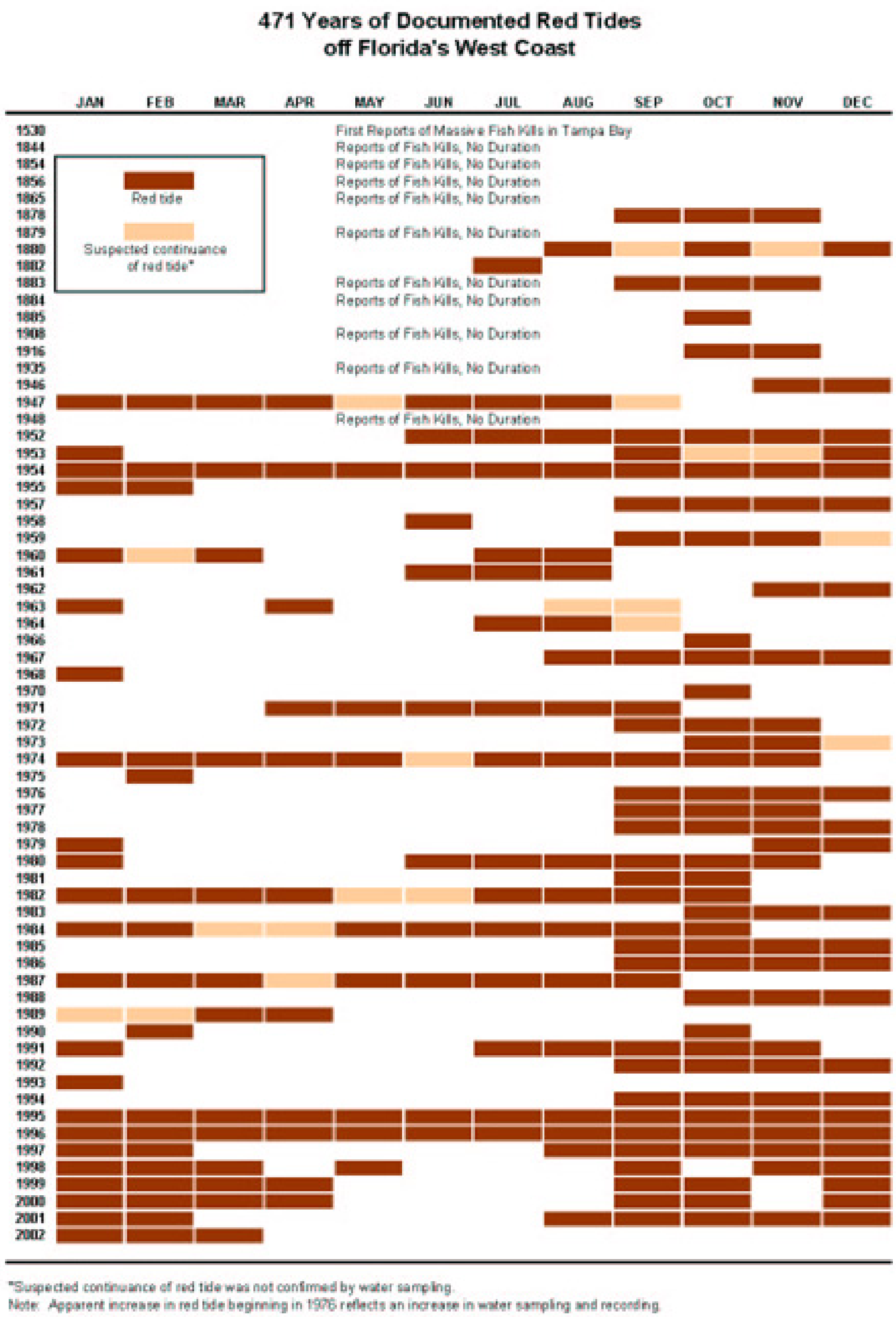
APPENDICES

Appendix 1. 471 years of documented red tide events off of Florida's west coast as shown on the FWRI website.



Appendix 2. Brevetoxin levels found in the blood serum samples from 57 double crested cormorants released from the Suncoast Seabird Sanctuary in April of 2006 after treatment for brevetoxicosis symptoms.

Identification number	Date	Toxin level (ng/mL)
HABB060417-031	Jan 9 2005	1.53
HABB060417-039	Sep 28 2005	2.44
HABB060417-014	Oct 16 2005	0
HABB060417-041	Oct 23 2005	4.43
HABB060417-006	Nov 7 2005	0
HABB060417-025	Nov 7 2005	3.82
HABB060417-053	Nov 8 2005	1.82
HABB060417-021	Nov 15 2005	4.48
HABB060417-044	Nov 16 2005	4.39
HABB060417-037	Nov 20 2005	3.27
HABB060417-059	Nov 21 2005	1.28
HABB060417-030	Nov 22 2005	1.68
HABB060417-024	Nov 26 2005	0
HABB060417-008	Nov 27 2005	1.28
HABB060417-011	Nov 28 2005	0
HABB060417-035	Nov 29 2005	2.95
HABB060417-034	Dec 2 2005	2.74
HABB060417-047	Dec 16 2005	3.14
HABB060417-023	Dec 18 2005	3.82
HABB060417-015	Dec 19 2005	2.12
HABB060417-026	Jan 1 2006	2.19
HABB060417-046	Jan 2 2006	3.61
HABB060417-051	Jan 2 2006	0
HABB060417-058	Jan 2 2006	3.53
HABB060417-018	Jan 3 2006	3.41
HABB060417-050	Jan 5 2006	1.58
HABB060417-048	Jan 6 2006	2.94
HABB060417-028	Jan 7 2006	3.31
HABB060417-055	Jan 27 2006	1.96
HABB060417-007	Jan 10 2006	0
HABB060417-056	Jan 10 2006	3.38
HABB060418-002	Jan 10 2006	4.31
HABB060417-013	Jan 11 2006	1.91
HABB060418-001	Jan 11 2006	0

Appendix 2 (Continued)

HABB060417-038	Jan 12 2006	1.83
HABB060417-054	Jan 12 2006	2.58
HABB060417-029	Jan 13 2006	1.65
HABB060417-032	Jan 14 2006	2.21
HABB060417-022	Jan 15 2006	2.03
HABB060417-017	Jan 16 2006	1.11
HABB060417-010	Jan 17 2006	0
HABB060417-043	Jan 19 2006	2.9
HABB060417-033	Jan 21 2006	2.72
HABB060417-040	Jan 21 2006	3.67
HABB060417-060	Jan 22 2006	0
HABB060417-012	Jan 24 2006	2.27
HABB060417-049	Jan 24 2006	4.28
HABB060417-052	Jan 24 2006	3.18
HABB060417-027	Jan 29 2006	1.45
HABB060417-045	Jan 29 2006	1.77
HABB060417-057	Jan 31 2006	1.43
HABB060417-016	Feb 7 2006	0
HABB060417-042	Feb 10 2006	4.51
HABB060417-009	Feb 21 2006	1.45
HABB060417-036	Feb 21 2006	3.25
HABB060417-019	Feb 28 2006	2.49
HABB060417-020	Feb 28 2006	2.48

Appendix 3. Specific results of samples taken for each of the 185 birds used in the study referenced by identification number. Values are in ng/g of brevetoxin concentration.

Identification number	Blood	Brain	Digestive tract	Fat	Gallbladder	Gi contents	Gonads	Gut contents	Heart	Intestinal contents	Kidney	Liver	Lung	Muscle	Si contents	Spleen	Stomach contents	Viscera
02010402																		0
02010602																		0
HABB031106-002	0																	
HABB031106-003	0																	
HABB031106-004	0																	
HABB031106-005	0																	
HABB031106-011	0																	
HABB031106-014												0		0			0	
HABB031106-015												0		0			0	
HABB040205-002		0							0	0	0	0	0	0				
HABB040205-003									0	22.81	0	19.4	0	0				
HABB040403-003									33.8			55.7					42.93	
HABB040706-001		0									0	0	0	0			0	0
HABB040709-003									0		0	0					0	
HABB040709-004									0			0					22.44	
HABB040709-005									0		0	0					0	
HABB040709-006		0		0			0		0	0	0	0	0	0			33.83	
HABB040714-004							0		0	26.08	0	0	33.1	14.06			32.56	
HABB040719-001		0					0		0	0	0	0	0	0		13.47	0	
HABB040722-009		0		0			0		0	0	0	0	0	0			0	
HABB040925-001									0		0	0	0	0			0	
HABB040925-002												0		0			7.66	
HABB040925-003												0					0	0
HABB040925-004											12.51	26.3		0			13.97	
HABB040925-005												40.5						0
HABB041014-007							0		0	0	16.21	13.6	0	0			0	
HABB041119-005		34.7							50.6	14.15	57.03	96.7	32.4	53.37		65.88	60.18	

Appendix 3 (Continued)

HABB041119-006	20.57				46.6	34.65	35.24	52.3	18.5	54.11		19.49
HABB050324-007	10.47	0		10.37	0	11.08	0	11.4	0	0	0	19.15
HABB050329-007								0				
HABB050526-003	17.67		240.52	51.27	20.7	68.16	38.99	61.8	60.9	55.59		182.59
HABB050526-004	0		32.38	0	0	0	0	0	0	0		0
HABB050526-005	26.19		510.69	87.13	51.7	194.85	69.26	137	20.1	90.95		163.82
HABB050526-006	0			10.38	0		8.04	7.53	0	0	34.97	0
HABB050601-003	15.63		424.48	61.11	65.3	45.17	45.5	85.9	10.7	77.99		39.32
HABB050601-004	20.98		948.92	56.35	51.2	72.7	64.21	98.6	12.5	96.86		61.37
HABB050603-001	13.18		418.13	59.32	39.9	67.14	39.26	122	18	56.27		442.91
HABB050603-002	28.17			75.65	81.6	171.39	76.05	82.4	22.9	61.13	63.8	99.49
HABB050608-001	18.61		467.76	76.71	35.7	116.57	51.12	108	14.9	45.53		161.53
HABB050608-002	12.19		584.46	82.59	68.3	218.09	41.32	73.4	14.9	56.35		42.96
HABB050609-012	12.09			33.54	55.4	8.94	18.32	58.1	0	45.03	12.86	61.9
HABB050609-013	0			10.3	0	88.32	0	0	0	0	0	0
HABB050614-015	7.58							13.6				0
HABB050614-016	0							0		11.31		0
HABB050614-017								23		0		
HABB050614-018	0							0		0		0
HABB050614-019	0							0		0		0
HABB050614-020	0						0	0		0		
HABB050614-023	0						18.64	23.4		0		
HABB050614-024							0	0	0	0		0
HABB050614-025	0						0	11.7	0			
HABB050615-001	0						0	0	0	0		257.83 / 48.13 / 1760.01
HABB050615-002	0						0	0	0	0		0
HABB050615-003	0						0	0	0	0		
HABB050615-004	0						0	0	0	0		31.68

Appendix 3 (Continued)

HABB050615-005		0		0							0	0	0	0		
HABB050630-010			243.83						15.3			138				
HABB050630-011		0					0		0	0	0	0	0	0	0	39.02
HABB050630-012		15.01	122.83						22.3			117		16.99		449.58
HABB050630-013		0					0		0	0	0	0	0	0	0	0
HABB050630-014		0					0		0	68.73	15.68	40.1	0	0		13.16
HABB050630-015			228.58									182				810.51
HABB050630-016		0							21	109.05	34.28	102	0	24.27	25.09	52.96 / 269.92
HABB050701-001		0							0	0	0	0	0	0	0	0
HABB050701-002		0							0	0	0	11.7	0	0	0	0
HABB050701-003		0							0	0	16.02	29.4	0	0	0	15.07 / 0
HABB050705-001		0		11.9					0	31.65	41.88	30.8	0	0		76.47 / 15.15 / 1095.98
HABB050705-002		0							0	0	0	25.5	0	0		0
HABB050705-003			270.76						36.4			227		31.99		727.22
HABB050705-004		0	0						0		0	0	0	0		0
HABB050705-005		0		0			0		0	0	0	0	0	0	0	0
HABB050705-006			0						0		0	42.6		0		
HABB050705-007		0							0	21.65	0	0	0	0	0	0
HABB050706-003		0							0	63	0	0	0	0	0	12.94
HABB051020-030												0	0			0
HABB051020-031												0	0			17.19
HABB051020-032					93.36	12.15	0		0		0	13.2	0	0		0
HABB051028-014					2099.21			932.09		2021.28	291.5	1044	18.9	142.92		653.74
HABB051028-015					897.11			2215.8		2800.73	398.2	1355	42.9	176.55		345.17
HABB051220-001	0 / 3.69															
HABB051220-002	0 / 4.09															
HABB051220-003	0															
HABB051220-004	1.82 / 3.88															

Appendix 3 (Continued)

HABB051220-005	3.92 / 0														
HABB051220-006	0														
HABB051220-007	0 / 2.05														
HABB051220-008	0														
HABB060112-001	1.74														
HABB060112-002	0 / 1.67														
HABB060112-003	4.15														
HABB060112-004	11.68														
HABB060112-005	6.98														
HABB060112-006	9.37														
HABB060112-007	7.05 / 0														
HABB060220-023	5.21														
HABB060303-001	12.31	0		20.02			0		0	0	0	0	0		18.36
HABB060303-006				34.45			0	17.25	0	0	0	18.5			8.61
HABB060307-001		23.5	121	718.43			102	2645.35	104.6	198	195	84.07		83.67	2310.32 / 4095.08 / 9988.62
HABB060322-013		64.82		519.13		55.74	35.5	114.74	64.91	147	12.9	39		82.04	525.23
HABB060322-014		8.02					4.93		6.07	11.6	0	8.17		12.25	33.07
HABB060322-015	0														
HABB060322-016	1.95														
HABB060322-017	1.17														
HABB060327-001								371.86		270		19.73			574.67
HABB060327-002		0					0	15.13	0	0	0	0			11.89
HABB060409-001								29.48		48.8	0				28.81
HABB060409-002						0	0	0	0	27.6	0	0		15.54	15.78
HABB060414-006	3.15				0				0	13.4	0	0			
HABB060414-007	4.23				0				0	0		0			
HABB060414-009				0				13.52		48.9					30.35
HABB060414-010								93.6		263	0				47.92

Appendix 3 (Continued)

HABB060414-011							12.07		196	0				39.78
HABB060414-016		16.13			23.73	35.4			106		84.45	56.63		39.8
HABB060414-019						23.4			96.9		0			4399.50 / 153.69
HABB060424-006							143.47		19.7		0			170.41
HABB060428-005		0		83.25		0	33.07	0	29.4	0	0			31.79
HABB060428-006						15.8	96.84		286	25.1	10.84			42.26
HABB060505-020			7.49			0	187.04		202		0			66.4
HABB060515-001						31.7	272.89		147		9.98			387.43
HABB060515-002				314.93		21.2	40.27	42.15	100	8.26	21.77		34.5	315.67
HABB060522-004						11.1	510.77	107.8	296		13.69			47.72
HABB060530-012				58.21	14.42	9.92	59.58	8.3	29.4	0	5.78		7.67	0
HABB060530-013		0			0	31.4	465.1	89.69	141		9.02			733.6
HABB060530-014		5.51				21.4	165.88	103.1	303	0	10.92			
HABB060530-016		0			0	0		0	0	9.14	0	0		0
HABB060530-017		0				0		0		0	0			
HABB060530-018	2.43	0		8.82		0		0	0	0	0	0		0
HABB060613-002						0	0	0	7.86		0			

Appendix 4. Identification numbers for each of the 185 birds used in the study with common name, collection date, region collected, history and miscellaneous comments listed.

Identification number	Species	Date	County	Comments
02010402	Common loon	1/10/02	Charlotte	pooled tissues, intestine, stomach, liver, kidney and spleen
02010602	Cormorant	1/10/02	Charlotte	pooled tissues, intestines, stomach, liver, kidney
HABB031106-002	Brown pelican	2/5/02	Monroe	
HABB031106-003	Brown pelican	2/13/02	Monroe	
HABB031106-004	Brown pelican	3/10/03	Monroe	
HABB031106-005	Great white Heron	10/29/01	Monroe	
HABB031106-011	Great white Heron	7/30/03	Monroe	
HABB031106-014	Egret	10/17/02	Monroe	pooled tissues
HABB031106-015	Great white Heron	7/30/03	Monroe	pooled tissues

Appendix 4 (Continued)

HABB040205-002	Laughing gull	1/30/04	Sarasota	
HABB040205-003	Sanderling	1/30/04	Sarasota	
HABB040403-003	Cormorant	3/29/04	Pinellas	
HABB040706-001	Brown pelican	5/12/04	Pinellas	found dead on Shell Key, trachea cut; stomach filled with parasites
HABB040709-003	Least tern	7/9/04	Pinellas	found on ground of parking lot
HABB040709-004	Least tern	7/9/04	Pinellas	found on ground of parking lot
HABB040709-005	Least tern	7/9/04	Pinellas	found on ground of parking lot
HABB040709-006	Laughing gull	5/27/04	Pinellas	no visual problems on outside of body; found dead on Shell Key
HABB040714-004	Royal tern	7/11/04	Pinellas	found dead on Shell Key

Appendix 4 (Continued)

HABB040719-001	Brown pelican	6/3/04	Pinellas	hit by car on the Skyway Bridge, seemed disoriented before impact, DOA
HABB040722-009	Laughing gull	7/20/04	Pinellas	found on shell key, disoriented, could not fly, drooping/dragging wings, died on way to rehab center, juvenile; found at 11:19 am, died 11:42 am
HABB040925-001	Least tern	6/20/04	Pinellas	least tern found dead at Autoway Pontiac in Clearwater, male, most likely died from fall from nest
HABB040925-002	Least tern	6/13/04	Pinellas	least tern left leg missing, covered in parasites, prob pushed from nest by sibling
HABB040925-003	Least tern	6/13/04	Pinellas	found on ground of parking lot

Appendix 4 (Continued)

HABB040925-004	Royal tern	8/22/04	Pinellas	royal tern #1 found on Shell Key transect. Found beak of another bird lodged in back. Very emaciated/no broken bones
HABB040925-005	Royal tern	8/22/04	Pinellas	royal tern #2 found on Shell Key transect/extremely emaciated/no broken bones
HABB041014-007	Laughing gull	10/3/04	Pinellas	laughing gull found on Shell Key, male, no apparent injuries
HABB041119-005	Cormorant	11/14/04	Lee	parasites in stomach, thin
HABB041119-006	Cormorant	11/14/04	Lee	broken left leg; heart enlarged, aspergillus looking spots in mouth, very red inside of mouth
HABB050324-007	White pelican	3/19/05	Lee	male/probable head impact injury/blood in nares, mouth and eyes, right side of head swollen.

Appendix 4 (Continued)

HABB050329-007	Whooping crane	3/27/05	Citrus	
HABB050526-003	Cormorant	3/8/05	Lee	found on Sanibel Island with brevetoxicosis symptoms/1.290 kg weight upon admit
HABB050526-004	Cormorant	3/9/05	Lee	head trauma, bloody eyes and mouth
HABB050526-005	Cormorant	3/9/05	Lee	1.195kg upon admit, found on Sanibel Island with brevetoxicosis symptoms
HABB050526-006	Herring gull	3/7/05	Pinellas	found dead at the Siesta Beach Access #5, white lesions under liver & on the stomach, lung & intestines
HABB050601-003	Cormorant	3/7/05	Lee	found alive on Sanibel Island, euthanized because of severe hypopoteinemia, showed marked ataxia, moderate head trauma & dull mentation

Appendix 4 (Continued)

HABB050601-004	Cormorant	3/7/05	Lee	found alive on Sanibel Island, 1.190 kg weight on admit, showed moderate ataxia, head tremor, slow blink, respiratory effort, bloodwork showed pcv 3%, TP 1.8g/dl
HABB050603-001	Cormorant	3/9/05	Lee	found alive on Sanibel Island, 1.195 kg weight upon admit, showed mild ataxia, tarry feces. bloodwork pvc 39%, TP 2.0 g/dl
HABB050603-002	Cormorant	3/4/05	Lee	found alive on Sanibel Island, 1.345 kg weight upon admit, showed moderate ataxia, head tremor, slow blink, bloodwork pcv 50%, TP 3g/dl
HABB050608-001	Cormorant	3/4/05	Lee	found alive on Sanibel Island, 1.330 kg weight upon admit, showed moderate ataxia & slow blink, bloodwork pcv 30%, TP 1.4 g/dl

Appendix 4 (Continued)

HABB050608-002	Cormorant	11/12/04	Lee	euthanized
HABB050609-012	Cormorant	3/10/05	Lee	found alive on Sanibel Island, euthanized, showed moderate ataxia, slight head tremor, tarry feces, pcv 33%, TP 2.4g/dl
HABB050609-013	Cormorant	5/24/05	Lee	DOA
HABB050614-015	Least tern	5/30/05	Pinellas	found on ground of parking lot
HABB050614-016	Least tern	5/30/05	Pinellas	found on ground of parking lot
HABB050614-017	Least tern	5/30/05	Pinellas	found on ground of parking lot
HABB050614-018	Least tern	5/30/05	Pinellas	found on ground of parking lot
HABB050614-019	Least tern	5/30/05	Pinellas	found on ground of parking lot

Appendix 4 (Continued)

HABB050614-020	Cormorant	5/5/05	Sarasota	found on Casey Key in Nokomis with propeller wounds on back, not using right leg, immature, indeterminate sex, 640 grams, GI loaded with parasites and lungs congested.
HABB050614-023	Northern gannet	6/3/05	Sarasota	adult male, 1448.9 grams, blood clot/hemmorhage at left femorotibial junction, bile in peritoneal cavity
HABB050614-024	Brown pelican	3/28/05	Pinellas	adult female, 1931.8 grams, tan clot/growth on subclavian, worms in gizzard
HABB050614-025	Northern gannet	5/20/05	Pinellas	adult male, 1590.9 g, low parasite load, slightly emaciated.
HABB050615-001	Brown pelican	6/11/05	Pinellas	male, second year adult, 2613.6 grams, emaciated.

Appendix 4 (Continued)

HABB050615-002	Osprey	6/10/05	Pinellas	immature female; 852.3 grams, found in backyard of home in Palm Harbor alive, emaciated, black liquid and twist tie in stomach.
HABB050615-003	Osprey	6/6/05	Pasco	mature female;1306.8 grams; found alive in backyard of home in Holiday zip code 34690, black oily substance in gizzard
HABB050615-004	Brown pelican	6/11/05	Pinellas	second year adult male, 2045.4 g, fishing line obstructing blood flow to leg, emaciated
HABB050615-005	Great blue heron	6/11/05	Pinellas	female; 2386.4 g, found alive on roadside
HABB050630-010	Green heron	6/1/05	Pinellas	male 63.8 grams, emaciated, some large intestine black
HABB050630-011	Great blue heron	6/1/05	Pinellas	1051.1 g upon admit, hemoabdomen, hemothorax - possible trauma from impact

Appendix 4 (Continued)

HABB050630-012	Green heron	6/1/05	Pinellas	male 85.2 g, yellow uric acid crystals throughout abdominal cavity and covering parts of outside body; liver infarct
HABB050630-013	Royal tern	6/1/05	Pinellas	mature male, 454.5 g, abscess in cheek, septic, heart hypoxic, one testicle necrotic
HABB050630-014	Laughing gull	6/18/05	Pinellas	mature female, 198.9 g, found in backyard of home in St. Petersburg alive, evidence of impact, jugular hematoma, hemmorhage in pecs, fractured humerus, necrotic oviduct, very thin
HABB050630-015	Green heron	6/1/05	Pinellas	male, 28.4 g upon admit, liver infarct, hypoxic heart
HABB050630-016	Great blue heron	6/27/05	Pinellas	female; 2686.4 g,found in backyard of private home in Largo, FL alive, systemic infection, strongest in lungs, also signs of impact/trauma with multiple hemmorhages, very thin

Appendix 4 (Continued)

HABB050701-001	Great blue heron	6/20/05	Pinellas	second year male, 1988.6 g, found roadside in St. Petersburg alive, hemmorhaging from pectoral muscle, abdomen, kidney, lungs, left femur, liver, possible impact trauma
HABB050701-002	Northern gannet	6/16/05	Pinellas	immature male, 1647.7 g upon admit, a few worms in GI, very thin
HABB050701-003	Cormorant	6/21/05	Pinellas	immature female, 965.9 g, very thin, overloaded with worms inside and on top of GI tract, mesentery scattered with hard yellow nodules
HABB050705-001	Brown pelican	6/25/05	Pinellas	male, 2329.5 g, found floating in water at St. Petersburg beach, multiple fractures to wing, several organs necrotic/hemmorhaging
HABB050705-002	Common loon	6/22/05	Pinellas	mature female, 2329.5 g, found at Caladesi Beach, growths in abdominal cavity

Appendix 4 (Continued)

HABB050705-003	Green heron	6/25/05	Pinellas	113.6 g, euthanized within 2 hours of admit, emaciated
HABB050705-004	Yellow crowned night heron	5/21/05	Pinellas	male, 511.4 g, euthanized within 3 hours of arrival, left leg inflamed/broken.
HABB050705-005	Cormorant	6/26/05	Pinellas	immature male, 1193.2 g, found alive at Indian Rocks Beach, growths under keel in peritoneum, many organs hyperemic.
HABB050705-006	Green heron	6/19/05	Pinellas	male, 113.6 g, found alive in yard of home in Madiera beach, liver and heart partly necrotic; brown lungs, fractured leg
HABB050705-007	Cormorant	6/18/05	Pinellas	immature female, 937.5 g, found alive roadside in Dunedin, parasite load high, septic abdomen
HABB050706-003	Cormorant	6/5/05	Pinellas	juvenile, multiple fractures, systematic infections

Appendix 4 (Continued)

HABB051020-030	Great egret	8/27/05	Pinellas	mature, 850 g, trauma, presumptive, resulting in coelomic hemorrhage and fracture, right ischium, subacute, moderate., ectoparasites and mites present., hemosiderosis, severe, liver, spleen, and kidney
HABB051020-031	Wood stork	8/31/05	Pasco	shaking and eyes rolling in head, septicemia, subacute, multifocal, severe resulting in necrotizing spleenitis, pneumonia, and cardiomyopathy, emaciation, severetracheitis, mild
HABB051020-032	Brown pelican	9/27/05	Pinellas	exhibited ascending paralysis
HABB051028-014	Laughing gull	8/28/05	Pinellas	juvenile
HABB051028-015	Laughing gull	8/28/05	Pinellas	juvenile
HABB051220-001	Cormorant	12/9/05	Pinellas	live sample
HABB051220-002	Cormorant	12/9/05	Pinellas	live sample

Appendix 4 (Continued)

HABB051220-003	Cormorant	12/9/05	Pinellas	live sample
HABB051220-004	Cormorant	12/9/05	Pinellas	live sample
HABB051220-005	Cormorant	12/9/05	Pinellas	live sample, had superficial hook puncture in chest
HABB051220-006	Cormorant	12/9/05	Pinellas	live sample
HABB051220-007	Cormorant	12/9/05	Pinellas	live sample but bird died later
HABB051220-008	Cormorant	12/9/05	Pinellas	live sample
HABB060112-001	Cormorant	1/11/06	Pinellas	mature adult
HABB060112-002	Cormorant	1/11/06	Pinellas	Immature juvenile
HABB060112-003	Cormorant	1/11/06	Pinellas	Immature juvenile
HABB060112-004	Cormorant	1/11/06	Pinellas	Immature juvenile
HABB060112-005	Cormorant	1/11/06	Pinellas	Immature juvenile
HABB060112-006	Cormorant	1/11/06	Pinellas	Immature juvenile
HABB060112-007	Cormorant	1/11/06	Pinellas	Immature juvenile

Appendix 4 (Continued)

HABB060220-023	Cormorant	1/13/06	Pinellas	
HABB060303-001	Cormorant	2/27/06	Pinellas	
HABB060303-006	Cormorant	1/8/05	Lee	euthanized, red tide symptoms, emaciated, GI filled with roundworm
HABB060307-001	Cormorant	8/19/05	Pinellas	juvenile female, 1560 g, red tide symptoms, convulsions, stomach full of roundworm and thread herring
HABB060322-013	Cormorant	8/17/05	Pinellas	juvenile male 1450g., malnourished, frayed feathers, roundworn
HABB060322-014	Cormorant	6/23/05	Pinellas	red tide symptoms, very thin, female juvenile 1136g upon admit

Appendix 4 (Continued)

HABB060322-015	Northern gannet	3/10/06	Pinellas	juvenile, generalized fungus infection
HABB060322-016	Brown pelican	3/21/06	Pinellas	
HABB060322-017	Cormorant	3/21/06	Pinellas	car impact, unbalanced, head weaving and hyperactive
HABB060327-001	Sanderling	2/20/05	Pinellas	found dead
HABB060327-002	Cormorant	6/18/05	Pinellas	female adult 1648g, parasites in GI and respiratory tract
HABB060409-001	sanderling	8/26/05	Pinellas	adult
HABB060409-002	Sora	3/18/05	Pinellas	
HABB060414-006	Common loon	3/21/06	Monroe	adult
HABB060414-007	Common loon	3/21/06	Monroe	adult
HABB060414-009	sanderling	10/4/05	Pinellas	
HABB060414-010	Sanderling	10/4/05	Pinellas	
HABB060414-011	Sanderling	10/4/05	Pinellas	

Appendix 4 (Continued)

HABB060414-016	Cormorant	2/23/06	Pinellas	
HABB060414-019	Royal tern	8/25/05	Pinellas	found dead, evidence of lightning strike
HABB060417-006	Cormorant	11/7/05	Pinellas	live sample
HABB060417-007	Cormorant	1/10/06	Pinellas	live sample
HABB060417-008	Cormorant	11/27/05	Pinellas	live sample
HABB060417-009	Cormorant	2/21/06	Pinellas	live sample
HABB060417-010	Cormorant	1/17/06	Pinellas	live sample
HABB060417-011	Cormorant	11/28/05	Pinellas	live sample
HABB060417-012	Cormorant	1/24/06	Pinellas	live sample
HABB060417-013	Cormorant	1/11/06	Pinellas	live sample
HABB060417-014	Cormorant	10/16/05	Pinellas	live sample
HABB060417-015	Cormorant	12/19/05	Pinellas	live sample
HABB060417-016	Cormorant	2/7/06	Pinellas	live sample

Appendix 4 (Continued)

HABB060417-017	Cormorant	1/16/06	Pinellas	live sample
HABB060417-018	Cormorant	1/3/06	Pinellas	live sample
HABB060417-019	Cormorant	2/28/06	Pinellas	live sample
HABB060417-020	Cormorant	2/28/06	Pinellas	live sample
HABB060417-021	Cormorant	11/15/05	Pinellas	live sample
HABB060417-022	Cormorant	1/15/06	Pinellas	live sample
HABB060417-023	Cormorant	12/18/05	Pinellas	live sample
HABB060417-024	Cormorant	11/26/05	Pinellas	live sample
HABB060417-025	Cormorant	11/7/05	Pinellas	live sample
HABB060417-026	Cormorant	1/1/06	Pinellas	live sample
HABB060417-027	Cormorant	1/29/06	Pinellas	live sample
HABB060417-028	Cormorant	1/7/06	Pinellas	live sample
HABB060417-029	Cormorant	1/13/06	Pinellas	live sample
HABB060417-030	Cormorant	11/22/05	Pinellas	live sample

Appendix 4 (Continued)

HABB060417-031	Cormorant	1/9/05	Pinellas	live sample
HABB060417-032	Cormorant	1/14/06	Pinellas	live sample
HABB060417-033	Cormorant	1/21/06	Pinellas	live sample
HABB060417-034	Cormorant	12/2/05	Pinellas	live sample
HABB060417-035	Cormorant	11/29/05	Pinellas	live sample
HABB060417-036	Cormorant	2/21/06	Pinellas	live sample
HABB060417-037	Cormorant	11/20/05	Pinellas	live sample
HABB060417-038	Cormorant	1/12/06	Pinellas	live sample
HABB060417-039	Cormorant	9/28/05	Pinellas	live sample
HABB060417-040	Cormorant	1/21/06	Pinellas	live sample
HABB060417-041	Cormorant	10/23/05	Pinellas	live sample
HABB060417-042	Cormorant	2/10/06	Pinellas	live sample
HABB060417-043	Cormorant	1/19/06	Pinellas	live sample
HABB060417-044	Cormorant	11/16/05	Pinellas	live sample

Appendix 4 (Continued)

HABB060417-045	Cormorant	1/29/06	Pinellas	live sample
HABB060417-046	Cormorant	1/2/06	Pinellas	live sample
HABB060417-047	Cormorant	12/16/05	Pinellas	live sample
HABB060417-048	Cormorant	1/6/06	Pinellas	live sample
HABB060417-049	Cormorant	1/24/06	Pinellas	live sample
HABB060417-050	Cormorant	1/5/06	Pinellas	live sample
HABB060417-051	Cormorant	1/2/06	Pinellas	live sample
HABB060417-052	Cormorant	1/24/06	Pinellas	live sample
HABB060417-053	Cormorant	11/8/05	Pinellas	live sample
HABB060417-054	Cormorant	1/12/06	Pinellas	live sample
HABB060417-055	Cormorant	1/7/06	Pinellas	live sample
HABB060417-056	Cormorant	1/10/06	Pinellas	live sample
HABB060417-057	Cormorant	1/31/06	Pinellas	live sample
HABB060417-058	Cormorant	1/2/06	Pinellas	live sample

Appendix 4 (Continued)

HABB060417-059	Cormorant	11/21/05	Pinellas	live sample
HABB060417-060	Cormorant	1/22/06	Pinellas	live sample
HABB060418-001	Cormorant	1/11/06	Pinellas	live sample
HABB060418-002	Cormorant	1/10/06	Pinellas	live sample
HABB060424-006	Brown pelican	7/7/05	Pinellas	live sample
HABB060428-005	Sandwich tern	7/28/05	Lee	live sample
HABB060428-006	Ruddy turnstone	10/1/05	Lee	adult, 74g
HABB060505-020	Sanderling	8/12/05	Pinellas	adult male 58g
HABB060515-001	Laughing gull	8/25/05	Pinellas	juvenile, 356g
HABB060515-002	Great blue heron	8/10/05	Pinellas	male, adult, 2125g
HABB060522-004	Green heron	7/5/05	Pinellas	female juvenile, 142g

Appendix 4 (Continued)

HABB060530-012	Northern gannet	6/17/05	Lee	male juvenile, euthanized
HABB060530-013	Royal tern	8/25/05	Pinellas	
HABB060530-014	Sanderling	9/24/05	Pinellas	
HABB060530-016	Yellow crowned night heron	5/1/06	Pinellas	
HABB060530-017	Yellow crowned night heron	5/1/06	Pinellas	
HABB060530-018	Great blue heron	5/2/06	Pinellas	
HABB060613-002	Black skimmer	7/7/05	Pinellas	euthanized, female, 227g

Appendix 5. Date, location and brief summary of *Karenia brevis* cell count data collected by FWRI.

Date	Description of detection and location of *Karenia brevis* cell counts
Oct-01	very low to low counts Sarasota south to Ft. Meyers
Nov-01	very low to medium counts St. Petersburg south to Ft. Meyers
Dec-01	very low to medium counts St. Petersburg south to Ft. Meyers
Jan-02	one patch of high counts in Tampa Bay during early part of the month with very low to medium counts south to Ft. Meyers
Feb-02	very low to medium counts along the south west Florida coast with high counts detected between Sarasota and Ft. Meyers towards the end of the month
Mar-02	not present St. Petersburg south to Sarasota, very low to medium counts Sarasota south to Ft. Meyers, not present all over by end of the month
Apr-02	low counts detected along Sarasota and in the Florida Keys several times during the month
May-02	very low counts detected between Sarasota and Ft. Meyers a few times during the month, not present all over by end of the month
Jun-02	very low counts off of St. Petersburg and Sarasota by the end of the month
Jul-02	very low counts off of St. Petersburg and Sarasota
Aug-02	very low to medium counts off of St. Petersburg and Sarasota
Sep-02	very low to high counts off of St. Petersburg and Sarasota, no presence everywhere by end of the month
Oct-02	very low counts between Sarasota and Ft. Meyers with medium counts off of St. Petersburg by the end of the month
Nov-02	very low to low counts Tarpon Springs south to Naples
Dec-02	very low to medium counts off of Sarasota and Naples

Appendix 5 (Continued)

Jan-03	very low to low counts from Naples south to Key West with high counts off of Naples and Ft. Meyers by the end of the month
Feb-03	very low to medium counts off of Ft. Meyers, Naples and in the Florida Keys
Mar-03	very low to medium counts between Sarasota and Naples with some high count patches between Ft. Meyers and Naples
Apr-03	very low to medium counts off of Sarasota south ot Ft. Meyers
May-03	very low counts detected from Tarpon Springs south to Sarasota and Ft. Meyers
Jun-03	very low to medium patches detected from Tarpon Springs south to Naples all month
Jul-03	very low to medium patches detected from tarpon Springs south to Naples all month
Aug-03	very low to medium patches detected from tarpon Springs south to Naples all month
Sep-03	very low to medium counts detected from St. Petersburg south to Sarasota
Oct-03	low counts detected from Sarasota south to Naples
Nov-03	very low counts detected from Sarasota south to Naples
Dec-03	very low counts off of Ft. Meyers
Jan-04	very low to medium counts from Tarpon Springs south to Sarasota
Feb-04	high to medium patched off of Sarasota and very low counts detected from Tarpon Springs to St. Petersburg
Mar-04	very low counts off of Tarpon Springs at beginning of month, no presence everywhere by end of month
Apr-04	very low count patchiness off of Sarasota at beginning of month, no presence anywhere by end of month
May-04	no presence

Appendix 5 (Continued)

Jun-04	no presence
Jul-04	no presence
Aug-04	no presence
Sep-04	no presence
Oct-04	very low counts detected off of Sarasota with very low to medium counts between Ft. Meyers and Naples
Nov-04	medium counts of off Ft. Meyers with medium to high count patchiness south of Naples, no presence anywhere by end of the month
Dec-04	very low to medium counts south of Naples with very low counts between Sarasota and Ft. Meyers, no presence anywhere by end of the month
Jan-05	very low to medium counts between St. Petersburg and Sarasota and off of Key West
Feb-05	low counts off of St. Petersburg, very low counts in the Florida Keys and high counts of off Sarasota
Mar-05	low to medium counts St. Petersburg south to Ft. Meyers with high count patches off of Sarasota
Apr-05	high count patches detected between St. Petersburg and Sarasota, very low to not present detected everywhere by the end of the month
May-05	medium to high counts detected St. Petersburg south to Sarasota
Jun-05	medium to high counts detected St. Petersburg south to Sarasota
Jul-05	low to medium counts from Tarpon Springs to St. Petersburg and high counts off of Sarasota
Aug-05	low to high counts detected from Tarpon Springs south to Sarasota all month
Sep-05	low to high count patches detected from Tarpon Springs south to Ft. Meyers all month

Appendix 5 (Continued)

Oct-05	high counts detected off of Sarasota with low to medium counts from Tarpon Springs south to Naples
Nov-05	very low counts off of Tarpon Springs, low to medium counts from St. Petersburg to Sarasota with a high count patch detected between Sarasota and Ft. Meyers
Dec-05	very low to medium counts from Tarpon Springs to Sarasota with a high patch off of Ft. Meyers and very low to medium counts all over the Florida Keys, no presence anywhere by the end of the month
Jan-06	medium counts off of St. Petersburg at beginning of month, very low counts off of Sarasota and Naples with no presence anywhere by end of month
Feb-06	very low counts of off St Petersburg and in the Florida Keys with no presence anywhere by the end of the month
Mar-06	no presence