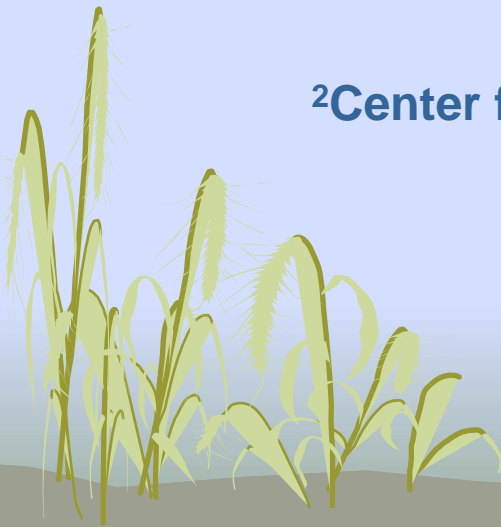


Brevetoxins in the Food Web and Relevance to Public Health

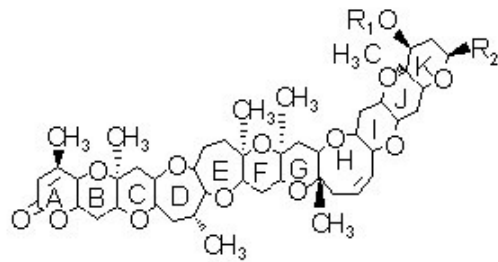
**Leanne J. Flewelling¹, Jan H. Landsberg¹,
and Jerome P. Naar²**

**¹Florida Fish & Wildlife Conservation Commission,
Florida Fish & Wildlife Research Institute, St. Petersburg, FL**

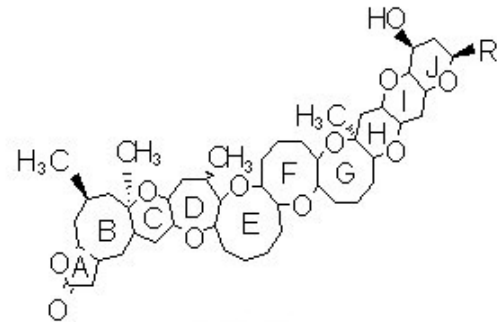
**²Center for Marine Science, University of North Carolina,
Wilmington, NC**



Brevetoxins



PbTx Type-2



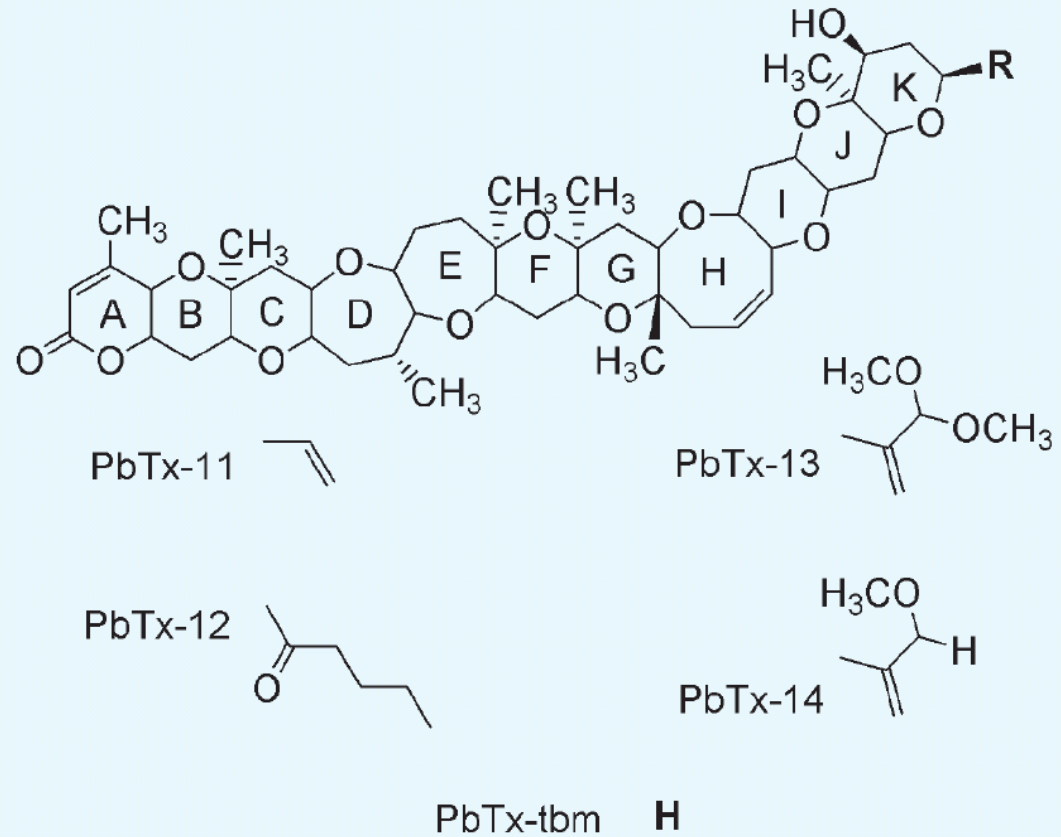
PbTx Type-1

	<u>R1</u>	<u>R2</u>		<u>R1</u>	<u>R2</u>
PbTx-2:	H	CH ₂ C(=CH ₂)CHO	PbTx-1:	H	CH ₂ CH(CH=CH ₂)CHO
PbTx-3:	H	CH ₂ C(=CH ₂)CH ₂ OH	PbTx-7:	H	CH ₂ CH(CH=CH ₂)CH ₂ OH
PbTx-5:	CH ₃ CO	CH ₂ C(=CH ₂)CHO			
PbTx-6:	H	CH ₂ C(=CH ₂)CHO			
		27,28 epoxide			
PbTx-8:	H	CH ₂ C(=CH ₂)COCH ₂ Cl			
PbTx-9:	H	CH ₂ CH(CH ₃)CH ₂ OH	PbTx-10:	H	CH ₂ CH(CH ₃)CH ₂ OH

- Family of neurotoxins produced by *Karenia brevis*, other *Karenia* spp., and some raphidophytes.
- Isolated in the 1970's (Baden and Mende 1979), structurally determined in the 1980's (Lin et al. 1981, Shimuzu et al. 1986).
- Separated into two "types" based on molecular backbone.
- Toxins were named PbTxs for *Ptychodiscus brevis* toxins in the 1980's.

Brevetoxins

- To date 14 different toxins have been isolated and fully characterized.
- PbTx-8, -13 and -14 are thought to be artifacts of extraction.
- PbTx-tbm is PbTx-2 with no side chain. Prevalent in senescent cultures.



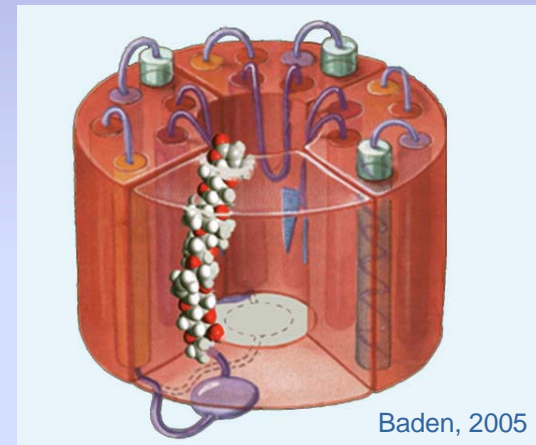
Baden, 2005

Toxicology of Brevetoxins in Mammals

Neurotoxic

Brevetoxins bind to site 5 on the α -subunit of voltage sensitive sodium channels (VSSCs).

Binding results in the persistent activation of the VSSC at normal resting potential and repetitive firing of nerves (neuronal, muscle, and heart).



Na⁺ channel α -subunit

Pulmonary effects

? Pulmonary receptor associated with a ligand-gated epithelial Na⁺ channel.

Immunosuppressant

? Cathepsin inhibition in macrophages.

Routes of Exposure

Dermal Contact

Swimming in red tides.

Effects of direct contact with toxin in the water are largely unknown.

Inhalation

Aerosolized toxins carried onshore in sea spray cause respiratory irritation.



Ingestion

Direct ingestion through drinking seawater or, for some animals, by filter-feeding.

Accumulation of toxin in the food web.

*Not restricted to times when *Karenia brevis* is present.*



Brevetoxins in Shellfish



Filter-feeding bivalves accumulate brevetoxins.

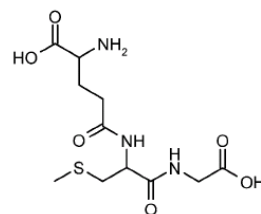
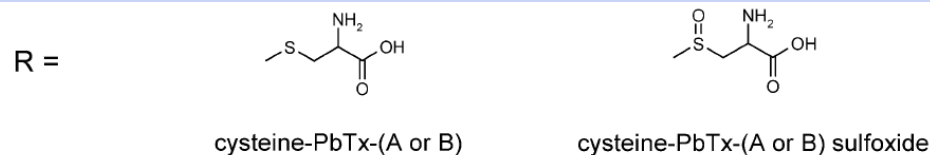
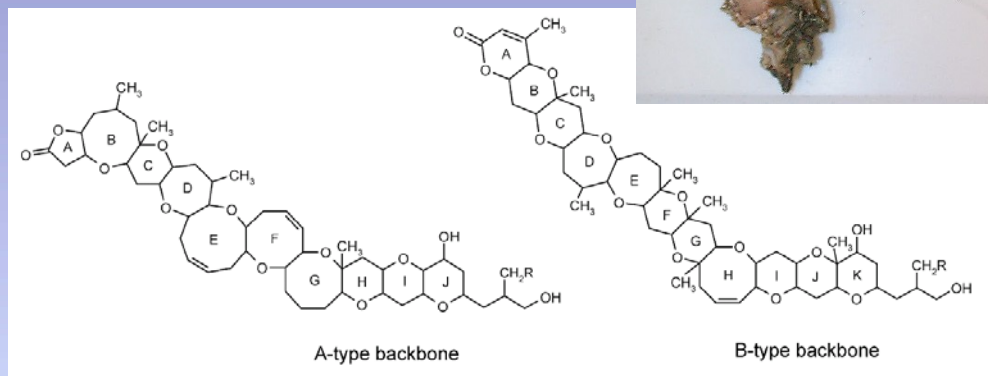
Ingestion of these shellfish leads to Neurotoxic Shellfish Poisoning (NSP).

Multiple brevetoxin derivatives (metabolites) have been isolated from toxic shellfish.

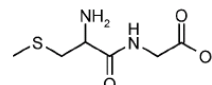
Metabolites are not produced by *K.brevis* but might be implicated in NSP.

PbTx-2, the main toxin produced by *K. brevis*, is not found in shellfish, but rather is rapidly metabolized.

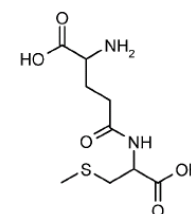
PbTx-3 is found in shellfish and is eliminated within a few weeks, largely unchanged.



glutathione-PbTx-(A or B)



cysteinylglycine-PbTx-(A or B)



γ -glutamylcysteine-PbTx-B

Fig. 1. Structures of the cysteine (and cysteine-peptide) adducts of brevetoxins as identified in the oyster by LC/MS/MS.

(Plakas et al. 2004)

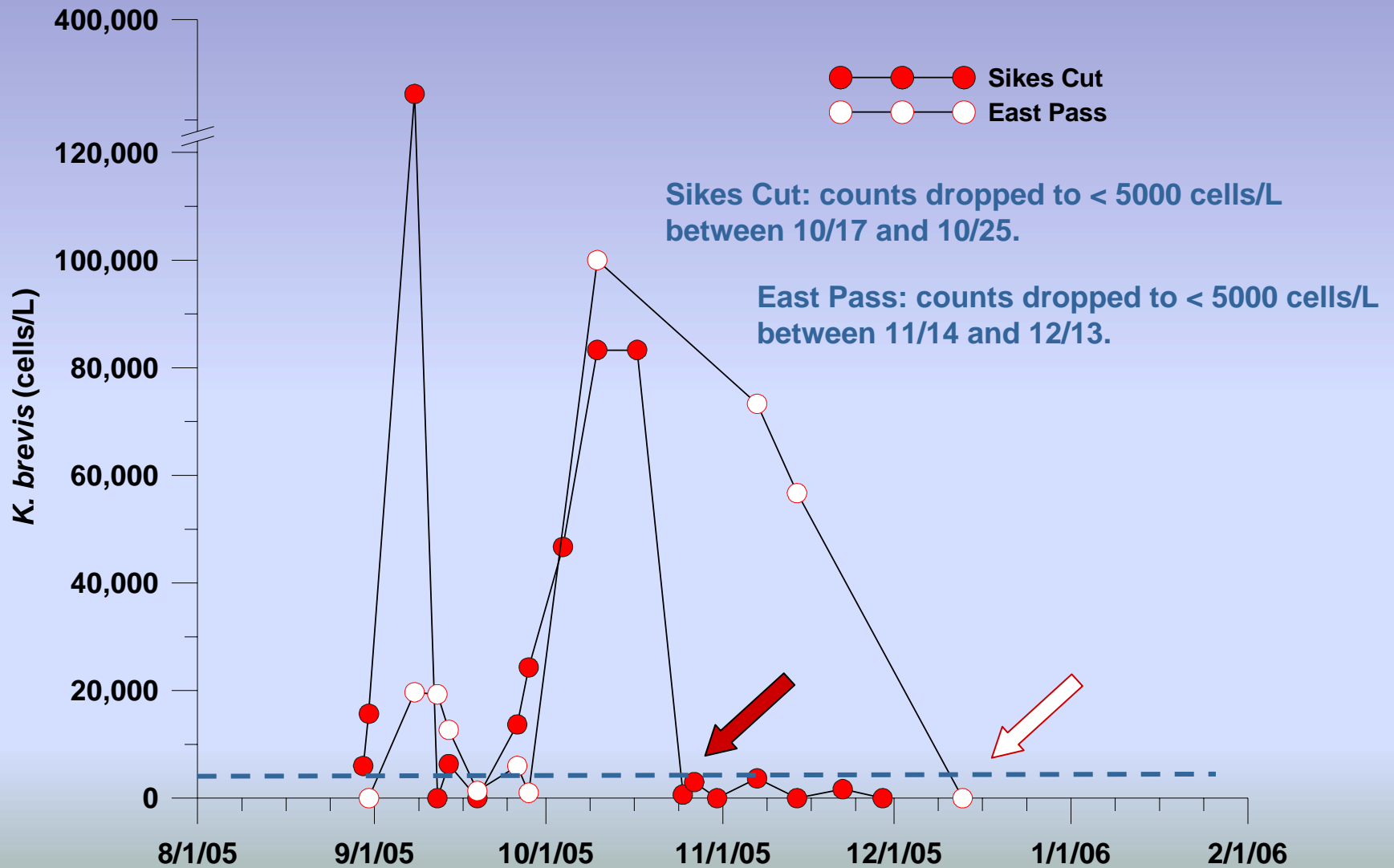
Metabolites can remain present much longer.

Apalachicola Oysters

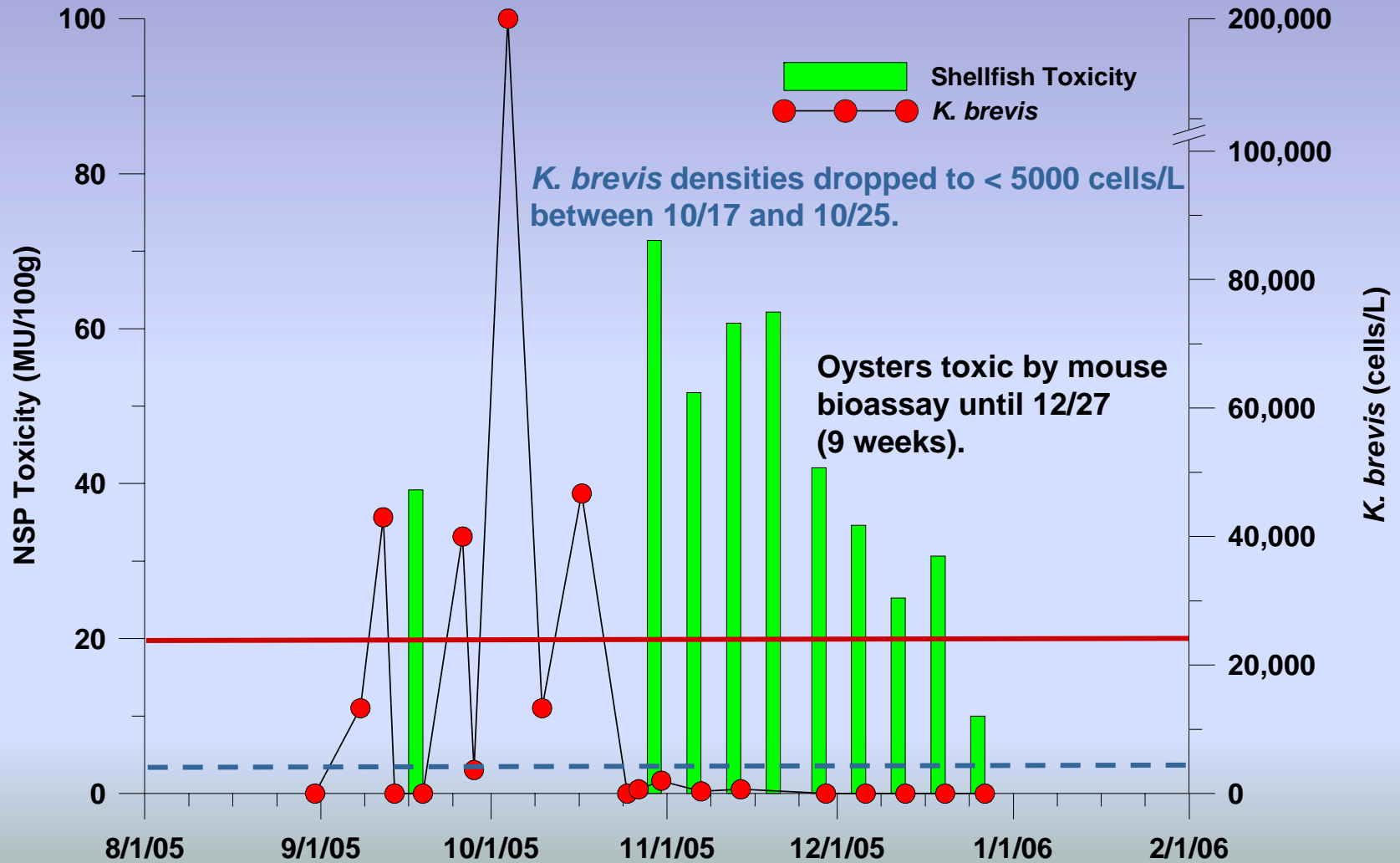


2005 Apalachicola Red Tide

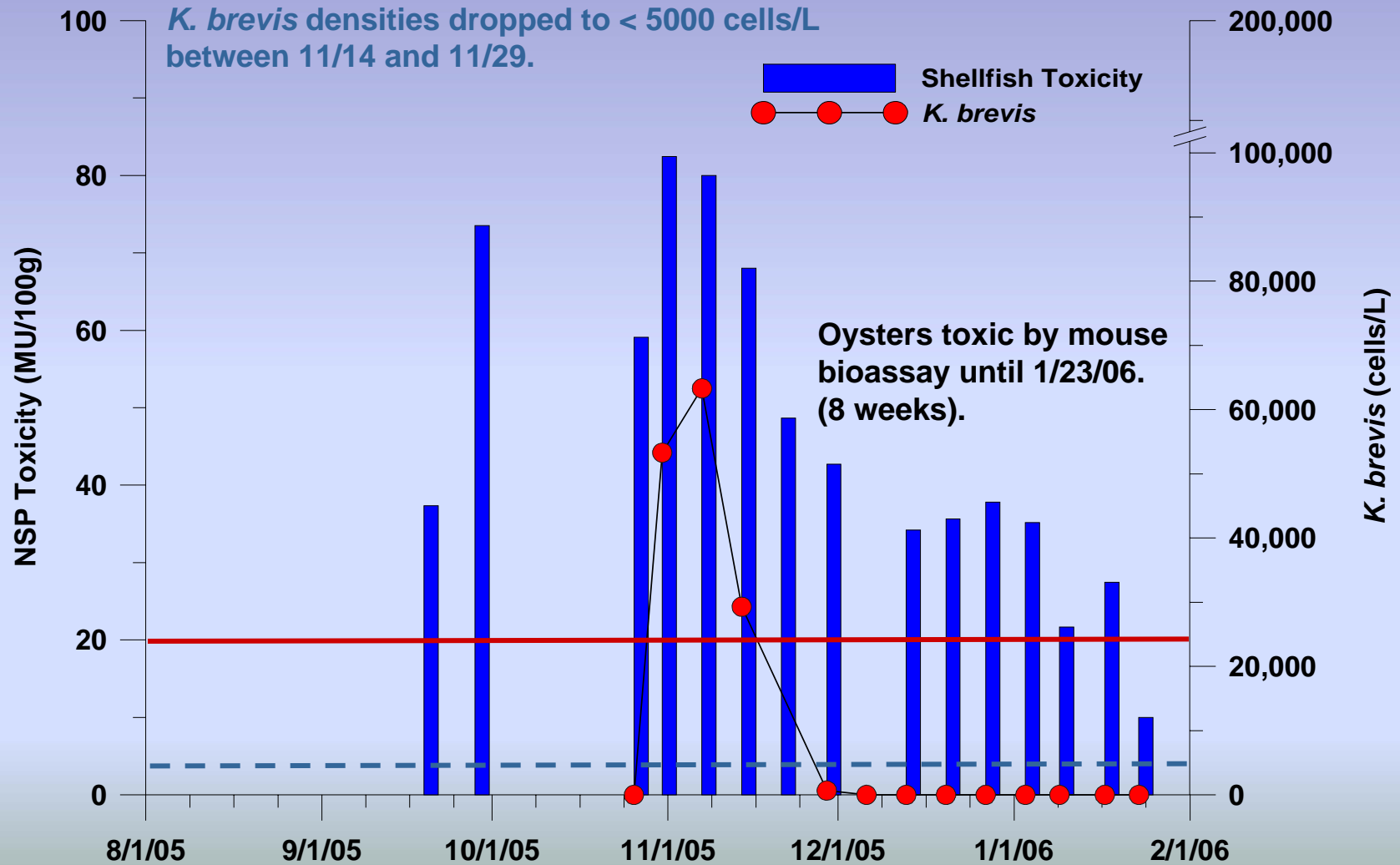
Karenia brevis cell counts



Cat Point Oysters



Platform Bar Oysters



Brevetoxin In Seafood

- **Oysters** and **clams** are the only seafood monitored for NSP in Florida.
- **Scallops** are not monitored. Scallop-related NSP does not occur for two reasons:
 1. People usually eat only the muscle which does not accumulate brevetoxin to dangerous levels.
 2. Scallops are less tolerant of brevetoxins than other bivalves. Scallop mortalities occur and recruitment is negatively impacted by *K. brevis* red tides.
- Other species of shellfish that are not monitored **can** become toxic and can potentially impact both human and wildlife health.

Smaller bivalves (e.g. **chione clams**, **coquinas**) can accumulate extremely high levels of brevetoxins.
- **Whelks** were implicated in an NSP event in 1996.
- **Conch** is suspected in a recent case currently under investigation.



What about fish?

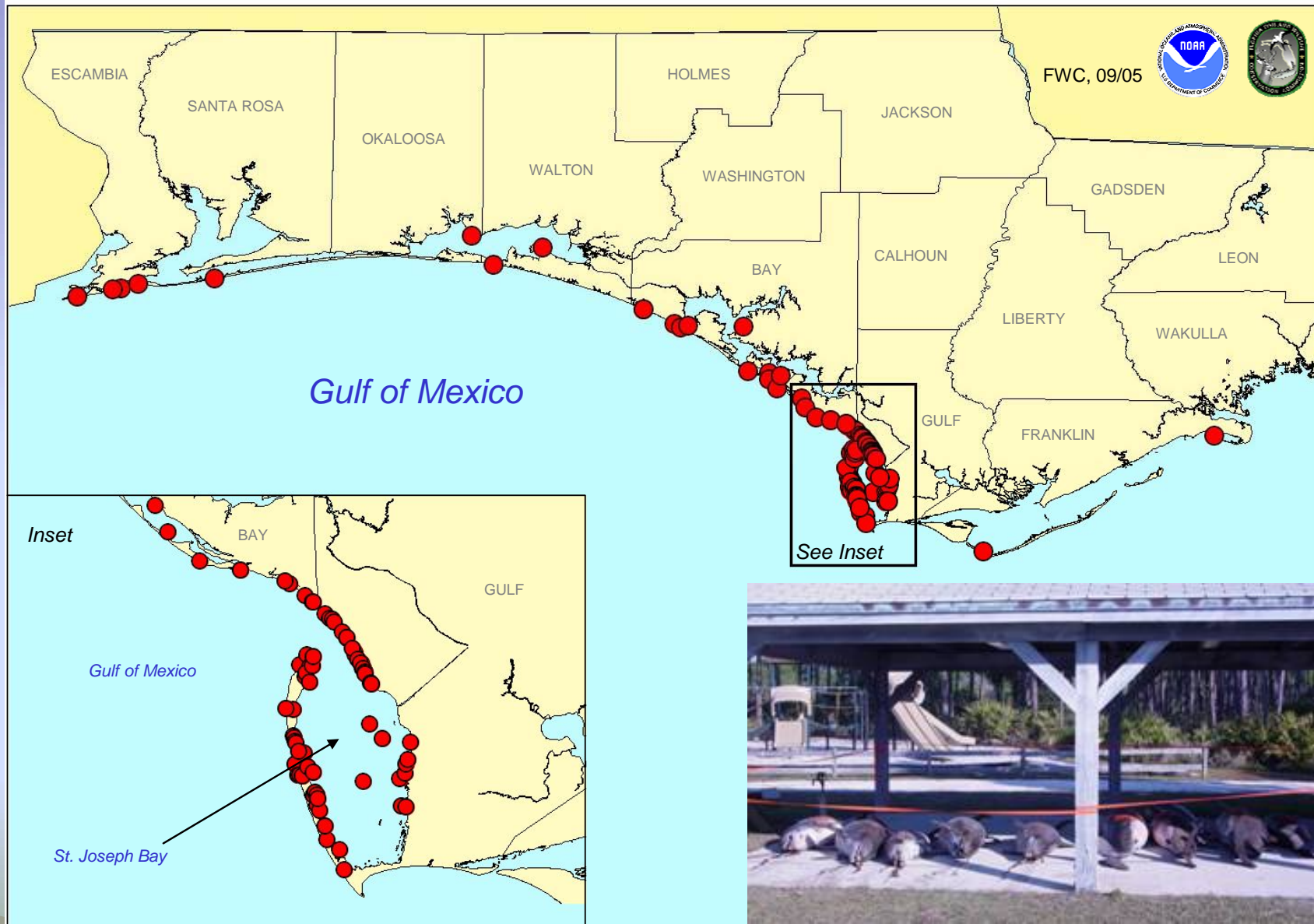
Ichthyotoxicity

- Fish kills in the Gulf of Mexico have been reported since as far back as 1844.
- Fish bioassay-guided fractionation was originally used to isolate the toxins.
- Accumulation in or food-web transfer by fish has not been regarded as a threat.

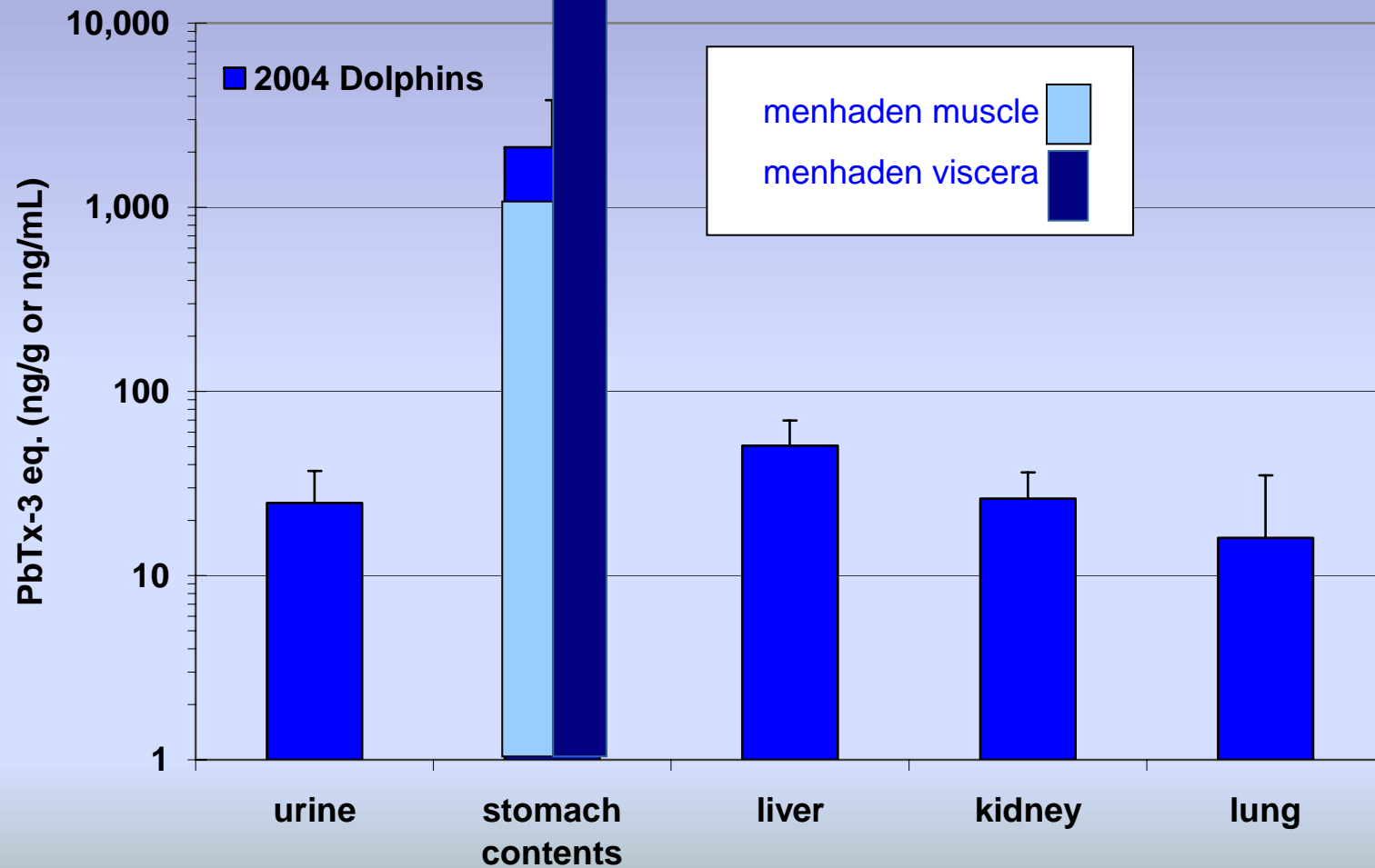


However, brevetoxin transfer through fish was hypothesized by Steidinger to explain the presence of brevetoxin found in some of the dolphins from the 1987-88 mortality as well as in some prey species.

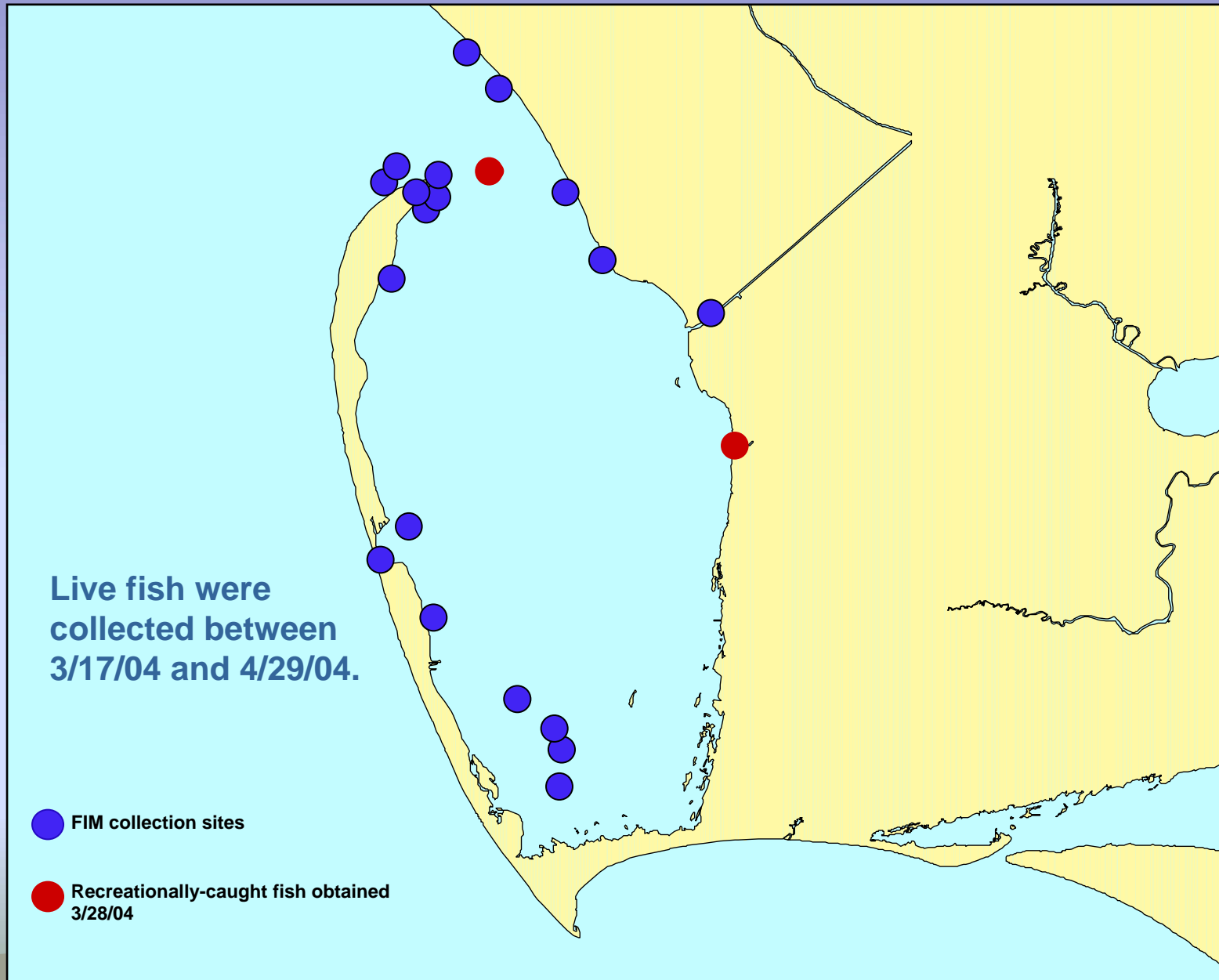
Dolphin Strandings (n=107) in the Florida Panhandle (March 10 - April 13, 2004)



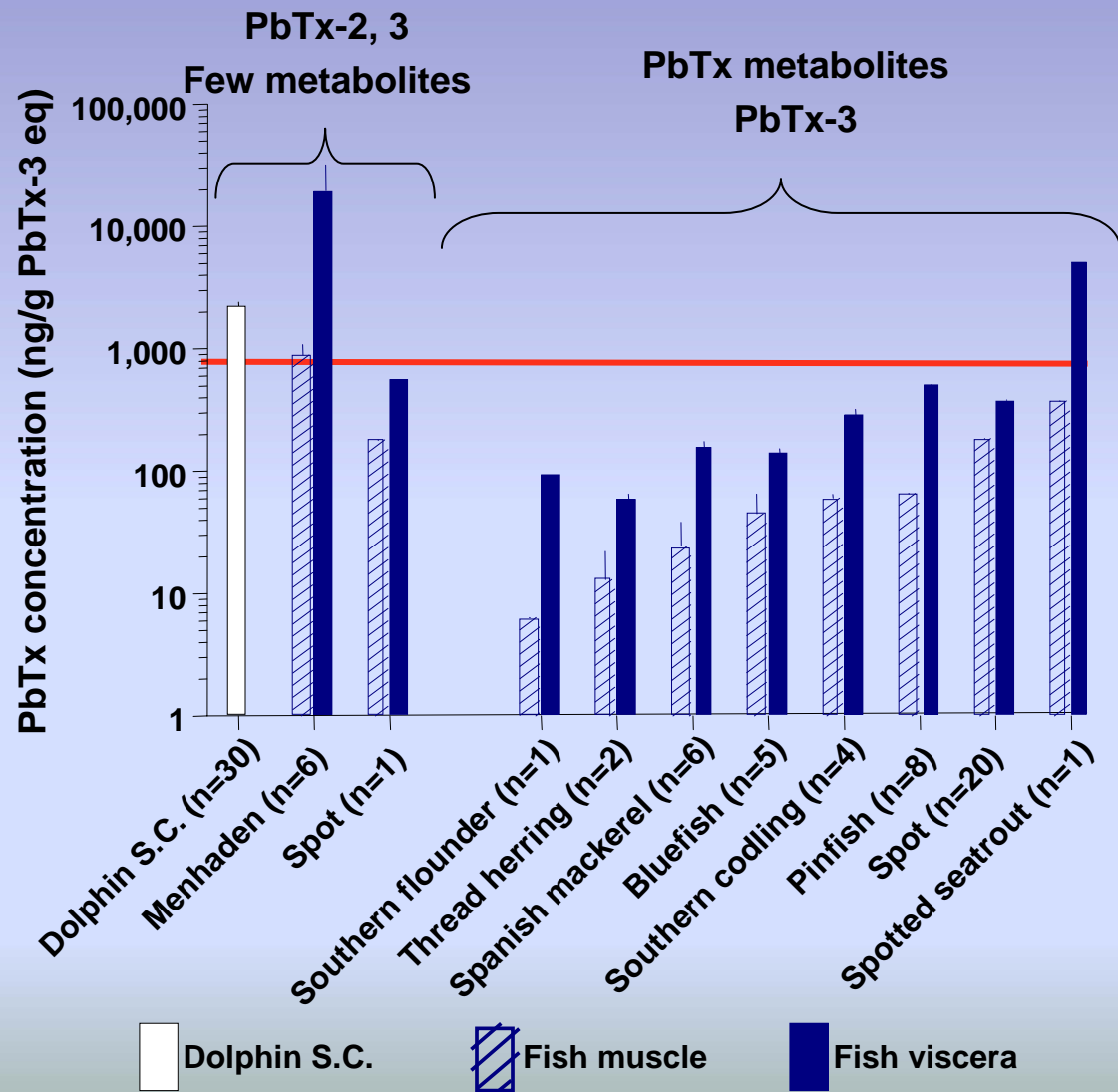
Brevetoxins In Dolphin Tissues



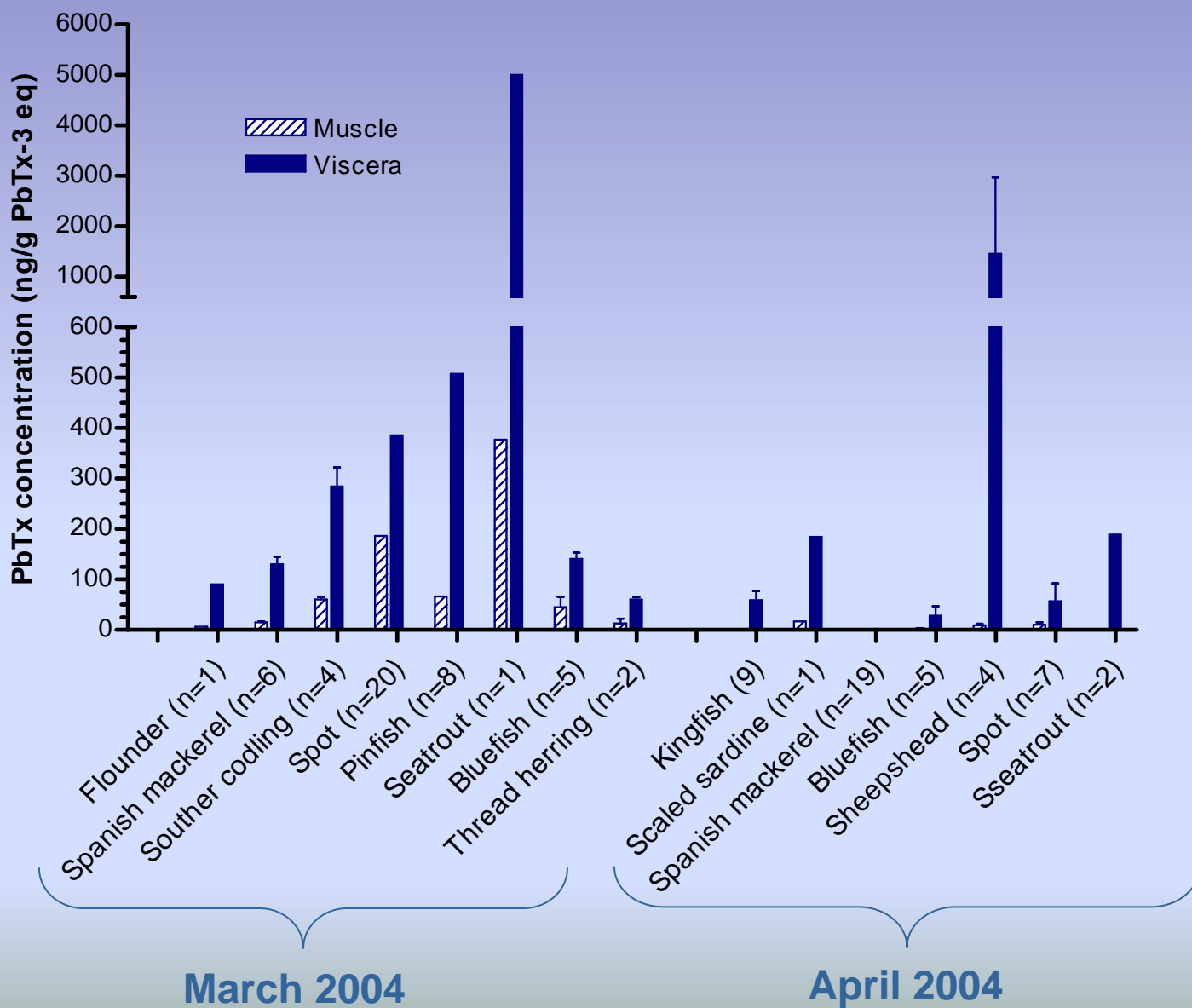
Brevetoxins in fish from St. Joseph Bay



Brevetoxins in fish from St. Joseph Bay



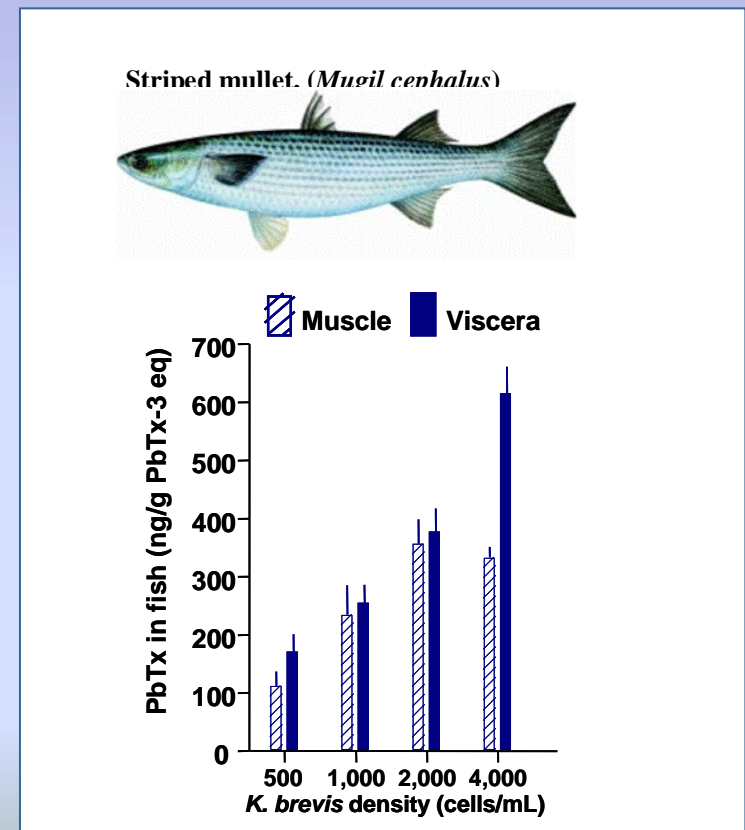
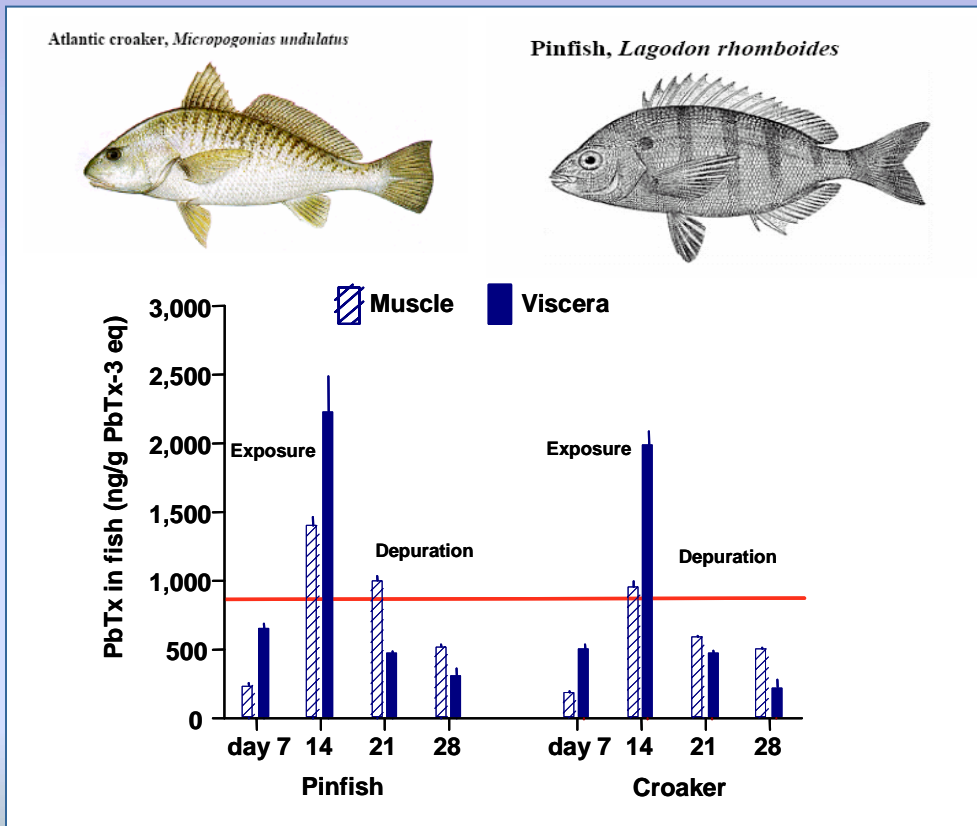
Brevetoxins In Fish From St. Joseph Bay



How Do Ichthyotoxic Compounds Accumulate in Fish?

Fish die when they are exposed to brevetoxins dissolved in seawater.

However, in a series of controlled exposure experiments, we demonstrated that fish can survive and accumulate brevetoxins when they feed on contaminated prey.



Fish can accumulate both parent brevetoxins and metabolites.

Brevetoxins in the Food Web and Relevance to Public Health

- Brevetoxins are present in multiple compartments of the aquatic food web.
- They can persist and present a health threat to both humans and aquatic animals.
- Oysters and clams are clearly a danger to humans during and after *K. brevis* blooms.
- Other species of shellfish less widely consumed can accumulate toxins as well and, on occasion, have lead to human illness.
- To date we have not found alarming levels of brevetoxins in the muscle of live fish.
- Internal organs of fish can be very toxic and should not be eaten.
- Chronic low-level exposure to brevetoxins and metabolites through shellfish and fish can occur, and the effects are not known.

Acknowledgements

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UNCW Center for Marine Science

FWC/FWRI Fisheries Independent Monitoring

Florida Department of Environmental Protection

Florida Department of Agriculture and Consumer Services, Division of Aquaculture