

Published in final edited form as:

*Harmful Algae*. 2010 September 1; 9(6): 620–635. doi:10.1016/j.hal.2010.05.002.

## Cyanobacterial Blooms and the Occurrence of the neurotoxin beta-N-methylamino-L-alanine (BMAA) in South Florida Aquatic Food Webs

Larry E. Brand<sup>1,\*</sup>, John Pablo<sup>2</sup>, Angela Compton<sup>1</sup>, Neil Hammerschlag<sup>1</sup>, and Deborah C. Mash<sup>2</sup>

<sup>1</sup>Division of Marine Biology and Fisheries and NSF/NIEHS Oceans and Human Health Center, Rosenstiel School of Marine and Atmospheric Science

<sup>2</sup>Department of Neurology, Miller School of Medicine University of Miami Miami, FL

### Abstract

Recent studies demonstrate that most cyanobacteria produce the neurotoxin beta-N-methylamino-L-alanine (BMAA) and that it can biomagnify in at least one terrestrial food chain. BMAA has been implicated as a significant environmental risk in the development of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS). We examined several blooms of cyanobacteria in South Florida, and the BMAA content of resident animals, including species used as human food. A wide range of BMAA concentrations were found, ranging from below assay detection limits to approximately 7000 µg/g, a concentration associated with a potential long-term human health hazard.

### Keywords

BMAA; cyanobacteria; Florida; harmful algal blooms; neurodegenerative disease; toxin

## 1. INTRODUCTION

There is a general consensus that Harmful Algal Blooms (HABs) are increasing worldwide (Smayda, 1990, Hallegraeff, 1993, Anderson et al., 2002, Glibert et al., 2005). This is primarily the result of an increasing human population generating increasing nutrient runoff from fertilizer, animal waste, sewage, and soil erosion (Nixon, 1995; Richardson and Jorgensen, 1996; Moffat, 1998; Heisler et al., 2008; Anderson et al., 2002, 2008). The increased mobilization of nutrients has led to the eutrophication, first of small water bodies such as ponds, lakes and rivers (Vollenweider, 1992a); then of estuaries such as Chesapeake Bay (Cooper and Brush, 1991; Harding and Perry, 1997); and more recently of large seas such as the Black Sea (Bodeanu, 1992; Mee, 1992; Cociasu et al., 1996), Baltic Sea (Larsson et al., 1985; Nehring, 1992), and Adriatic Sea (Vollenweider et al., 1992b; Justic et al., 1995); and of continental

© 2010 Elsevier B.V. All rights reserved.

\*Corresponding author. Tel.: +1-305-421-4138; fax: +1-305-421-4600 LBrand@rsmas.miami.edu .

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

shelf areas such as the Mississippi River delta (Turner and Rabalais, 1991, 1994; Justic et al., 1995).

Blooms of cyanobacteria appear to also be increasing worldwide in response to increased nutrient inputs (Chorus and Bartram, 1999; Paerl, 2008). Cyanobacteria have the ability to produce a wide array of secondary metabolites (Moore, 1996; Welker and von Dohren, 2006; Sivonen and Borner, 2008), many of which are noxious or toxic to animals and/or humans (Carmichael and Falconer, 1993; Chorus and Bartram, 1999; Carmichael, 2001; Carmichael et al., 2001; Ibelings et al., 2008; Falconer, 2008; Pilotto, 2008; Stewart et al., 2008). Many of these toxins are dermatotoxins, hepatotoxins, or neurotoxins, but only a few species of cyanobacteria are known to produce each of these toxins. Recently Cox et al. (2005) have produced laboratory results that suggest that virtually all cyanobacteria species produce the neurotoxin beta-N-methylamino-L-alanine (BMAA). The data demonstrate a 500-fold variation in the amount of BMAA produced among the cyanobacteria species examined, but how much of this variation is genetic and how much is environmental-physiological is not known at the present time. Metcalf et al. (2008) and Esterling and Downing (2008) have also presented data suggesting that most cyanobacteria produce BMAA in the environment.

In vitro studies have shown that BMAA is toxic to neurons at concentrations as low as 10-30 nM (Weiss and Choi, 1988; Weiss et al., 1989; Rao et al., 2006; Lobner et al., 2007). BMAA is structurally similar to glutamate and binds to glutamate receptors (Richter and Mena, 1989; Copani et al., 1990; Smith and Meldrum, 1990; Allen et al., 1995; Mash, 2008). Abnormal stimulation of these receptors may play a role in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (Takahashi et al., 1997). BMAA has also been shown to be neurotoxic in a variety of animal models (Spencer et al., 1987; Karamyan and Speth, 2008).

BMAA, as an amino acid, is water soluble, not lipid soluble, and this has a low octanol-water partition coefficient (Yunger and Cramer, 1981). It therefore would not be expected to biomagnify up the food chain (Mackay, 1982; Connolly and Pedersen, 1988; Arnot and Gobar, 2006; Kelly et al., 2007). However, unlike the more moderate bioaccumulation observed with most water soluble cyanobacteria toxins (Xie et al., 2005; Ibelings and Chorus, 2007; Ibelings and Havens, 2008; Funari and Testai, 2008), Cox et al. (2003), Banack and Cox (2003), Murch et al. (2004), and Banack et al. (2006) have demonstrated a 10,000-fold biomagnification of free BMAA and 50-fold biomagnification of total BMAA in a food chain in Guam from symbiotic cyanobacteria to cycads to fruit bats (Pteropids, also known as "flying foxes"). Cox and Sacks (2002) hypothesized that the increased consumption of these fruit bats by one ethnic group of people in Guam, the Chamorros, in the 1940s led to a 100-fold increase in the development of Amyotrophic Lateral Sclerosis (ALS)-Parkinsonism dementia complex in the Chamorros living in Guam. The observation of high concentrations of BMAA in the autopsied brains of Chamorros who died of these neurodegenerative diseases and the absence of BMAA in the age-matched non-neurological control brains of Canadians who died of other causes suggested a possible link between BMAA and neurodegenerative diseases (Murch et al., 2004). Recently Pablo et al. (2009) have demonstrated high concentrations of BMAA in Americans who died of Alzheimer's disease or ALS, but little or no BMAA in the brains of age-matched non-neurological controls, or in cases of Huntington's disease, a genetic disorder. These data suggest that the unusual Guam situation is not unique, and that BMAA may biomagnify in other food chains, enter the human diet, and potentially trigger neurodegenerative disease.

Together, these recent studies suggest that BMAA from cyanobacteria could be involved in neurodegenerative diseases. As BMAA is not lipophilic, it would not be expected to biomagnify, yet the indirect evidence suggests that it can biomagnify in at least certain

circumstances. To determine if BMAA is present and can biomagnify in aquatic food chains, BMAA concentrations were analyzed in animals collected from water bodies in South Florida (Fig. 1) known to have blooms of cyanobacteria.

## 2. METHODS

### 2.1 Sampling methods and sites

Water samples were collected on a roughly monthly basis at numerous stations in Florida Bay (Fig. 2), Biscayne Bay (Fig. 3), and the Caloosahatchee River (Fig. 4). Sample tissue to be analyzed for BMAA content were collected from various frozen animals that had been collected for a variety of other research projects in these water bodies.

### 2.2 Cyanobacteria abundance

Cyanobacteria abundance in water samples was estimated by measuring concentrations of phycocyanin. Water samples were filtered through GF/F glass fiber filters and the filters were frozen at  $-20^{\circ}\text{C}$  until extracted. These filters were extracted overnight with a phosphate buffer (0.05M  $\text{H}_2\text{KPO}_4$ , 0.05M  $\text{HK}_2\text{PO}_4$ ; pH 6.5; with 0.01% (v/v) mercaptoethanol added) at  $5^{\circ}\text{C}$  and analyzed for phycocyanin with a SPEX Fluorolog-3 spectrofluorometer, using 580 nm excitation and 640 nm emission. The spectrofluorometer was calibrated using pure phycocyanin.

### 2.3 Water flow and nutrients

Water flow and nutrient data were obtained from the hydrometeorological and water quality databases of the South Florida Water Management District and the Lee County Environmental Laboratory.

### 2.4 Extraction and measurement of BMAA

Quantitation of BMAA was performed on frozen, unfixed tissues by a modification of previously validated methods (Cox et al., 2003; Banack and Cox, 2003). All tissue samples were analyzed and assayed in duplicate. Briefly, frozen-pulverized liquid nitrogen submerged (Model# 36903-10, Cole-Parmer, Quebec, Canada) tissues (1:10 w/v) were hydrolyzed overnight in 6 M HCl (1:10 w/v) at  $110^{\circ}\text{C}$ . Particulate matter was removed in 500  $\mu\text{l}$  aliquots by ultrafiltration (Ultrafree-MC, Millipore) at  $15,800 \times g$ . Two hundred microliters of the extract was vacuum centrifuged in a speed-vac (Thermo-Savant SC250DDA Speed Vac Plus with a Savant refrigerator trap RVT 4104). The lyophilized residue was resuspended in 200  $\mu\text{l}$  0.1 M trichloroacetic acid (TCA) then washed with 100  $\mu\text{l}$  of chloroform for extraction of any residual lipids. The aqueous layer was then transferred to a fresh tube and the chloroform layer was discarded. Samples (5  $\mu\text{l}$ ) and standards were derivatized with 6-aminoquinolyl-N-hydrosuccinimidyl carbamate (AQC) using the AccQ-Fluor reagent (Waters Corp, Millford, MA; WAT052880). BMAA was separated from the protein amino acids by reverse-phase elution (Waters Nova-Pak C18 column,  $3.9 \times 300$  mm using 140 mM sodium acetate, 5.6 mM triethylamine, pH 5.7 (mobile phase A), and 58% acetonitrile in water (mobile phase B) at 1 ml per min at  $37^{\circ}\text{C}$ . The elution gradient (30 min) was as follows: time 0 = 75% A; 2 min = 75% A curve 6; 17 min = 63% A curve 7; 18.5 min = 100% B curve 6; 23.5 min = 100% B curve 6; 25 min = 75% A curve 6; 30 min = 75% A. Samples were run in duplicate followed by an AQC blank (containing borate buffer and AQC tag) to ensure that there was no carryover between samples. BMAA was quantified by detection of the AQC fluorescent tag (Waters 2475 Multi  $\lambda$ -Fluorescence Detector) with excitation at 250 nm and emission at 395 nm. Experimental samples were compared to standard spiked similar matrix negative for endogenous BMAA, containing commercial (Sigma B-107; >95% purity) or authentic standards of BMAA (99% purity). The percentage of recovery of BMAA was >94%.

Identification of a BMAA peak detected by reverse phase HPLC was verified by LC/MS/MS using product ion mode in a triple quadrupole system. The frozen (unfixed) sample was hydrolyzed for 18 hours in 6N HCL at 110°C (Fountoulakis et al., 1998) and then dried to remove HCl in a Thermo-Savant SC250DDA Speed Vac Plus (Waltham, MA). The sample was reconstituted in dilute HCl (20 mM) and derivatized with AQC, which increased the molecular weight of the BMAA analyte from 118 to 458. Individual compounds were eluted from the column with a gradient elution of 140 mM sodium acetate buffer, 5.6 mM triethylamine, pH 5.7 (mobile phase A) and 52% acetonitrile (mobile phase B) with a flow rate of 1.0 ml/min. Separation was achieved by liquid chromatography (Thermo model Surveyor LC, San Jose, CA) using a Thermo Hypersil GOLD 100 × 2.1 mm, 3 μm particle column at 0.28 ml/min using the eluents of 0.1% formic acid (v/v) in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B) with the following gradient: time 0 = 100% A, 2 min = 100% A, 16 min = 80% A, 20 min = 2% A, 22 min = 2% A, 24 min = 81% B, 26 min = 81% A, 27 min = 100% A, 31 min = 100% A. Nitrogen gas was supplied to the H-ESI probe (heated electrospray ionization) with a nebulizing pressure of 25 psi and a vaporizing temperature of 300°C. The mass spectrometer was operated in the positive electrospray ionization (ESI) mode under the following conditions: nebulizing pressure of 25 psi, vaporizing temperature of 300°C, capillary temperature set at 280°C, capillary offset of 35, tube lens offset of 94, source collision energy of 5v. The protonated molecular ion of double derivatized BMAA (m/z 459) was used as the precursor ion for SRM (single reaction monitoring) analysis. Three transitions were monitored: 459 to 119 (collision energy of 19v), 459 to 171 (collision energy of 32v), 459 to 289 (collision energy of 16v). The ratios of these three product ions were compared to the ratios of the product ions created by injection of AQC-derivatized pure BMAA standard (synthesized and then triple crystallized by Peter Nunn, University of Portsmouth, U.K.) into the triple quadrupole LC/MS/MS under the same conditions.

## 2.5 Measurement of Glutamate

The effects of BMAA are purported to affect glutamate-associated mechanisms (Rao et al., 2006; Lobner et al, 2007). To validate the methods used for amino acid hydrolysis/extraction, we quantified the area ratio of glutamate (rt = 2.47 min) compared to an internal standard (istd) norleucine (rt = 18.91 min.) using standard curves of the specific matrix taken from various species. Briefly, increasing amounts (0-250 ng/injection) of glutamate along with one concentration (50ng/injection) of istd were spiked into 0.1 mg tissue/injection and analyzed by HPLC as per methods similarly described for BMAA.

## 3. RESULTS

### 3.1 BMAA Extraction-Quantitation Method Validation

Using HPLC we were able to detect significant concentrations of BMAA in various marine samples taken from various locations of the South Florida marine ecosystems (see Fig. 5 for a representative chromatogram). Initial assays using BMAA-free matrix spiked with increasing amounts of L-BMAA were conducted to determine if there were any differences in the lower limits of detection (LOD) and lower limits of quantitation (LOQ) for the extraction/instrumental method on the various matrices assayed. Results from these assays demonstrated an increasing threshold for detection in matrix dependent on origin (buffer < mollusc < teleost fish < crustacean), and a recovery range of 94-98% (Fig. 6). The average linear dynamic range (1-250 ng) for BMAA spiked standard curves in matrix (Grey snapper, *Lutjanus griseus*, muscle) gave an  $R^2$  value of 0.998 ( $P < 0.0001$ ). The presence of BMAA from marine samples found to have positive HPLC results were confirmed using triple quadrupole LC/MS/MS (Fig. 7). The application of triple quadrupole LC/MS/MS has four checks to verify the identity of the BMAA peak: (i) a specific single parent mass [molecular weight (MW): 459] is selected in the first quadrupole with all other masses excluded from the second quadrupole; (ii) the

column retention time of the peak is unique; (iii) collision-induced dissociation of product ions in the second quadrupole must be all detected in the third quadrupole; (iv) the product ion ratios must match repetitive runs of the standard injected at a similar concentration within 5% variation. All four of these LC/MS/MS checks were verified with ratios of the product ions (MW: 289, 171, 119) of BMAA matching the daughter ion ratios of the derivatized standard (Fig. 7).

### 3.2 Measurement of Glutamate

HPLC analysis of glutamate was conducted on select marine samples (mollusc (1 sample), crustacean (2 samples), and teleost fish (2 samples)) as an internal control and reference value for comparison to BMAA concentrations. To determine the significance of the BMAA concentrations, averages of glutamate and BMAA concentrations were converted to mole units and the calculated ratios gave a range of 18-45 for the measured glutamate to BMAA concentrations (Table 1). Glutamate measured in these samples were in good agreement with previous estimates (Dunn et al., 1949), providing validation for the amino acid extraction methods described here.

### 3.3 Florida Bay

A large bloom of cyanobacteria developed in north central Florida Bay in the 1980s, which has persisted ever since (Brand, 2002). Although there are no quantitative data before 1989, many fishers and other boaters who were frequently in Florida Bay were quoted by DeMaria (1996) as observing algal blooms and water “discoloration” beginning in 1981 and increasing thereafter. They stated “In 1981, the water got dirtier, the blooms grew, and the seagrass started dying.” and “From 1981 to 1986, the decline [of the bay] was gradual. In 1987, the bay started to collapse quickly.”

The bloom is located primarily in northcentral Florida Bay and is primarily cyanobacteria (Fig. 8). It occurs in an area where high phosphorus in the west (Fig. 9) mixes with high nitrogen in the east (Fig. 10). It has been hypothesized that the phosphorus in western Florida Bay is from natural phosphorite deposits tens of meters below being transported by geothermal circulation to the surface (Top et al., 2001; Brand, 2002). Phosphorus is low in eastern Florida Bay because there are no phosphorite deposits in that area and the calcium carbonate that is the primary mineral of the Florida peninsula chemically scavenges phosphate from seawater (DeKanel and Morse, 1978; Kitano et al., 1978). This occurs in both groundwater flowing through limestone as well as shallow surface water in which calcareous sediments are resuspended. The nitrogen in eastern Florida Bay, on the other hand, is from the decomposition of nitrogen-rich organic peat in the northern Everglades as a result of the drainage of the Everglades and conversion into agricultural fields (now called the Everglades Agricultural Area), primarily sugar cane (Brand, 2002). Figure 11 shows the high nitrogen and phosphorus in this water in the north and the gradual decline to the south. Plants scavenge phosphorus from the water as it flows to the south through the Everglades, but they cannot scavenge all of the nitrogen because of the initial high N:P ratio (Brand, 2002). Diversion of this nitrogen-rich water in the 1980s into eastern Florida Bay (Fig. 12) led to the blooms of cyanobacteria developing in northcentral Florida Bay where nitrogen from the east meets phosphorus from the west (Brand, 2002). Because of the highly seasonal nature of the rainfall and runoff in South Florida, cyanobacteria abundance is low during the dry season and high during the wet season (Fig. 13). Florida Bay is a major nursery area for shrimp, and critical to the Florida shrimping industry. Samples of pink shrimp in Florida Bay have high concentrations of BMAA (Table 2). Grey snapper collected south of the bloom have either no or only moderate concentrations of BMAA (Table 1).



### 3.4 South Biscayne Bay-Eastern Florida Bay

South Biscayne Bay-Eastern Florida Bay normally has extremely low concentrations of phosphorus (Fig. 14A) because of calcium carbonate scavenging (Brand, 2002), and low abundance of cyanobacteria (Fig. 14C). In the summer of 2005, large areas of mangroves in this area were destroyed in a road widening project (Fig. 3), and the organic material was buried in the surrounding sediments of South Biscayne Bay-Eastern Florida Bay. The subsequent anaerobic conditions and acidification of the calcium carbonate sediments released large amounts of phosphorus (Figs. 14B, 15). As phosphorus was the limiting nutrient in this area, the additional phosphorus led to large blooms of cyanobacteria (Figs. 14D, 15) that persisted for approximately two years. Samples of fish and crustaceans collected just north of this area in the spring and summer of 2007 (Fig. 3) as the bloom was on the decline show some species with no BMAA and others with very high concentrations (Table 3).

### 3.5 Caloosahatchee River

Some of the nutrient rich water from the Everglades Agricultural Area is pumped into Lake Okeechobee and then to the west down the Caloosahatchee River (Fig. 1, Brand, 2002). Because of the phosphorite deposits, waters along the western side of the Florida peninsula tend to be rich in phosphorus and thus nitrogen limited (Brand, 2002). High concentrations of nitrogen flow out of Lake Okeechobee and down the Caloosahatchee River (Fig. 16) and large blooms of cyanobacteria develop (Fig. 17). Moderate amounts of BMAA were found in molluscs and high concentrations were found in fish in the river (Table 4).

## 4. DISCUSSION

Because the animal samples were collected for other research projects unrelated to our research on cyanobacteria blooms and the BMAA hypothesis, the spatial and temporal patterns of animal samples are not optimal for examining the hypothesized link between cyanobacteria blooms and BMAA accumulation in animals. Nevertheless, these initial results indicate that high concentrations of BMAA can accumulate in some aquatic animals in areas of cyanobacteria blooms. Not enough samples have been analyzed to discern any clear patterns, but some possibilities do appear.

In south Biscayne Bay, there was large variation in the BMAA concentrations between individuals of a species in pink shrimp, blue crabs, mojarra, band tail puffers, least puffers, and bluestriped grunts (Table 3). The replicability of our methods suggest that these differences are real and not the result of methodological problems. The cause of this variation is unknown but several possibilities exist. As these animals were collected from an area north of the bloom, depending on their migration patterns, some animals may have spent considerable time in the bloom (or the organisms in their diet did so) while others may have spent little or no time in the bloom. While our research focussed on planktonic cyanobacteria, benthic cyanobacteria and cyanobacteria epiphytic on seagrass and macroalgal blades are also present. In these shallow waters, microalgal abundance per square meter is typically around 20 times higher on the sediment surface than in the water column above (Brand and Suzuki, 1999). Microhabitat differences in the relative abundance of these different groups of cyanobacteria and their BMAA content might account for the differences observed. Genetic differences between individuals in their ability to accumulate BMAA are also a possibility (discussed below), just as there are genetic differences among humans in their susceptibility to develop neurodegenerative diseases. Whatever the cause of this variation, it must be kept in mind when examining data on only a few individuals of a species.

Many crustaceans appear to have high concentrations of BMAA. Pink shrimp in Florida Bay (Table 2) have high concentrations of BMAA, comparable to those found in the fruit bats of

Guam (3556 µg/g; Cox et al., 2003). Pink shrimp and blue crabs in south Biscayne Bay have a wide range of concentrations (Table 3). All three species of fish from the Caloosahatchee River (Table 4) have high concentrations of BMAA similar to that of the fruit bats of Guam. The filter feeding molluscs in the Caloosahatchee River and estuary have only moderate amounts of BMAA (Table 4). Grey snapper in Florida Bay south of its cyanobacteria bloom have moderate amounts of BMAA (Table 2), while grey snapper in Biscayne Bay north of its cyanobacteria bloom have none (Table 3).

In south Biscayne Bay, a wide range of BMAA concentrations was observed among the 17 species examined. A perusal of the data does not reveal a classical biomagnification pattern, as top carnivores such as barracuda and grey snapper show no BMAA, while species near the bottom of the food chain, such as pink shrimp, pufferfish, and sea bream have relatively high concentrations. A pattern that does appear, however, is a tendency for higher concentrations of BMAA in species that feed on the benthos, and lower concentrations in species that feed on the plankton. An exception to this pattern is pinfish, which primarily feed on benthic vegetation, but had no BMAA. No BMAA was found in anchovies, silversides, needlefish, and barracuda that feed in the water column, while high concentrations were found in pink shrimp, blue crabs, pufferfish, and cowfish that feed on the bottom. This could be an indication that benthic cyanobacteria produce more BMAA than planktonic species, but we have no direct information on this. A perusal of the data of Cox et al. (2005) does suggest that such a pattern may exist.

At the present time, we do not know why cyanobacteria produce BMAA and thus, what environmental patterns may exist. Similarly, we do not know the mechanism of bioaccumulation of this amino acid. Because BMAA is water soluble, the standard mechanism for biomagnification of lipophilic compounds is not plausible. One possibility is differential uptake and excretion. Because BMAA is structurally similar to glutamate in the presence of bicarbonate as a carbamate (Weiss et al., 1988), neutral acid uptake enzymes in the gastrointestinal tract and elsewhere may take up BMAA from dietary sources, but enzymes involved in the turnover, degradation, and excretion of amino acids may not be able to recognize BMAA and eliminate it. A differential in uptake vs. excretion enzymes may be one possible mechanism for the large differences observed between individuals and species in this study.

Ultimately there are many sources of variation that could account for the results shown here. The laboratory data of Cox et al. (2005) have shown a 500-fold variation in the amount of BMAA produced by cyanobacteria. Much of this is likely the result of genetic differences between species, but some is likely due to physiological differences in response to environmental differences. As a result, one would expect microhabitat variation among benthic and epiphytic cyanobacteria, and differences between planktonic and benthic communities. As BMAA propagates up the food chain, differential uptake and excretion will cause varying amounts of biomagnification. The age of animals could be important, with older animals accumulating more BMAA. The time course of BMAA exposure over the lifetime of an animal could be important, depending on the ability of an animal to slowly or quickly excrete BMAA after short term exposure. While it is clear that most cyanobacteria produce BMAA, we cannot, at this time, be sure that there are no other sources of BMAA such as heterotrophic bacteria.

Based upon this study and other data, it appears that the situation in Guam is not unique, and that high concentrations of BMAA do occur in parts of aquatic food webs. To enhance the chances of finding BMAA in aquatic animals, this study focussed on areas of unnatural blooms of cyanobacteria caused by nutrient enrichment by human activities. Cyanobacteria are of course a significant part of natural aquatic habitats unaffected by human activities. Cyanobacteria are approximately 50% of the phytoplankton community of the open ocean covering over half of the earth (Li et al., 1983; Itturiaga and Mitchell, 1986). It is plausible that humans have been exposed to some level of BMAA throughout their evolutionary history. The

increase in cyanobacteria blooms as a result of human activities is probably increasing this exposure, but by how much is not yet known. Our research is now expanding from this initial exploratory analysis to determine the distribution of BMAA in aquatic food webs and seafood in both natural ecosystems and eutrophic ecosystems.

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS) are also increasing (Kokmen, et al., 1993; Gauthier, 1997; Chio, 2005). Increased longevity alone may not account for all of this increase. Heritability of these diseases is low, accounting for less than 10% of cases. While certain genetic factors are known to influence susceptibility to these diseases, some unknown environmental factors probably play a major role. BMAA may be a significant environmental factor. Cyanobacteria and the presence of BMAA in portions of aquatic food webs used as human food could be a significant human health hazard.

## 5. CONCLUSIONS

These data on BMAA concentrations in animals in South Florida waters indicate that the situation in Guam is not unique. These data further suggest that BMAA could be found in high concentrations in aquatic animals in many areas of the world where cyanobacteria blooms occur. This possibility includes not only natural water bodies, but also nutrient rich habitats such as aquaculture ponds and flooded rice fields that have high concentrations of cyanobacteria (Paerl and Tucker, 1995; Massout, 1999; Kankaanpaa et al., 2005; Zimba, 2008). Agricultural soils can also have high concentrations of cyanobacteria (Ramsey and Ball, 1983; Shimmel and Darley, 1985; Whitton, 2000).

As an increasing human population on the earth increases the flux of sewage, agricultural fertilizer, animal wastes, and eroded soil into various aquatic habitats, it is predicted that blooms of cyanobacteria will increase. It is predicted that human exposure to cyanobacteria and BMAA will increase, leading to a possible increased incidence of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS).

## Acknowledgments

We would like to acknowledge John Cassani, Maria Criales, John Lamkin, and Aswani Voley for some of the animal samples used in this study, and Margaret Basile for help in the laboratory. Nutrient and water flow data were obtained from the South Florida Water Management District and Lee County Environmental Laboratory. We thank Lora Fleming for reviewing an earlier draft of this paper. We would like to acknowledge the Cove Point Foundation, National Science Foundation (#OCE0742285 and #OCE0432368) and National Institute of Environmental Health Sciences (#1 P50 ES12736) for their financial support of this research. Partial support for this study was provided by gifts in kind from the Institute for Ethnomedicine.

## REFERENCES

- Allen CN, Omelchenko I, Ross SM, Spencer P. The neurotoxin beta-N-methylamino-L-alanine (BMAA) interacts with the strychnine-insensitive glycine modulatory site of the N-methyl-D-aspartate receptor. *Neuropharm* 1995;34:651–658.
- Anderson DM, Glibert PM, Burkholder JM. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 2002;25:704–726.
- Anderson DM, et al. Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. *Harmful Algae* 2008;8:39–53. [PubMed: 19956363]
- Arnot JA, Gobas FAPC. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Env. Rev* 2006;14:257–297.
- Banack SA, Cox PA. Biomagnification of cycad neurotoxins in flying foxes. *Neurol* 2003;61:387–389.
- Banack SA, Murch SJ, Cox PA. Neurotoxic flying foxes as dietary items for the Chamorro people, Marianas Islands. *J Ethnopharm* 2006;106:97–104.



- Bodeanu, N. Algal blooms and the development of the main phytoplanktonic species at the Romanian Black Sea littoral in conditions of intensification of the eutrophication process. In: Vollenweider, RA.; Marchetti, R.; Viviani, R., editors. *Marine Coastal Eutrophication*. Elsevier; Amsterdam: 1992. p. 891-906.
- Brand, LE. The transport of terrestrial nutrients to South Florida coastal waters. In: Porter, JW.; Porter, KG., editors. *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys*. CRC Press; Boca Raton, Florida: 2002. p. 353-406.
- Brand, LE.; Suzuki, M. Distribution of benthic chlorophyll in Florida Bay sediments. 1999 Florida Bay and Adjacent Marine Systems Science Conference; Key Largo, FL. Nov. 1999; 1999. p. 129
- Carmichael WW. Health effects of toxin producing cyanobacteria: The cyanoHABS. *Human Ecol. Risk Assess* 2001;7:1393-1407.
- Carmichael, WW.; Falconer, IR. Diseases related to freshwater blue-green algal toxins, and control measures. In: Falconer, IR., editor. *Algal Toxins in Seafood and Drinking Water*. Academic Press; 1993. p. 187-209.
- Carmichael WW, Azevedo MFO, An JS, Molica RJR, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Egelsham GK. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. *Env. Health Persp* 2001;109:663-668.
- Chio A. Mortality trends in ALS: an increasingly intricate puzzle. *The Lancet Neurol* 2005;4:453-454.
- Chorus, I.; Bartram, J. *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*. E&FN Spon; London: 1999.
- Cociasu A, Dorogan L, Humborg C, Popa L. Long-term ecological changes in Romanian coastal waters of the Black Sea. *Mar. Poll. Bull* 1996;32:32-38.
- Connolly JP, Pedersen CJ. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Env. Sci. Tech* 1988;22:99-103.
- Cooper SR, Brush GS. Long-term history of Chesapeake Bay anoxia. *Science* 1991;254:992-995. [PubMed: 17731522]
- Copani A, Canonico PL, Nicoletti F. Beta-N-methylamino-L-alanine (L-BMAA) is a potent agonist of "metabotropic" glutamate receptors. *Eur. J. Pharmacol* 1990;181:327-328.
- Cox PA, Sacks OW. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease in Guam. *Neurology* 2002;58:956-9. [PubMed: 11914415]
- Cox PA, Banack SA, Murch SJ. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proc. Natl. Acad. Sci. USA* 2003;100:13380-13383. [PubMed: 14612559]
- Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B. Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid. *Proc. Natl. Acad. Sci. USA* 2005;102:5074-5078. [PubMed: 15809446]
- DeKanel J, Morse JW. The chemistry of orthophosphate uptake from seawater onto calcite and aragonite. *Geochim. Cosmochim. Acta* 1978;42:1335-1340.
- DeMaria, K. Changes in the Florida Keys Marine Ecosystem based upon Interviews with Experienced Residents. *The Nature Conservancy and Center for Marine Conservation*; 1996. p. 105
- Dunn MS, Camien MN, Eiduson S, Malin RB. The nutritive value of canned foods. I. Amino acid content of fish and meat products. *J. Nutr* 1949;39:177-185. [PubMed: 15392424]
- Esterhuizen M, Downing TG.  $\beta$ -N-methylamino-L alanine (BMAA) in novel South African cyanobacterial isolates. *Ecotox. Env. Safety* 2008;71:309-313.
- Falconer, IR. Health effects associated with controlled exposures to cyanobacterial toxins. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 607-612. p. 950
- Fountoulakis M, Lahm H-W. Hydrolysis and amino acid composition analysis of proteins. *J Chromatogr* 1998;826:109-134.
- Funari E, Testai E. Human health risk assessment related to cyanotoxins exposure. *Crit. Rev. Tox* 2008;38:97-125.
- Gauthier S, Panisset M, Nalbantoglu J, Poirier J. Alzheimer's disease: current knowledge, management and research. *Can. Med. Assoc. J* 1997;157:1047-1052. [PubMed: 9347775]

- Glibert PM, Seitzinger S, Heil CA, Burkholder JM, Parrow MW, Codispoti LA, Kelly V. The role of eutrophication in the global proliferation of harmful algal blooms. *Oceanogr* 2005;18:198–209.
- Hallegraeff GM. A review of harmful algal blooms and their apparent global increase. *Phycologia* 1993;32:79–99.
- Harding LW, Perry ES. Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. *Mar. Ecol. Prog. Ser* 1997;157:39–52.
- Heisler J, et al. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* 2008;8:3–13.
- Ibelings BW, Chorus I. Accumulation of cyanobacterial toxins in freshwater “seafood” and its consequences for public health: A review. *Env. Poll* 2007;150:177–192.
- Ibelings, BW.; Havens, KE. Cyanobacterial toxins: A qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 675-732.p. 950
- Ibelings, BW.; Havens, K.; Codd, GA.; Dyble, J.; Landsberg, J.; Coveney, M.; Fournie, JW.; Hilborn, ED. Ecosystem effects workgroup report. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 655-674.p. 950
- Itturiaga R, Mitchell BG. Chroococcoid cyanobacteria: A significant component in the food web dynamics of the open ocean. *Mar. Ecol. Progr. Ser* 1986;28:291–297.
- Justic D, Rabalais NN, Turner RE, Dortch Q. Changes in nutrient structure of river-dominated coastal waters: Stoichiometric nutrient balance and its consequences. *Est. Coast. Shelf Sci* 1995;40:339–356.
- Kankaanpaa HT, Holliday J, Schroder H, Goddard TJ, von Fister R, Carmichael WW. Cyanobacteria and prawn farming in northern New South Wales, Australia – a case study on cyanobacteria diversity and hepatotoxin bioaccumulation. *Tox. Appl. Pharm* 2005;203:243–256.
- Karamyan VT, Speth RC. Animal models of BMAA neurotoxicity: A critical review. *Life Sci* 2008;82:233–246. [PubMed: 18191417]
- Kelly BC, Ikonou MG, Blair JD, Morin AE, Gobas FAPC. Food web-specific biomagnifications of persistent organic pollutants. *Science* 2007;317:236–238. [PubMed: 17626882]
- Kitano Y, Okumura M, Idogaki M. Uptake of phosphate ions by calcium carbonate. *Geochem. J* 1978;12:29–37.
- Kokmen E, Beard CM, O’Bien PC, Offord KP, Kurland LT. Is the incidence of dementing illness changing? *Neurol* 1993;43:1887–1892.
- Larsson U, Elmgren R, Wulff F. Eutrophication and the Baltic Sea: Causes and Consequences. *Ambio* 1985;14:9–14.
- Li WK, Rao DV, Harrison WG, Smith JC, Cullen JJ, Irwin B, Platt T. Autotrophic picoplankton in the tropical ocean. *Science* 1983;219:292–295. [PubMed: 17798278]
- Lobner D, Piana PM, Salous AK, Peoples RW.  $\beta$ -NMethylamino-L-alanine enhances neurotoxicity through multiple mechanisms. *Neurobiol. Dis* 2007;25:360–366. [PubMed: 17098435]
- Mackay D. Correlation of bioconcentration factors. *Env. Sci. Tech* 1982;16:274–278.
- Mash DC. Cyanobacterial toxins in neurodegeneration. *Cont. Life Learn. Neurol* 2008;14:138–149.
- Massout, L. Cyanobacteria management in aquaculture ponds: A review. In: Charpy, L.; Larkum, AWD., editors. *Marine Cyanobacteria. Bulletin de L’Institut Oceanographique* 19, Monaco Musee Oceanographique; 1999. p. 579-584.
- Mee LD. The Black Sea in Crisis: A need for concerted international action. *Ambio* 1992;21:278–285.
- Metcalf JS, Banack SA, Lindsay J, Morrison LF, Cox PA, Codd GA. Co-occurrence of  $\beta$ -N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990-2004. *Env. Microbiol* 2008;10:702–708. [PubMed: 18237305]
- Moffat AS. Global nitrogen overload problem grows critical. *Science* 1998;279:988–989.
- Moore RE. Cyclic peptides and depsipeptides from cyanobacteria: A review. *J. Ind. Microbiol* 1996;16:134–143. [PubMed: 8730577]
- Murch SJ, Cox PA, Banack SA. A mechanism for slow release of biomagnified cyanobacterial neurotoxins and neurodegenerative disease in Guam. *Proc. Nat. Acad. Sci* 2004;101:12228–11131. [PubMed: 15295100]

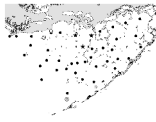
- Murch SJ, Cox PA, et al. Occurrence of beta-methylamino-l-alanine (BMAA) in ALS/PDC patients from Guam. *Acta Neurol. Scand* 2004;110:267–269. [PubMed: 15355492]
- Nehring, D. Eutrophication in the Baltic Sea. In: Vollenweider, RA.; Marchetti, R.; Viviani, R., editors. *Marine Coastal Eutrophication*. Elsevier; Amsterdam: 1992. p. 673-682.
- Nixon SW. Coastal eutrophication: A definition, social causes, and future concerns. *Ophelia* 1995;41:199–220.
- Pablo J, Banack SA, Cox PA, Johnson TE, Papapetropoulos S, Bradley WG, Buck A, Mash DC. Cyanobacterial neurotoxin BMAA in ALS and Alzheimer disease. *Acta Neurol. Scand.* 2009 DOI 10.1111/J.1600-0404.2008.01150.x.
- Paerl, H. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater – marine continuum. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 217-237.p. 950
- Paerl HW, Tucker CS. Ecology of blue-green algae in aquaculture ponds. *J. World Aq. Soc* 1995;26:109–131.
- Pilotto, LS. Epidemiology of cyanobacteria and their toxins. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 639-649.p. 950
- Ramsay AJ, Ball KT. Estimation of algae in New Zealand pasture soil and litter by culturing and by chlorophyll a extraction. *N. Z. J. Sci* 1983;26:493–503.
- Rao SD, Banack SA, Cox PA, Weiss JH. BMAA selectively injures motor neurons via AMPA/kainate receptor activation. *Exp. Neurol* 2006;201:244–252. [PubMed: 16764863]
- Richardson, K.; Jorgensen, BB. Eutrophication in Coastal Marine Ecosystems. *Coastal and Estuarine Studies*. Vol. Vol. 52. American Geophysical Union; 1996. Eutrophication: Definition, history and effects; p. 1-19.
- Richter KE, Mena EE. L-beta-methylaminoalanine inhibits [3H]glutamate binding in the presence of bicarbonate ions. *Brain Res* 1989;492:385–588. [PubMed: 2568879]
- Smith SE, Meldrum BS. Receptor site specificity for the acute effects of beta-N-methylamino-alanine in mice. *Eur. J. Pharmacol* 1990;187:131–134. [PubMed: 1980247]
- Shimmel KM, Darley WM. Productivity and density of soil algae in an agricultural system. *Ecology* 1985;66:1439–1447.
- Sivonen, K.; Borner, T. Bioactive compounds produced by cyanobacteria. In: Herrero, A.; Flores, E., editors. *The Cyanobacteria: Molecular Biology, Genomics and Evolution*. Caister Academic Press; 2008. p. 159-197.p. 484
- Smayda, TJ. Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Graneli, E.; Sundstrom, B.; Edler, L.; Anderson, DM., editors. *Toxic Marine Phytoplankton*. Elsevier Science; 1990. p. 29-40.
- Spencer PS, Nunn PB, Hugon J, Ludolph AC, Ross SM, Roy DN, Robertson RC. Guam amyotrophic lateral sclerosis-Parkinsonism-dementia linked to a plant excitant neurotoxin. *Science* 1987;2237:517–522. [PubMed: 3603037]
- Stewart, I.; Seawright, AA.; Shaw, GR. Cyanobacterial poisoning in livestock, wild mammals and birds – an overview. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 613-637.p. 950
- Takahashi M, Billups B, Rossi D, Sarantis M, Hamann M, Attwell D. The role of glutamate transporters in glutamate homeostasis in the brain. *J. Exp. Biol* 1997;200:401–409. [PubMed: 9050249]
- Top Z, Brand LE, Corbett RD, Burnett W, Chanton J. Helium as a tracer of groundwater input into Florida Bay. *J. Coastal Res* 2001;17:859–868.
- Turner RE, Rabalais NN. Changes in Mississippi River water quality this century: Implications for coastal food webs. *Bioscience* 1991;41:140–147.
- Turner RE, Rabalais NN. Coastal eutrophication near the Mississippi river delta. *Nature* 1994;368:619–621.
- Vollenweider, RA. Coastal marine eutrophication: Principles and control. In: Vollenweider, RA.; Marchetti, R.; Viviani, R., editors. *Marine Coastal Eutrophication*. Elsevier; Amsterdam: 1992a. p. 1-20.

- Vollenweider, RA. Eutrophication, structure and dynamics of a marine coastal system: results of a ten-year monitoring along the Emilia-Romagna coast (Northwest Adriatic Sea). In: Vollenweider, RA.; Marchetti, R.; Viviani, R., editors. *Marine Coastal Eutrophication*. Elsevier; Amsterdam: 1992b. p. 63-106.
- Weiss JH, Choi DW.  $\beta$ -N-methylamino-L-alanine neurotoxicity: requirement for bicarbonate as a cofactor. *Science* 1988;241:973–975. [PubMed: 3136549]
- Weiss JH, Kaoh JY, Choi DW. Neurotoxicity of beta-N-methylamino-L-alanine (BMAA) and beta-N-oxalylamino-L-alanine (BOAA) on cultured cortical neuron. *Brain Res* 1989;497:64–71. [PubMed: 2551452]
- Welker M, von Dohren H. Cyanobacterial peptides – Nature’s own combinatorial biosynthesis. *FEMS Microbiol. Rev* 2006;30:530–563. [PubMed: 16774586]
- Whitton, BA. Soils and rice fields. In: Whitton, BA.; Potts, M., editors. *The Ecology of Cyanobacteria*. Kluwer Academic Publishers; 2000. p. 233-255.p. 669
- Xie L, Xie P, Guo L, Li L, Miyabara Y, Park H-D. Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. *Env. Tox* 2005;20:293–300.
- Yunger LM, Cramer RD. Measurement and correlation of partition coefficients of polar amino acids. *Mole. Pharm* 1981;20:602–608.
- Zimba, PV. ARS research on harmful algal blooms in SE USA aquaculture impoundments. In: Hudnell, HK., editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer; 2008. p. 577-578.p. 950

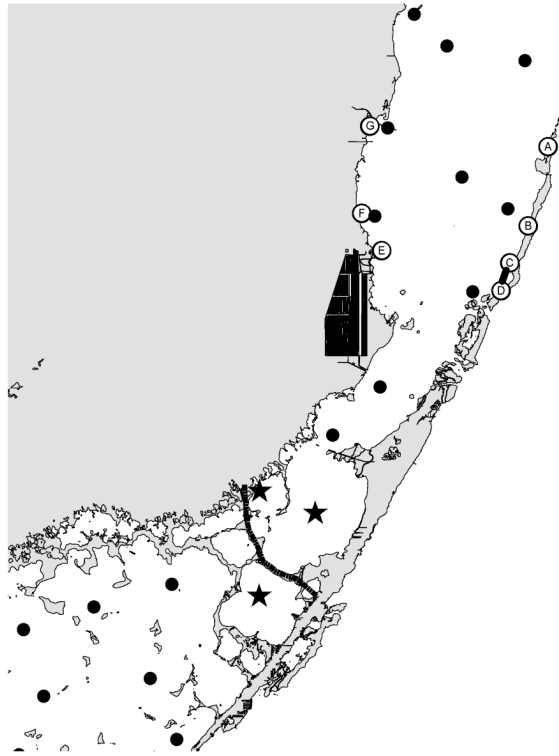


**Figure 1.**  
Map of major water bodies in South Florida. Lines of parallel bars indicate general watershed boundaries of the Everglades.

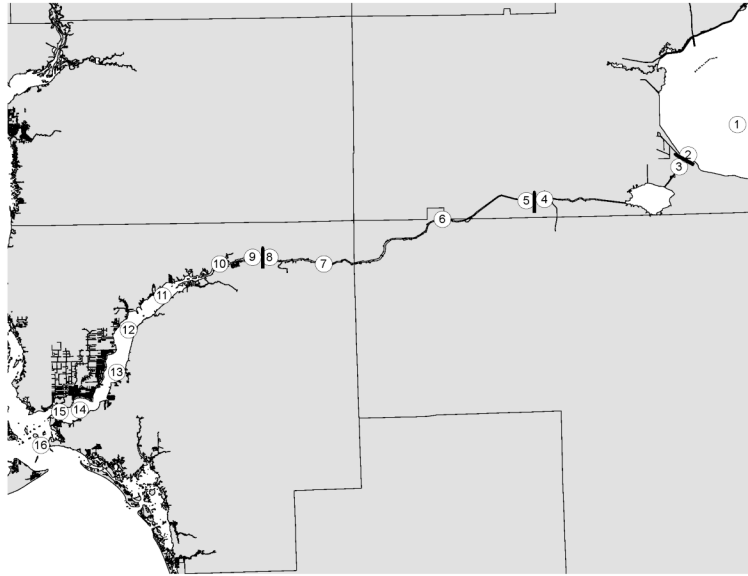




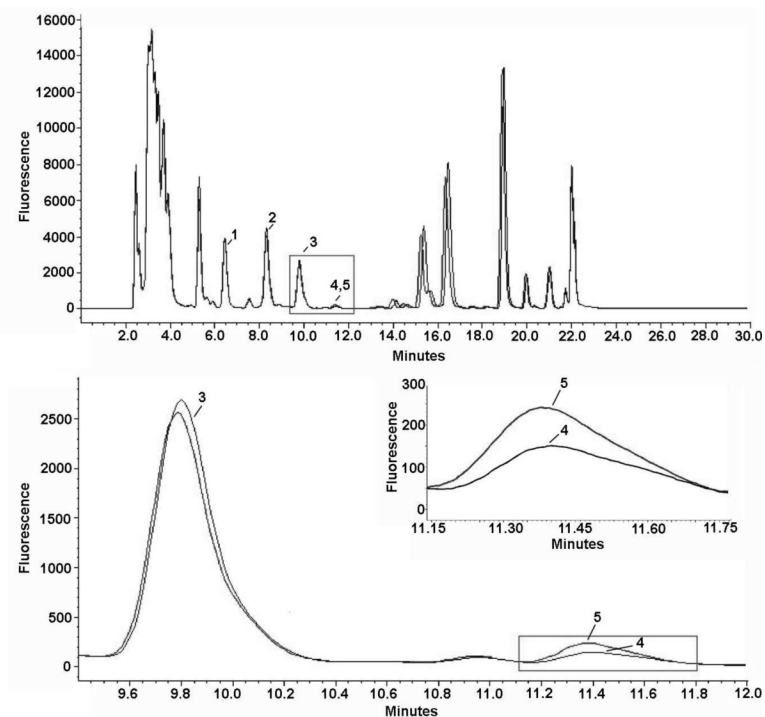
**Figure 2.**  
Location of sampling stations in Florida Bay. Dots are water samples. Stars are water samples used in Figure 13. Letters are where animals were collected.



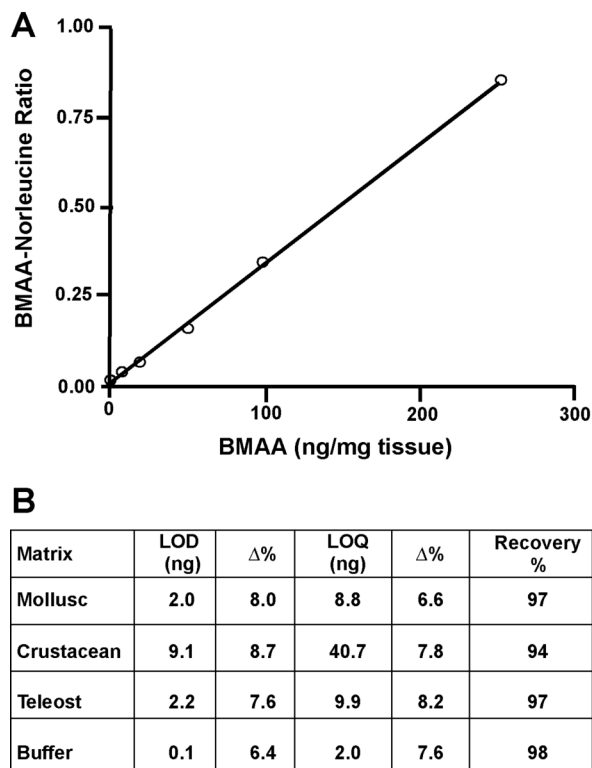
**Figure 3.** Location of sampling stations in eastern Florida Bay-south Biscayne Bay. Dots are water samples. Stars are water samples used in Figure 15. Letters are where animals were collected. Parallel bars show area where mangrove trees were destroyed.



**Figure 4.** Location of sampling stations along the Caloosahatchee River. Circles are water sampling stations. Bars are dams on the river.



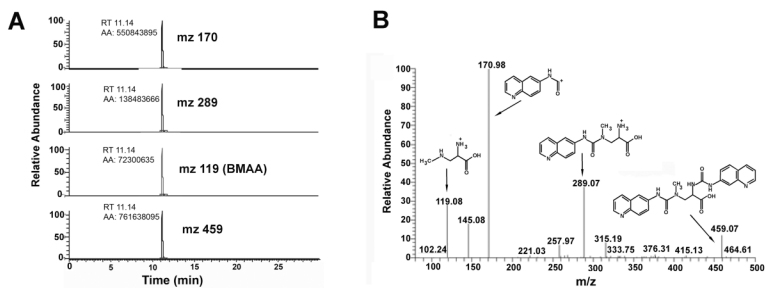
**Figure 5.** HPLC identification of beta-N-methylamino-L-alanine (L-BMAA) extracted from blue crab (*Callinectes sapidus*). A) Representative chromatogram of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) tagged amino acids in protein bound extract of muscle from a blue crab (blue trace) taken from Biscayne Bay. Peaks indicated by numbers are the amino acids closest to BMAA: L-tyrosine (1), L-valine (2), and L-methionine(3). The sample chromatographic peak of ACQ-BMAA (4) was confirmed by comparison (black trace, inset) to a co-run identical sample spiked with a known quantity of L-BMAA (100 ng). B) Matrix spiked with L-BMAA and 2,4 DAB synthetic standards. Although DAB is a structural isomer of BMAA, these results demonstrate two distinct chromatographic peaks separated by a later retention time for AQC-tagged DAB.



**Figure 6.**

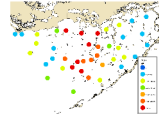
Calibration of the HPLC assay of beta-N-methylamino-L-alanine (BMAA) A. Hydrolyzed protein matrix from a control (BMAA-negative) specimen of Grey snapper (*Lutjanus griseus*) was spiked with increasing amounts of L-BMAA and compared to spiked norleucine (istd). Neutralized samples derivatized with AQC were analyzed by HPLC without further treatment. Each point represents an average of three runs assayed in duplicate. The response was linear up to 250 ng/mg,  $r^2 = 0.998$ ). B. Validation parameters and results summary from case study of specimens in different phyla. The limit of detection (LOD) and quantitation (LOQ) were validated in HCl extracts of specific matrix protein and compared to buffer blanks to ensure specificity and sensitivity of the quantitation limits. Recovery was determined in control matrix extracts spiked with BMAA prior to acid hydrolysis and compared to the same extract with BMAA added immediately before derivatization with AQC as described in the methods. %CV, Percent Coefficient of variation; %Δ, Percent deviation of the mean from target value.



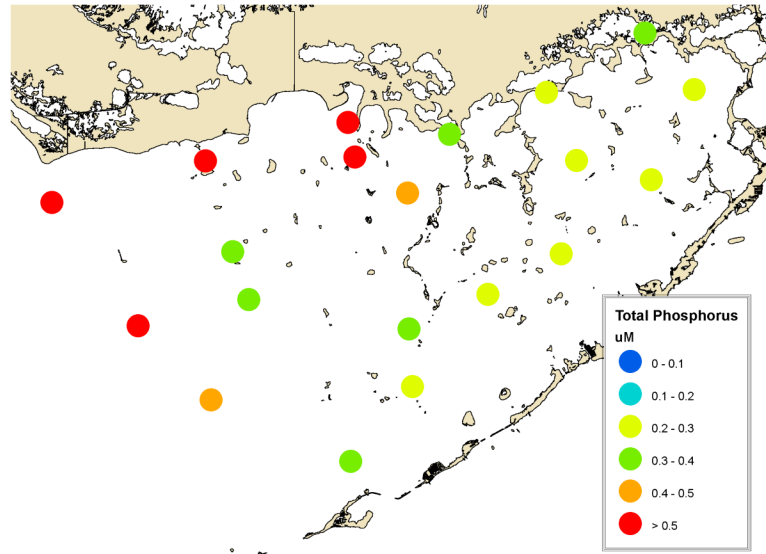


**Figure 7.**

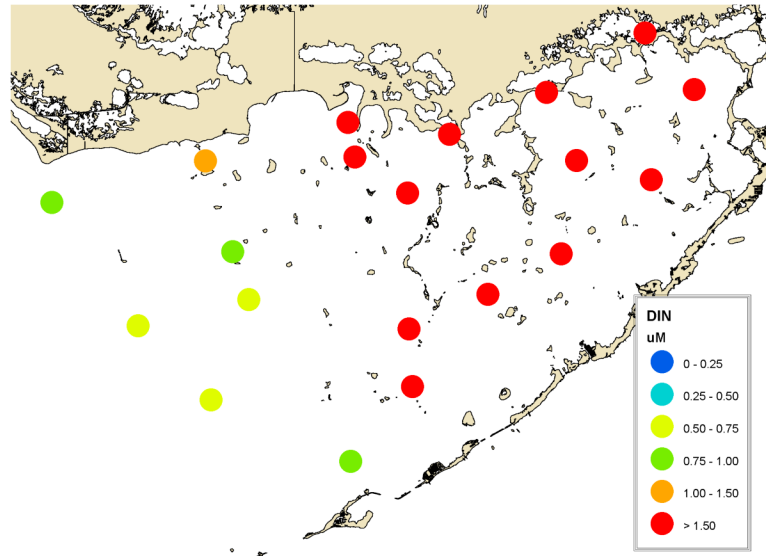
LC/MS/MS identification and verification of L-BMAA in blue crab from Biscayne Bay. A) Triple quadrupole LC/MS/MS verification of BMAA in a representative marine sample (blue crab). Ion chromatograms of product ion from collision induced dissociations of  $m/z$  459. The chromatography of the three major ions produced are: (1) protonated AQC derivative fragment ( $m/z$  171) is the quantitation ion, (2) the protonated-BMAA AQC fragment ( $m/z$  289) is the first qualifier ion with a ratio of 38% and (3) the protonated-BMAA fragment ( $m/z$  119) is the second qualifier ion with a ratio of 27%. B) Full product ion scan of an injection of AQC derivatized BMAA (217 fmole) in LC/MS/MS. Spectrum shows the dissociation of  $m/z$  459 at 20 V.



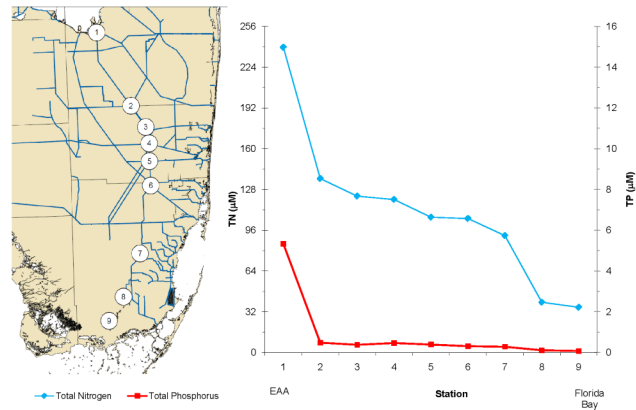
**Figure 8.**  
Average abundance of cyanobacteria in Florida Bay in 2000-2008 measured monthly as phycocyanin concentration.



**Figure 9.** Average distribution of total phosphorus in Florida Bay in 2000-2008. Monthly data from the South Florida Water Management District.

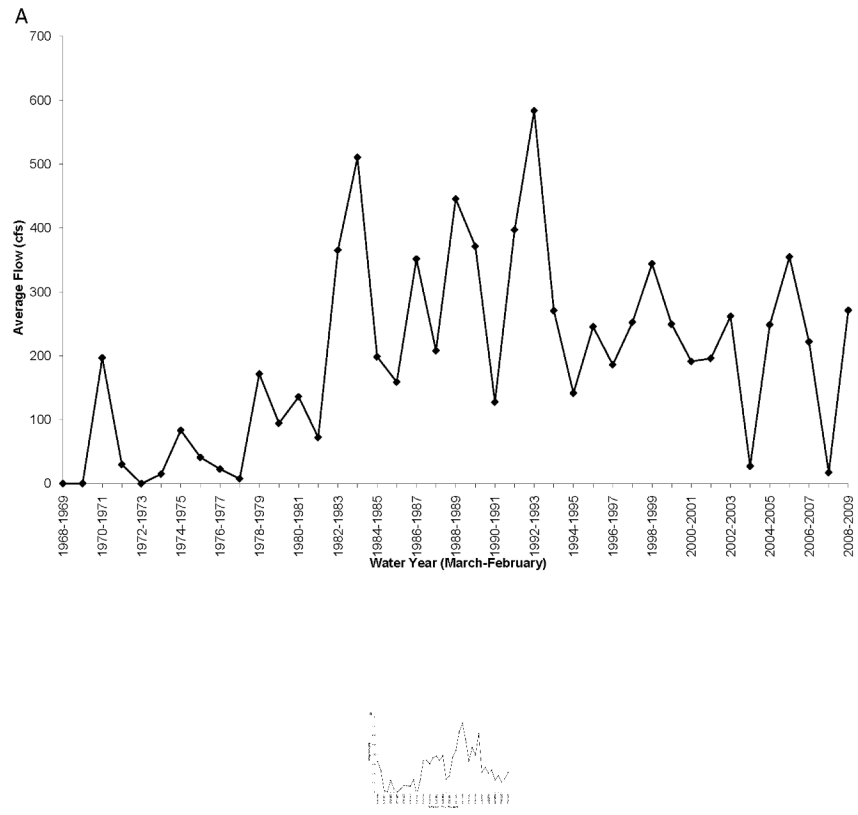


**Figure 10.** Average distribution of inorganic nitrogen (nitrate, nitrite, and ammonia) in Florida Bay in 2000-2008. Monthly data from the South Florida Water Management District.

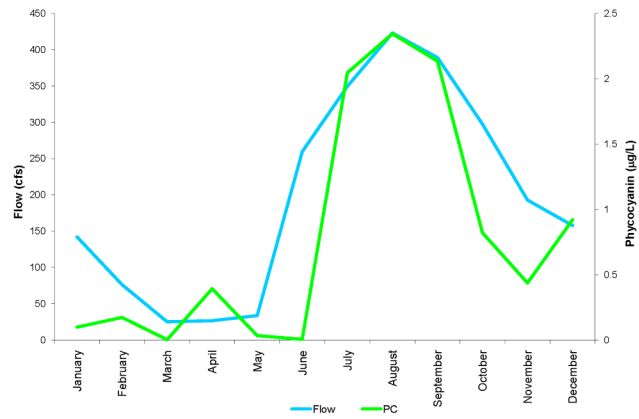


**Figure 11.** Transect of average nitrogen and phosphorus concentrations in 2008 from the Everglades Agricultural Area (EAA) through the Everglades into Florida Bay. Note that the scales for nitrogen and phosphorus are adjusted to the Redfield ratio of 16:1. These data show that phosphorus is the limiting nutrient in the Everglades. Monthly data from the South Florida Water Management District.

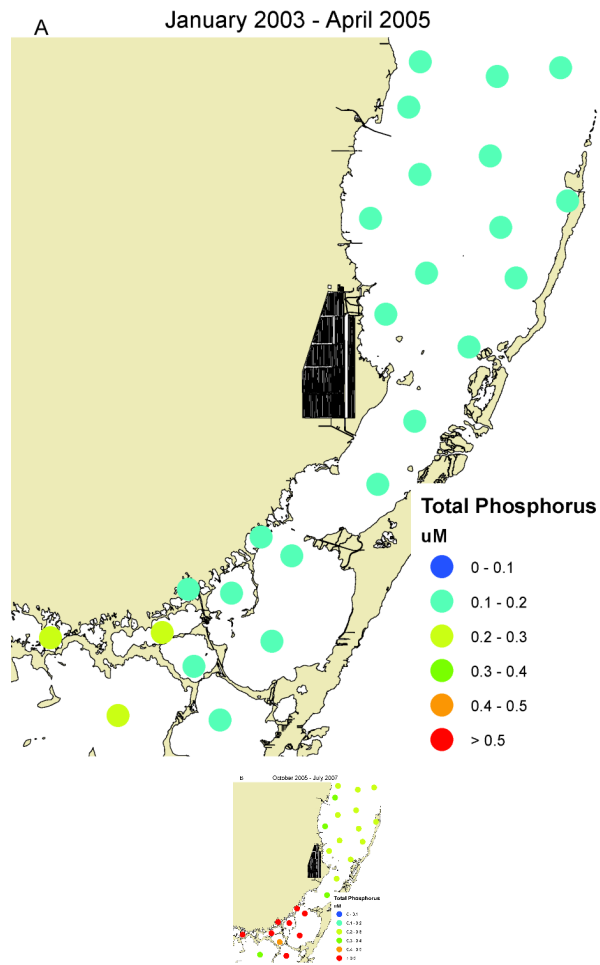


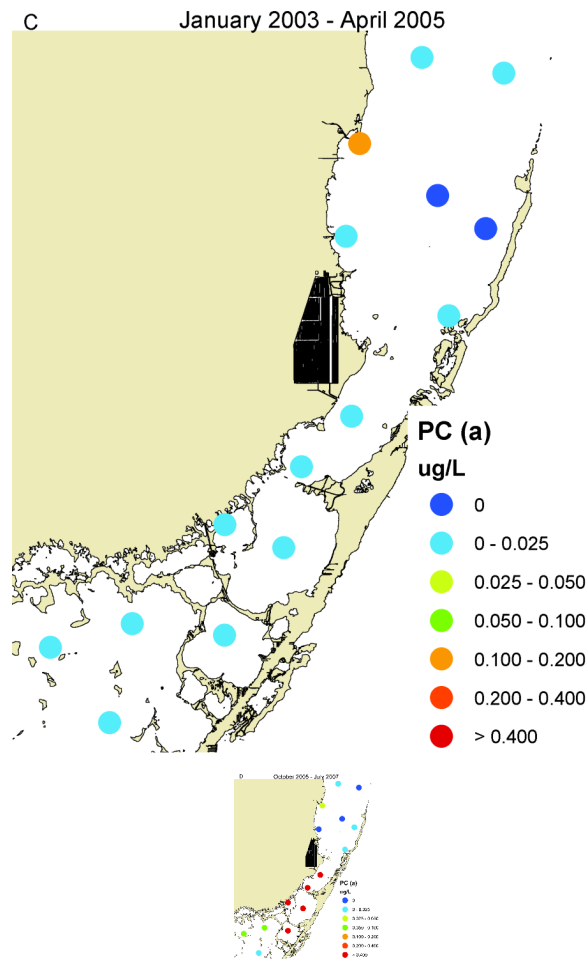


**Figure 12.** Long term changes in water flow from the Everglades Agricultural Area to Florida Bay, as measured at S151 (A) and Taylor Slough (B) (locations shown in Fig. 1). Data from the South Florida Water Management District.



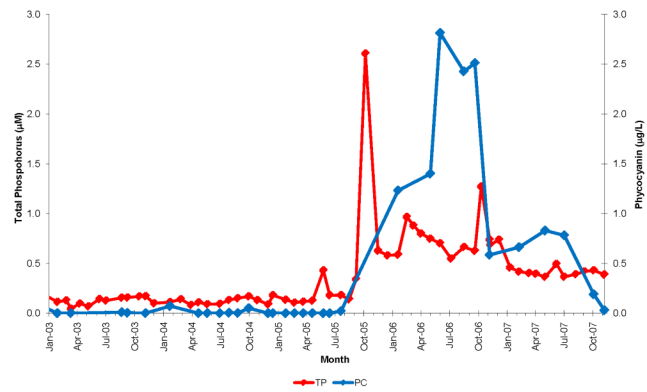
**Figure 13.** Average seasonal flow of water into Florida Bay and concentrations of cyanobacteria (measured as phycocyanin) over the 2000 to 2008 time period. Flow data from the South Florida Water Management District.



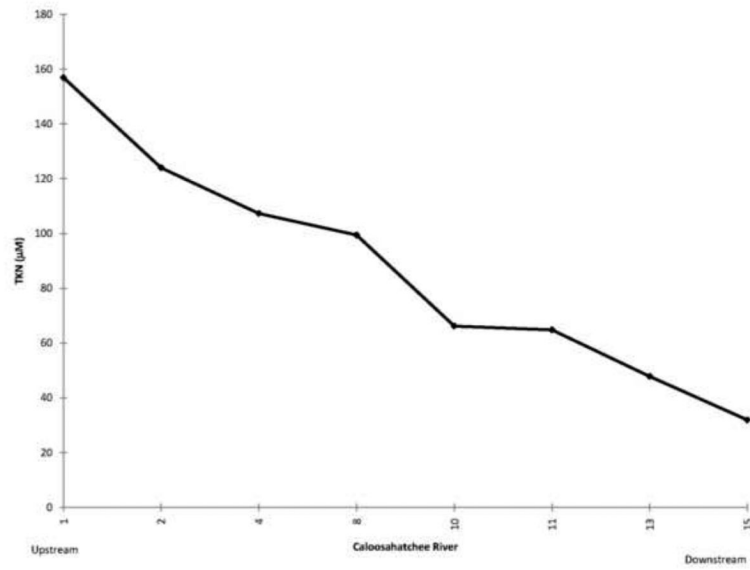


**Figure 14.**

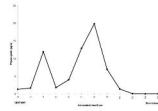
Concentrations of total phosphorus and cyanobacteria (measured as phycocyanin) in eastern Florida Bay-south Biscayne Bay. A. Total phosphorus before mangrove destruction. B. Total phosphorus after mangrove destruction. C. Cyanobacteria before mangrove destruction. D. Cyanobacteria after mangrove destruction. Total phosphorus concentrations from South Florida Water Management District.



**Figure 15.** Time course of total phosphorus and cyanobacteria (measured as phycocyanin) at locations (stars) shown in Fig. 3 in the area of mangrove destruction. Total phosphorus data from South Florida Water Management District.



**Figure 16.** Average concentrations of total nitrogen along the Caloosahatchee River from Lake Okeechobee to Sanibel Island May-December, 2008. Monthly data from South Florida Water Management District.



**Figure 17.** Average concentrations of cyanobacteria (measured as phycocyanin) along the Caloosahatchee River from Lake Okeechobee to Sanibel Island May-December, 2008.

## Glutamate Concentrations in Aquatic Samples

Table 1

Common Name (Species)	Glutamate (MW 146)		BMAA (MW 119)		Glut:BMAA (molar ratio)	
	µg/g	µmole/g (%Tot Prot) <sup>‡</sup>	µg/g	µmole/g (%Tot Prot)		
Blue crab ( <i>Callinectes sapidus</i> )	52,045	356	2,286	19	0.7	18.6
Oyster ( <i>Pinctada margaritifera</i> )	14,050	96	275	2	0.1	41.6
Puffer fish ( <i>Sphoeroides parvus</i> )	60,870	417	1,907	16	0.6	26.0
Shrimp ( <i>Panaeus duorarum</i> )	64,264	440	1,156	10	0.2	45.3
White perch ( <i>Pomoxis annularis</i> )	51,878	355	1,806	15	0.5	23.4

Concentrations were determined using a modification of the HPLC-AQC method and compared to an identical sample spiked with a known amount of glutamate. Glutamate/BMAA ratios were determined by conversion of the average concentrations by weight for each analyte to their molar concentrations.

<sup>‡</sup> Reference value for average glutamate concentration in Teleost fish muscle is 14.6% of total protein (Dunn et al., 1949)

Dunn MS, Camien MN, Eiduson S, and Malin RB (1949). The nutritive value of canned foods. I Amino acid content of fish and meat products. *Journal of Nutrition* 39:177-185.



Table 2

Concentrations of BMAA in animals in Florida Bay.

Sample	Species	Scientific name	Station	Location	Date	tissue type	Total BMAA (ug/g)		
							Assay 1	Assay 2	Assay 3
MC1	pink shrimp	<i>Panaeus duorarum</i>	A	Murray Key	6/25/2008	muscle	1890	1247	1361
MC2	pink shrimp	<i>Panaeus duorarum</i>	B	Joe Kemp Key	8/24/2008	muscle	3042	2860	
JL2	grey snapper	<i>Lutjanus griseus</i>	C	24.8500 N 80.8330 W	10/12/2008	muscle	ND		
JL4	grey snapper	<i>Lutjanus griseus</i>	C	24.8500 N 80.8330 W	10/12/2008	muscle	ND		
JL1	grey snapper	<i>Lutjanus griseus</i>	C	24.8500 N 80.8330 W	10/12/2008	muscle	20		
JL3	grey snapper	<i>Lutjanus griseus</i>	C	24.8500 N 80.8330 W	10/12/2008	muscle	53		
JL5	grey snapper	<i>Lutjanus griseus</i>	C	24.8500 N 80.8330 W	10/12/2008	muscle	188		

Table 3

Concentrations of BMAA in animals in Biscayne Bay.

Sample	Species	Scientific name	Station	Location	Date	tissue type	Total BMAA (ug/g)		
							Assay 1	Assay 2	Assay 3
NH34	pink shrimp	Panaeus duorarum	C-D transect	Elliot Key	summer, 2007	muscle	55		
NH14	pink shrimp	Panaeus duorarum	A	25.5150N 80.1795W	spring, 2007	muscle	181	362	457
NH28	pink shrimp	Panaeus duorarum	F	25.4653N 80.3359W	2/12/2007	muscle	942		
NH17	blue crab	Callinectes sapidus	G	25.5314N 80.3286W	spring, 2007	muscle	ND		
NH24	blue crab	Callinectes sapidus	F	25.4653N 80.3359W	summer, 2007	muscle	303	212	
NH31	blue crab	Callinectes sapidus	C-D transect	Elliot	summer, 2007	muscle	357	324	
NH4	blue crab	Callinectes sapidus	A	25.5150N 80.1795W	spring, 2007	muscle	4979	3037	
NH16	blue crab	Callinectes sapidus	E	25.4369N 80.3193W	spring, 2007	muscle	6976	6557	
NH20	spider crab	Clupeoides	C-D transect	Elliot Key	summer, 2007	muscle	ND	ND	
NH33	anchovy-sardine	Anchoa sp.	C-D transect	Elliot Key	summer, 2007	muscle	ND	ND	
NH15	hardhead silverside	Atherinomorinus stipes	A	25.5150N 80.1795W	spring, 2007	muscle	ND	ND	ND
NH27	hardhead silverside	Atherinomorinus stipes	F	25.4653N 80.3359W	2/12/2007	muscle	ND	ND	ND
NH38	hardhead silverside	Atherinomorinus stipes	C-D transect	Elliot Key	Summer, 2007	muscle	ND		
NH9	pinfish	Lagodon rhomboides	G	25.5314N 80.3286W	spring, 2007	muscle	ND	ND	
NH36	pinfish	Lagodon rhomboides	C-D transect	Elliot Key	summer, 2007	muscle	ND		
NH6	mojarra	Gerreidae	D	25.4060N 80.2196W	spring, 2007	muscle	ND		
NH21	mojarra	Gerreidae	C-D transect	Elliot Key	Elliot	muscle	ND	ND	
NH25	mojarra	Gerreidae	F	25.4653N 80.3359W	spring, 2007	muscle	154		
NH3	mojarra	Gerreidae	A	25.5150N 80.1795W	spring, 2007	muscle	567	456	
NH8	grey snapper	Lutjanus griseus	D	25.4060N 80.2196W	spring, 2007	muscle	ND		
NH12	grey snapper	Lutjanus griseus	A	25.5150N 80.1795W	spring, 2007	muscle	ND	ND	
NH40	grey snapper	Lutjanus griseus	C-D transect	Elliot Key	summer, 2007	muscle	ND		
NH2	school master snapper	Lutjanus apodus	A	25.5150N 80.1795W	spring, 2007	muscle	ND	ND	
NH11	band tail puffer	Spherooides spengleri	A	25.5150N 80.1795W	spring, 2007	muscle	ND	ND	ND
NH29	band tail puffer	Spherooides spengleri	F	25.4653N 80.3359W	spring, 2007	muscle	455	417	
NH18	least puffer	Spherooides parvus	G	25.5314N 80.3286W	spring, 2007	muscle	194	213	
NH10	least puffer	Spherooides parvus	A	25.5150N 80.1795W	spring, 2007	muscle	7351	6523	
HN30	scrawled cowfish	Acanthostracion quadricornis	A	25.5150N 80.1795W	1/20/2007	muscle	34	47	36

Sample	Species	Scientific name	Station	Location	Date	tissue type	Total BMAA (ug/g)		
							Assay 1	Assay 2	Assay 3
NH23	sea bream	<i>Archosargus rhomboidalis</i>	B	25.4550N 80.1965W	summer, 2007	muscle	484	636	
NH39	sea bream	<i>Archosargus rhomboidalis</i>	C-D transect	Elliot Key	summer, 2007	muscle	2349	2091	
NH37	sailors choice grunt	<i>Haemulon parra</i>	C-D transect	Elliot Key	summer, 2007	muscle	242	203	
NH7	sailors choice grunt	<i>Haemulon parra</i>	D	25.4060N 80.2196W	spring, 2007	muscle	722	674	
NH32	bluestriped grunt	<i>Haemulon sciurus</i>	C-D transect	Elliot Key	summer, 2007	muscle	ND		
NH1	bluestriped grunt	<i>Haemulon sciurus</i>	A	25.5150N 80.1795W	spring, 2007	muscle	1723	1568	
NH5	bluestriped grunt	<i>Haemulon sciurus</i>	D	25.4060N 80.2196W	spring, 2007	muscle	3776	2945	
NH19	redfin needlefish	<i>Strongylura notata</i>	G	25.5314N 80.3286W	spring, 2007	muscle	ND		
NH26	redfin needlefish	<i>Strongylura notata</i>	F	25.4653N 80.3359W	2/12/2007	muscle	ND	ND	
NH13	great barracuda	<i>Sphyraena barracuda</i>	A	25.5150N 80.1795W	spring, 2007	muscle	ND		
NH22	great barracuda	<i>Sphyraena barracuda</i>	B	25.4550N 80.1965W	summer, 2007	muscle	ND	ND	
NH35	great barracuda	<i>Sphyraena barracuda</i>	C-D transect	Elliot Key	summer, 2007	muscle	ND		

Table 4

Concentrations of BMAA in animals in the Caloosahatchee River.

Sample	Species	Scientific name	Station	Location	Date	tissue type	Total BMAA (ug/g) Assay 1	Assay 2
LB31	paper pondshell mussel	Utterbackia imbecillis	2	26.8319N 81.0901W	9/26/2008	mantle muscle	261	251
LB28	oyster	Crassostrea virginica	11	26.6769N 81.8444W	8/14/2008	mantle muscle	305	281
JC4	bowfin	Amia calva	8	26.7291N 81.7284W	1/24/2008	head	554	609
JC5	bowfin	Amia calva	8	26.7291N 81.7284W	1/24/2008	tail	2559	2245
JC7	alligator gar	Atractosteus spatula	8	26.7291N 81.7284W	1/24/2008	head	2140	2290
JC8	alligator gar	Atractosteus spatula	8	26.7291N 81.7284W	1/24/2008	tail	857	797
JC9	alligator gar	Atractosteus spatula	8	26.7291N 81.7284W	1/24/2008	central body	1207	990
JC1	largemouth bass	Micropterus salmoides	8	26.7291N 81.7284W	1/24/2008	head	1130	949
JC2	largemouth bass	Micropterus salmoides	8	26.7291N 81.7284W	1/24/2008	tail	2388	2245