Appendix 2B-4: Preliminary Report on Florida Bay Mercury Screening Study (Monitoring the Effect of Restoration and Enhanced Freshwater Flows on Biogeochemistry and Bioaccumulation of Mercury in Florida Bay)

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KEY FINDINGS AND OVERALL ASSESSMENT

Since 1995 Florida Bay has been under a health advisory recommending limited consumption of fish caught in the bay due to elevated levels of mercury. Yet, little is known about the sources of mercury contamination to Florida Bay. Limited studies suggest that surface water inflows from the mainland may contribute significant loads of total mercury (THg) and methylmercury (MeHg) to eastern Florida Bay. However, it is equally plausible that atmospheric deposition of inorganic mercury to the bay is sufficient to feed *in situ* production of enough MeHg to account for the observed mercury bioaccumulation. Because of constraints in the ability to predict the effects of increased Everglades inflows to eastern Florida Bay, two existing programs were integrated into a single, multi-agency study in 2000 to assess the impact of hydrologic restoration on the bay's mercury problem. Since 2000, surface water, sediment and fishes have been collected from 12 stations along two transects into eastern Florida Bay and one station in Whipray basin and were analyzed for THg and MeHg. Preliminary results reveal levels of mercury in certain gamefish from northeastern Florida Bay continue to exceed 0.5 parts per million (ppm), which is the criterion for limited-consumption advisories. Gradients in surface water and sediment implicate runoff from the mainland, *in situ* production within the mangrove ecotone, and *in situ* production within the bay itself as significant sources of MeHg. When completed, results of this study should improve the ability to make informed decisions about the management of Everglades inflows for the restoration of the sport fishery and the protection of fish-eating wildlife in Florida Bay.

INTRODUCTION

In the Everglades Forever Act (EFA) Ch. 373.4592, F.S, the Florida Legislature recognized that improved water supply and hydroperiod management were crucial elements to overall revitalization of the Everglades ecosystem, including Florida Bay. With the EFA, the legislature also recognized that the Everglades ecosystem must be restored both in terms of water quantity and water quality and must be preserved and protected in a long-term and comprehensive manner. To meet these management objectives and those set forth in the Central and Southern Florida (C&SF) Project Comprehensive Review Study (Restudy), the South Florida Water Management District (District) and the U.S. Army Corps of Engineers (USACE) are involved in a massive, collaborative restoration program in South Florida. The program, known as the Comprehensive Everglades Restoration Plan (CERP), has the potential to alter water deliveries, water quality, and circulation within Florida Bay. This appendix reports on a program to investigate how these changes might affect Florida Bay's mercury problem. In essence this monitoring program attempts to evaluate the fate and biogeochemistry of mercury over a large, regional scale that includes two unique ecosystems – the freshwater Everglades and the estuarine Florida Bay – and is complicated by how these two systems interact.

BACKGROUND

Fish surveys conducted in the late 1980s and early 1990s in Taylor Slough and eastern Florida Bay by the Florida Game and Fresh Water Fish Commission (FGFWFC) and the Florida Department of Environmental Protection (FDEP) routinely found mercury concentrations exceeding 0.5 ppm, and often 1.0 ppm (Strom and Graves, 1995; Adams and McMichael, 2001; Strom and Graves, 2001; T. Lange, personal communication). On October 6, 1995 the FDEP, the Florida Department of Health and Rehabilitative Services, and Everglades National Park (ENP or Park) issued a joint health advisory recommending limited consumption of select fish species in Florida Bay due to elevated levels of mercury. More recent surveys of mercury in fish from Florida Bay report concentrations of up to 0.78 ppm wet weight (3.9 ppm dry weight; Kannan et al., 1998). Further, elevated levels of mercury have been found in native and transplanted oysters (*Crassostrea virginica*) at the mouth of Taylor River in Little Madeira Bay (Goodman et al., 1999). Likewise, oysters collected from Joe Bay under the National Oceanic and Atmospheric Administration (NOAA) Mussel Watch Project have consistently shown elevated mercury concentrations relative to national averages (Cantillo et al., 1997). More recently, Evans and Crumley (in review) report that levels of mercury in spotted sea trout (*Cynoscion nebulosus*) from eastern Florida Bay typically exceed Florida's no-consumption level of 1.5 ppm. Finally, mercury also appears to be bioaccumulating in fish-eating birds that forage in the bay. A study of sick or injured birds at the Florida Keys Wild Bird Center from 1994 through 1997 found mercury concentrations of up to 250 ppm in the livers of double-crested cormorants (Sepulveda et al., 1998). These levels are three times higher than liver concentrations reported for great blue herons from the Everglades (Sundlof et al., 1994).

Florida Bay's mercury problem was given a regional perspective by a mercury survey of fishes from the Gulf of Mexico and its estuaries (Ache et al. 2000). This survey, which was funded by the U.S. Environmental Protection Agency's (USEPA's) Gulf of Mexico Program, identified two mercury "hot spots": Lavaca Bay, Texas, and Florida Bay. Lavaca Bay received industrial discharge during the 1960s from a chlor-alkali facility managed by the Aluminum Company of America (ALCOA; for details see Santschi et al., 1999). While Lavaca Bay had a

known source of mercury, at present little is known about the sources of mercury contamination to Florida Bay.

Only two small-scale studies have been conducted to investigate mercury in surface water flows from the mainland. Kannan et al. (1998) reported high concentrations of both THg and MeHg (3.0 to 7.4 ng/L and < 0.002 to 2.3 ng/L, respectively) in filtered water samples collected from canals and creeks flowing into eastern Florida Bay. A USEPA follow-up study reported MeHg in filtered samples from these same creeks at lower concentrations: 0.395 ng/L during the wet season, and up to 0.575 ng/L in the dry season (Lores et al., 1998). Results from these limited studies suggest that surface water flows from the mainland might make a significant contribution of THg and MeHg to eastern Florida Bay. This conclusion is consistent with the findings of a study of sediment cores from the bay. Based on levels of ^{210}Pb and Hg in sediment cores, Kang et al. (2000) concluded that Hg flux from runoff dominated over atmospheric input at many Florida Bay sites. However, they also found a distinct spatial pattern with accumulation of excess Hg from runoff near Taylor Slough that was two to six times greater than more remote (open bay) sites in eastern and southwestern Florida Bay.

Accordingly, to assess the impact of the C-111 and Modified Water Delivery projects, as well as future projects associated with the CERP, the District initiated a one-year, scoping-level study to establish baseline data on seasonal mercury loading and MeHg bioaccumulation in eastern Florida Bay. In late 2000 this baseline study was integrated with an ongoing NOAA program to assess the impact of hydrologic restoration on the bay's mercury problem. This two-year, multi-agency study was funded almost entirely through a grant from NOAA's South Florida Ecosystem Restoration Prediction and Modeling Program (SFERPM). This appendix reports the preliminary results from that study.

CONCEPTUAL MODEL

The principal pathway for mercury exposure to humans is through the consumption of marine fish and fish products (Rolfus and Fitzgerald, 1995). Yet, there remains a paucity of data on the cycling and fate of mercury in estuarine and marine systems. **Figure 1** depicts a generalized conceptual model of mercury flux and cycling in the freshwater/estuarine transition zone (for reviews, see Gilmour and Henry, 1991; Horvat et al., 1999; Langer et al., 2001). To a large extent, the model is based on information gathered in freshwater Everglades studies. Accordingly, flux rates (i.e., arrow sizes) will be revised when additional site-specific information becomes available.

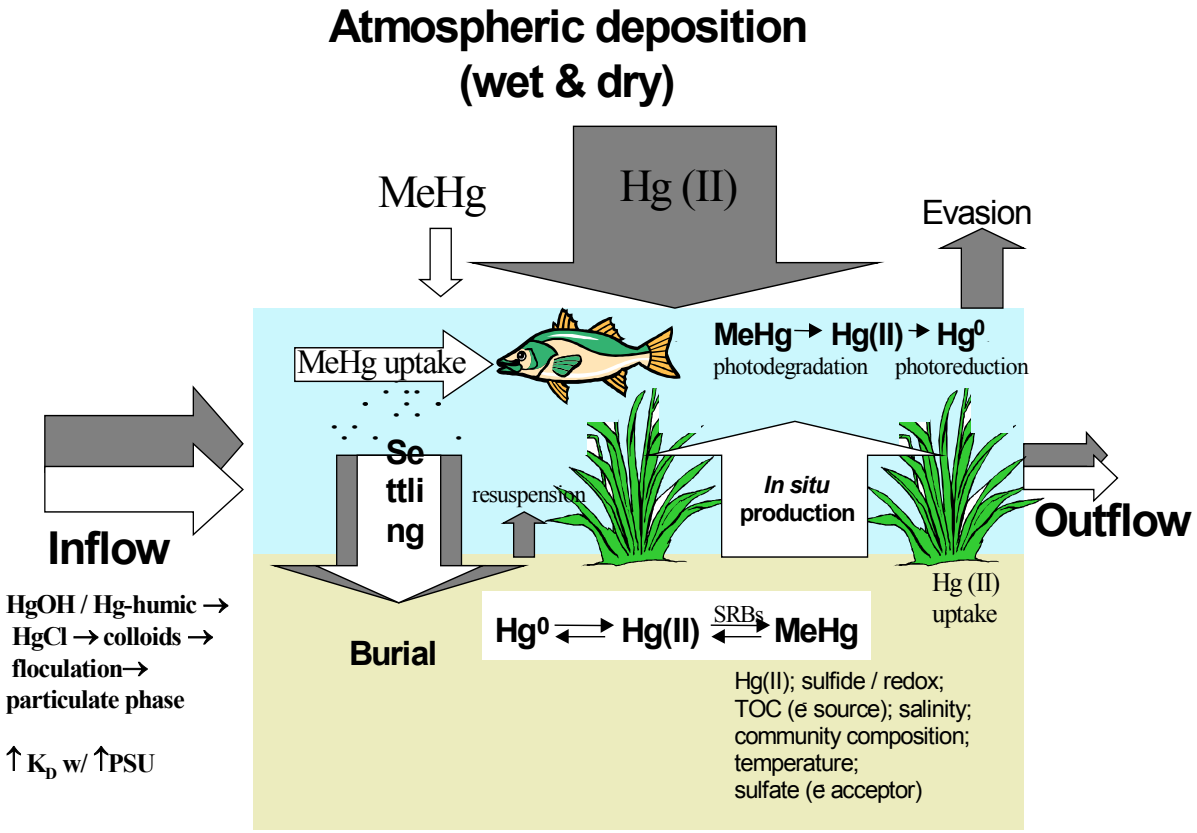

Figure 1. Generalized mercury cycle in the freshwater/marine transition zone

The limited studies done on estuaries, to date, have shown mercury cycling to be complex and highly variable, often differing from one estuary to another (Horvat et al., 1999). In some estuaries, dissolved mercury behaves conservatively, while in others it behaves non-conservatively. Processes that aid in the flux of materials within the transition zone include diffusive flux of dissolved species from sediments, porewater advection, bioturbation and biological transport, sediment suspension, tidal action (including possible drainage of porewaters during ebb tide), tidal pumping, creek flow and overland flow. Sources of inorganic mercury and MeHg to estuaries include direct atmospheric deposition, watershed runoff, groundwater discharge and, in the case of MeHg, *in situ* sedimentary production (i.e., conversion of inorganic to organic).

Mercury species in surface water discharges to estuaries likely undergo a competitive process between organic binding in solution, chloro-complexation, particle sorption, floculation of organic mercury colloids (Cossa et al., 1988; for review, see Horvat et al., 1999). Colloidal ligands, particularly those with thio functional groups, likely play an important role in mercury cycling in estuaries. Both quantitative and qualitative differences in dissolved organic carbon

(DOC) as a possible ligand or chelating agent, especially within the mangrove transition zone (Dittmar et al., 2001; Lacerda et al., 2001), likely influence the rate of influx and stability of mercury species from upstream sources (LeRoux et al., 2001). However, inorganic mercury and MeHg show different distribution patterns in the particle, colloidal and truly dissolved phases, which likely influence residence time in the water column. Further, phase association may vary with land use due to differences in carrier phases in runoff.

The amount of inorganic mercury that is methylated, not the total amount of mercury present, is the critical factor that determines whether a system will have a significant ecological problem. This is best illustrated by a study by Hines et al. (2000) that reported up to 322 ng THg/L, but only 0.6 ng MeHg/L in the Idrija River downstream of the second-largest mercury mine in the world. A variety of geochemical and biological factors play a role in controlling the rate of net mercury methylation, including concentrations of Hg(II), sulfate, sulfide, DOC, pH, organic carbon content and community composition (for review see Gilmour and Henry, 1991; Gilmour and Capone, 1987; Olson and Cooper, 1976; King et al., 2000). While most studies have focused on mercury methylation by *Desulfovibrio desulfuricans*, King et al. (2000) recently reported that different phylogenetic groups of sulfur-reducing bacteria (SRB) methylate mercury at different rates.

Until recently, it was thought that mercury methylation was inhibited in marine environments (for review see Langer et al., 2001) due to high sulfides (Berman and Bartha, 1986; Compeau and Bartha, 1987; Gilmour and Henry, 1991; Gilmour et al., 1998; Benoit et al., 1998) and salinities (Blum and Bartha, 1980; Compeau and Bartha, 1987; Barkey et al., 1997). However, recent studies have demonstrated methylation activity in the presence of sulfide at millimolar range (King et al., 2000; Langer et al., 2001). Langer et al. (2001) report highest methylation rates at the surface within the redox transition zone, decreasing below this zone where sulfide can build up and bind to the inorganic Hg. They also argue that sulfide-oxidizing bacteria could be methylating Hg.

Determining the source of the MeHg that is driving the bioaccumulation in eastern Florida Bay is a fundamental concern, i.e., runoff from the mainland versus *in situ* sedimentary production within the bay. If the rate of *in situ* sedimentary production of MeHg is determined to be significant, then it will be crucial to discover how far out in the bay this production is controlled by inorganic mercury in runoff (i.e., indirect atmospheric deposition) versus direct atmospheric deposition of inorganic mercury, particularly considering that there may be differences in bioavailability. If mainland runoff of either THg or MeHg is a significant forcing function out in the bay, then increased surface water deliveries to eastern Florida Bay could worsen the mercury problem by increasing loading. Alternatively, the possibility must also be considered that increased surface water deliveries and attendant changes in porewater chemistry within the bay (e.g., salinity, redox conditions, and sulfide concentrations) have the potential to alter the rate of *in situ* methylation in the bay.

STUDY SITE AND METHODS

Florida Bay is a large (1,800 sq km), shallow (< 2 m) estuarine system at the southern tip of the Florida Everglades. Freshwater flow through the Everglades and into Florida Bay is a key avenue by which the two ecosystems interact. Samples were collected along two transects into Florida Bay (**Figure 2**) under appropriate state and federal permits. The first transect begins in the C-111 basin and extends south through Joe Bay and Trout Creek into the bay. The second transect follows the flow path of Taylor Slough out through Little Madeira and into the bay. This

flowpath is expected to experience increased flows resulting from efforts associated with the C-111 and Modified Water Delivery projects. One site in Whip Ray basin was also sampled as a reference site. Efforts were made to co-locate these sites with existing water quality monitoring sites.

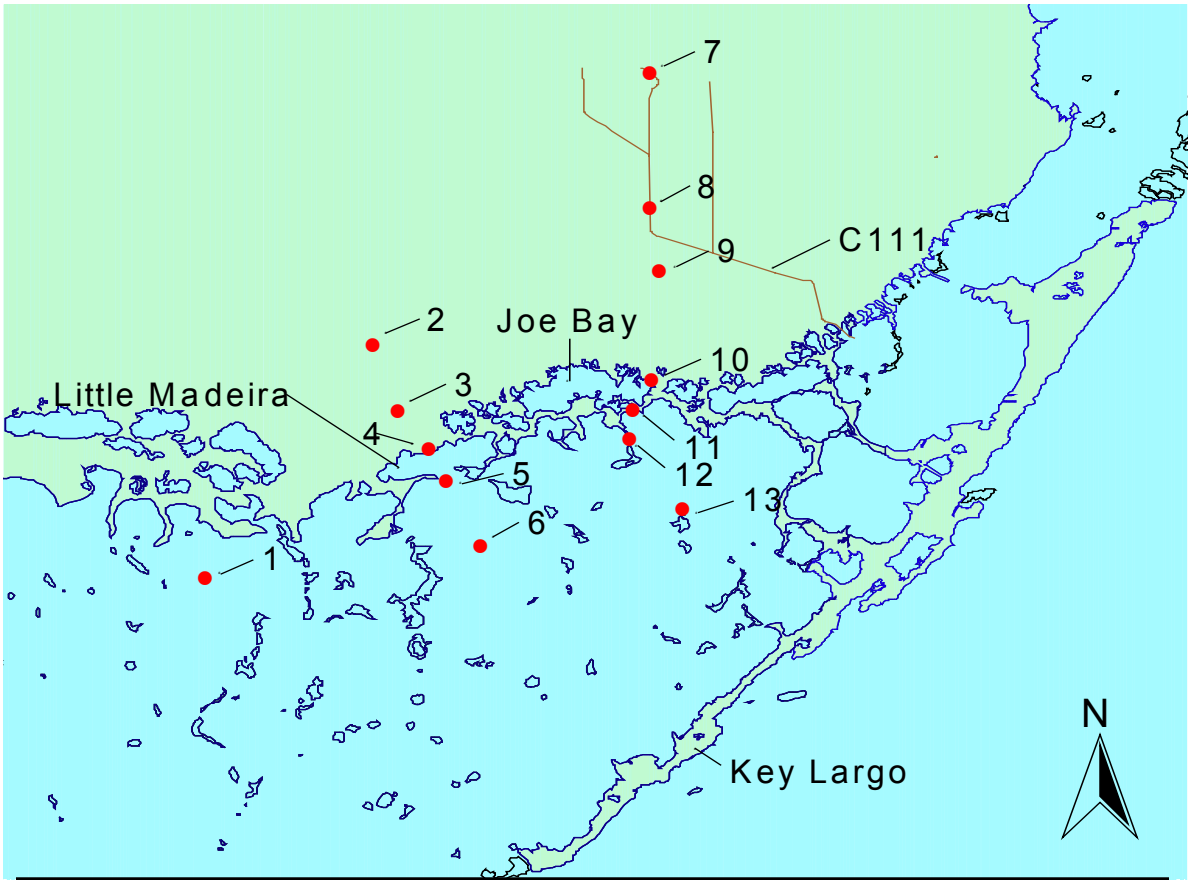

Figure 2. Map of sampling sites in and around Florida Bay

Surface water, sediment, and fish were collected using methods adapted from standard operating procedures developed for the Everglades Mercury Screening Program. Briefly, **after** employing a clean-hands/dirty-hands technique, duplicate samples of filtered and unfiltered surface water were collected from mid-depth using a peristaltic pump and ultra-clean Teflon sampling train. The samples were immediately shipped to a contracted laboratory for determination of total mercury (THg), total dissolved mercury (THgF), mono-methylmercury (MeHg) and dissolved mono-methylmercury (MeHgF). Dissolved species were operationally defined as material passing through a 0.45 µm Meissner capsule filter.

Additionally, temperature, conductivity, salinity, turbidity, and dissolved oxygen (DO) were measured at a minimum of three depths (0.1 m from the bottom, mid-depth, and 0.1 m from the surface) at each location using a YSI 6920 multi-parameter water quality monitor.

Sediment cores (4-cm depth) were collected in triplicate at each location using clean Butyrate core tubes. The cores were composited to form a single sample, which was then homogenized and analyzed for both THg and MeHg (based on dry weight). At each location, attempts were also made to collect representative, small-size forage fish species, medium-size prey fish species, and large-bodied predatory fish species. Small-size forage fish species (fresh and marine; mosquitofish, *Gambusia* spp.; sailfin molly, *Poecilia latipinna*; rainwater killifish, *Lucania parva*; anchovies, *Anchoa mitchilli*, killifishes, *Fundulis* spp.; sheepshead minnow, *Cyprinodon variegatus*; silversides, *Menidia* spp.) were collected using long-handled dip nets, seine nets, cast nets (1/4 in mesh size) and/or throw traps (species varied along the gradient). Small forage fish (n = up to 100 individuals) were pooled by species, homogenized, and then treated as a composite sample from each site. Medium-size prey fish species (freshwater and marine; mayan ciclid, *Cichlasoma urpophthalmus*; sunfish, *Lepomis* spp.; mojarra, *Eucinostomus* spp.; mullet, *Mugil* spp.) were collected in replicate (n = 5) by cast net or hook-and-line; whole fish were then homogenized (i.e., with stomach contents) using a commercial meat grinder or food processor with stainless steel blades. At each site, attempts were also made to collect large-bodied predatory fish species, including gamefish (fresh and marine; largemouth bass, *Micropterus salmoides*; spotted seatrout, *Cynoscion nebulosus*; redfish, *Sciaenops ocellatus*; gray snapper, *Lutjanus griseus*; crevalle jack, *Caranx hippo*; gafftopsail catfish, *Bagre marinus*; common snook, *Centropomus undecimalis*), in replicate (n = 5) by hook-and-line. Fillets of these fish were then analyzed for THg.

Surface water samples were analyzed for THg and MeHg, and sediments were analyzed for MeHg by Frontier Geosciences and CEBAM Analytical, Inc., both of Seattle. THg analysis was carried out using EPA method 1631 (EPA-821-R-99-005). In brief, all mercury in the water sample was oxidized to Hg(II) using 0.2N bromine monochloride solution. After oxidation, hydroxylamine hydrochloride was added to inhibit further reaction and destroy free halogens. Hg(II) was reduced to volatile Hg(0) by the addition of stannous chloride. The Hg(0) was then separated from solution by purging with nitrogen and concentration onto a gold-coated sand trap. The trapped Hg was thermally desorbed from the gold trap and was determined using cold vapor atomic fluorescence spectroscopy. Following co-distillation into pure water, MeHg was determined by aqueous phase ethylation using sodium tetraethyl borate (sodium tetraethyl borate converts nonvolatile monomethyl Hg to gaseous methyl ethyl Hg), followed by purge-and-trap on a Carbotrap™. The trap was then thermally desorbed into an isothermal GC column for peak separation and was then quantified by cold vapor atomic fluorescence spectroscopy (Bloom, 1989).

THg concentrations in fish tissues and sediments were determined by the Florida Department of Environmental Protection (FDEP) Chemistry Laboratory, CEBAM Analytical, and at the NOAA Laboratory using a modified version of EPA method 245.6. The mercury in the sample was first oxidized to Hg(II) using a combination of potassium permanganate and potassium persulfate. Hydroxylamine hydrochloride was then added to reduce excess oxidizing reagents. The mercuric ions in solution were reduced to Hg(0) using stannous chloride and were purged into an atomic absorption spectrometer. A subsample of collected fish will be analyzed under contract for stable carbon and nitrogen isotopes by gas chromatography/isotope ratio mass spectrometry.

MeHg was extracted from a sub-sample of large fish collected in both the freshwater drainages of the Everglades and from within western Florida Bay and were analyzed for $\delta^{13}C$

with the expectation that there will be about a 16 percent difference between the two. MeHg will also be extracted and similarly analyzed from gamefish collected within eastern Florida Bay, where mercury concentrations are high. In all samples, MeHg will be extracted as above and isolated cryogenically. Carbon isotope ratios will be determined in the extracted MeHg by isotope ratio mass spectrometry by methods modified from Chanton et al. (1992).

RESULTS AND DISCUSSION

Six sampling transects have been completed: December 2000, March, June, September, and December 2001 and March 2002. Water and sediment have been analyzed for MeHg and THg, and fish have been analyzed for THg from these transects. Combined with data from earlier transects of February and July 2000, results from the samples reveal a pattern of elevated MeHg concentrations in water and sediments in the mangrove transition zone of Little Madeira Bay (sites 3 and 4) and Joe Bay (sites 9 and 10), where the Everglades runoff mixes with saline bay waters (**Figures 2**, **3**, and **4**). This suggests a local source of MeHg formation in this region. Florida's prolonged drought has resulted in limited freshwater flows into this area and has also restricted sampling at site 2 in Taylor Slough and site 9 in the Joe Bay drainage. Because of the drought, these sites have either been dry or inaccessible by airboat.

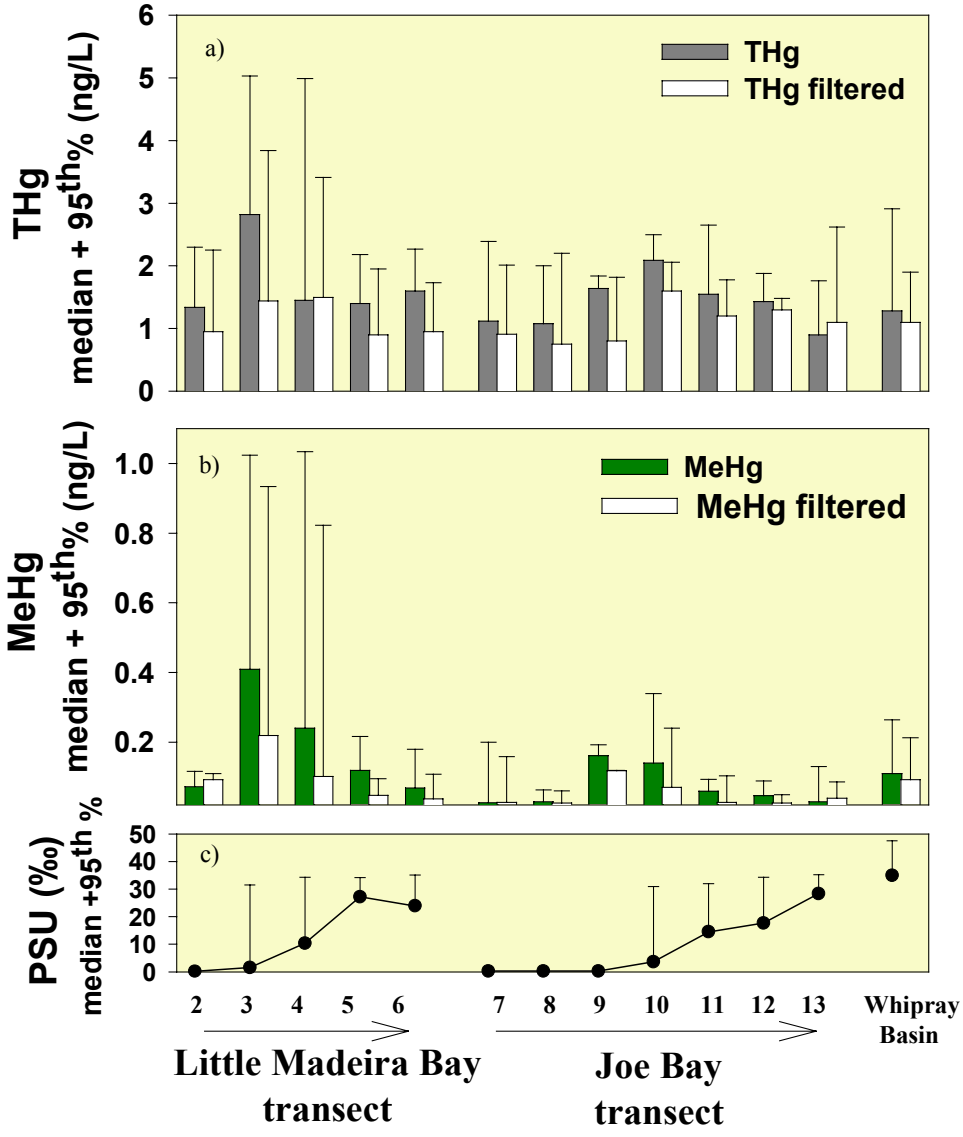

Figure 3. Median concentrations of total mercury (a) and methylmercury (b) in surface water. Lower panel shows median practical salinity units of water at time of collection

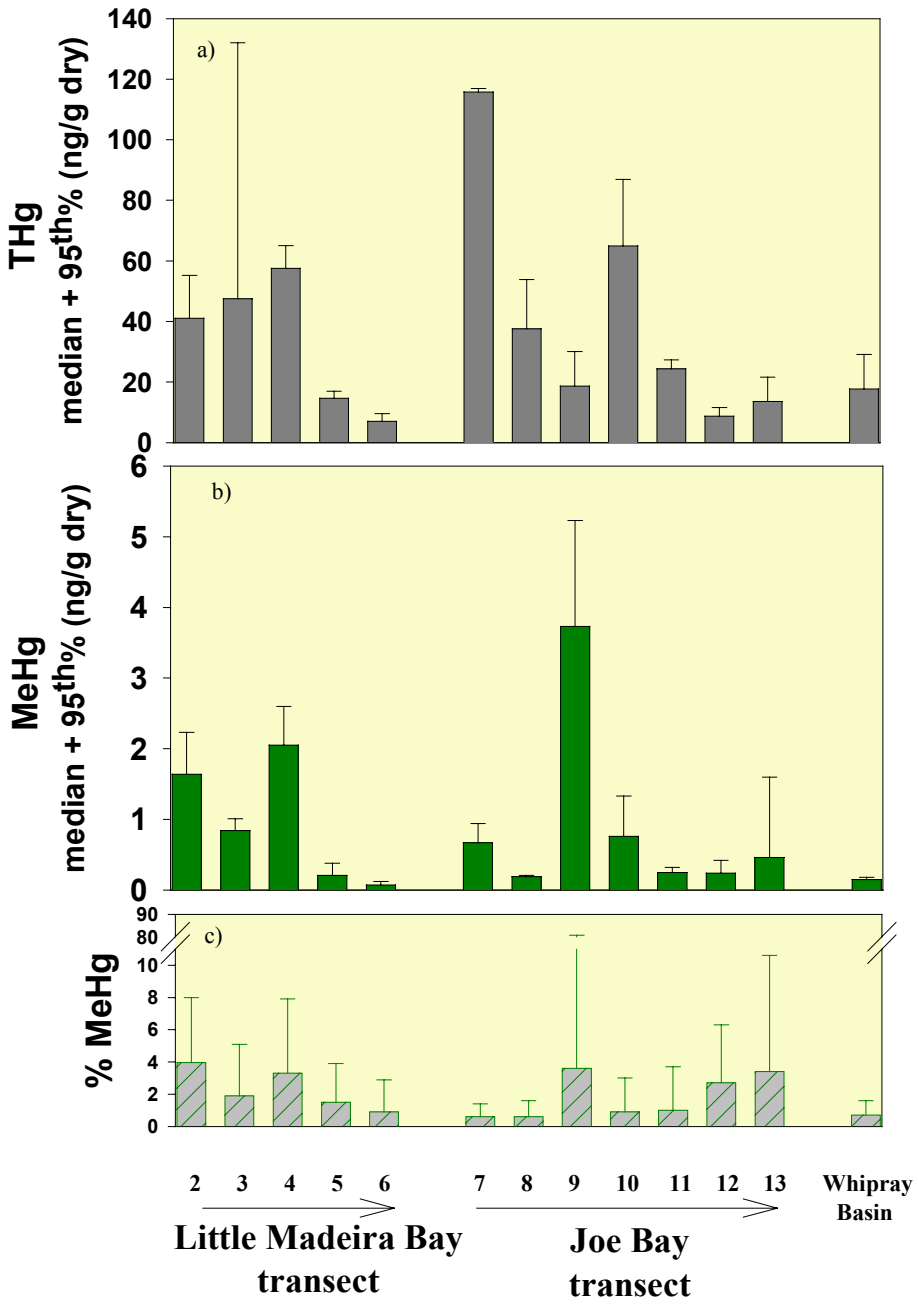

Figure 4. Median concentrations of THg (a) and MeHg (b) in sediments. Lower panel shows percent of total that was in the methyl form

Mercury methylation rates have been measured to identify areas of active MeHg production that could enter the food web. Intact sediment cores collected in November 2001 from 11 sites were spiked with inorganic ^{202}Hg tracer and incubated to allow mercury to be methylated by resident microbes. The cores were then cut, frozen and shipped to David Krabbenhoft at the USGS Mercury Research Laboratory in Wisconsin, where they were then analyzed for total mercury and MeHg by ICP-MS. The fraction of THg that was methylated is shown in **Figure 5**. Surprisingly, the degree of methylation was higher in many of the outer bay sites than in mangrove and freshwater wetland sites. The THg concentrations in Florida Bay are lower than in the mangrove and freshwater sites so that the rates of MeHg formation are comparable.

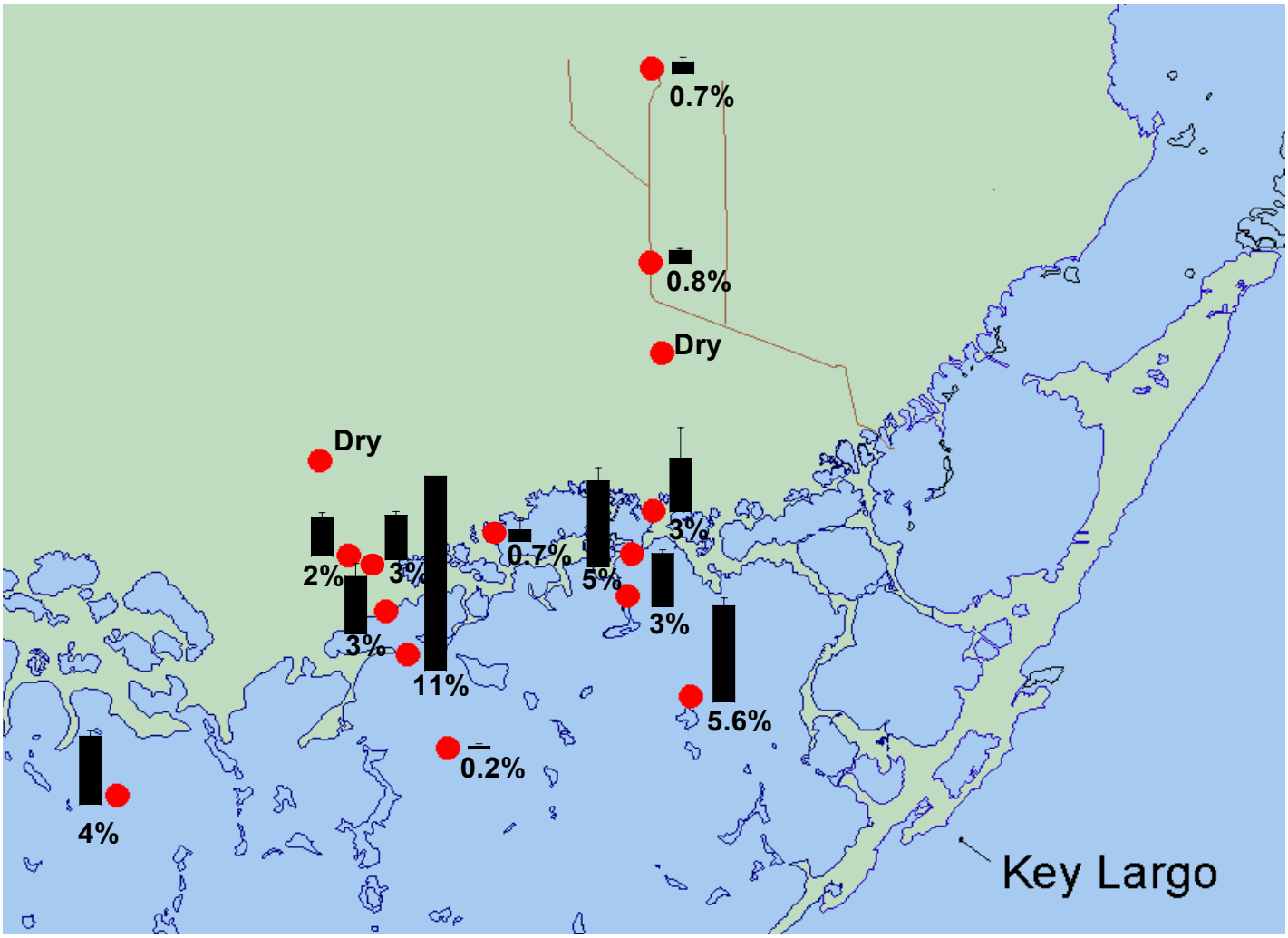

Figure 5. Net methylation of ^{202}Hg in 0 to 4 cm sediment cores incubated for 24 hours

Sufficient samples have been obtained to begin to identify seasonal patterns in mercury concentrations in forage fish inhabiting the estuary (**Figure 6**). The lowest MeHg concentrations occur in the dry season of mid-winter, when flows are minimal (**Figure 7**). MeHg concentrations in water rise when freshwater flow increases in response to summer rains. Concomitantly, THg concentrations in silversides (*Menidia* spp.), a fast-growing, pelagic forage fish, also increase at this time, suggesting higher exposure of parts of the food chain to MeHg. This linkage between MeHg concentrations in water and THg concentrations in fish is strongest at sites within the mangrove transition zone, where the highest MeHg concentrations in water and sediments have been observed. Plans to extend these temporal observations backward in time by analyzing small forage fish collected from this zone during the period of 1994 to 1998, and provided by Jerry Lorenz from his Ph.D. research at the University of Miami, are in preparation (Lorenz is currently with Florida Keys Audubon). Further analyses of these patterns in relationship to summer rains, atmospheric deposition of mercury, and seasonal reflooding of Everglades marshes are planned.

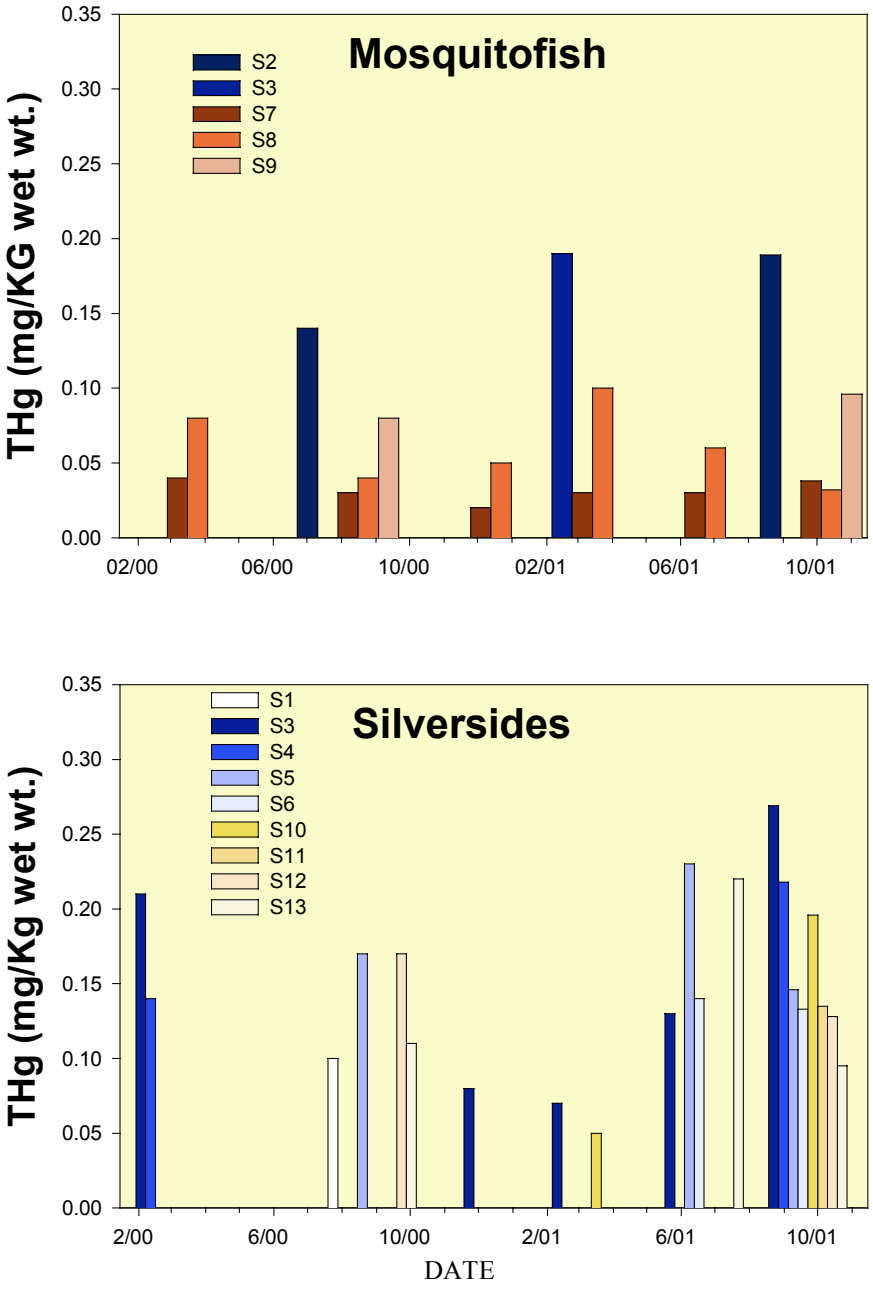

Figure 6. Concentration of THg in forage fish collected at various sites over time

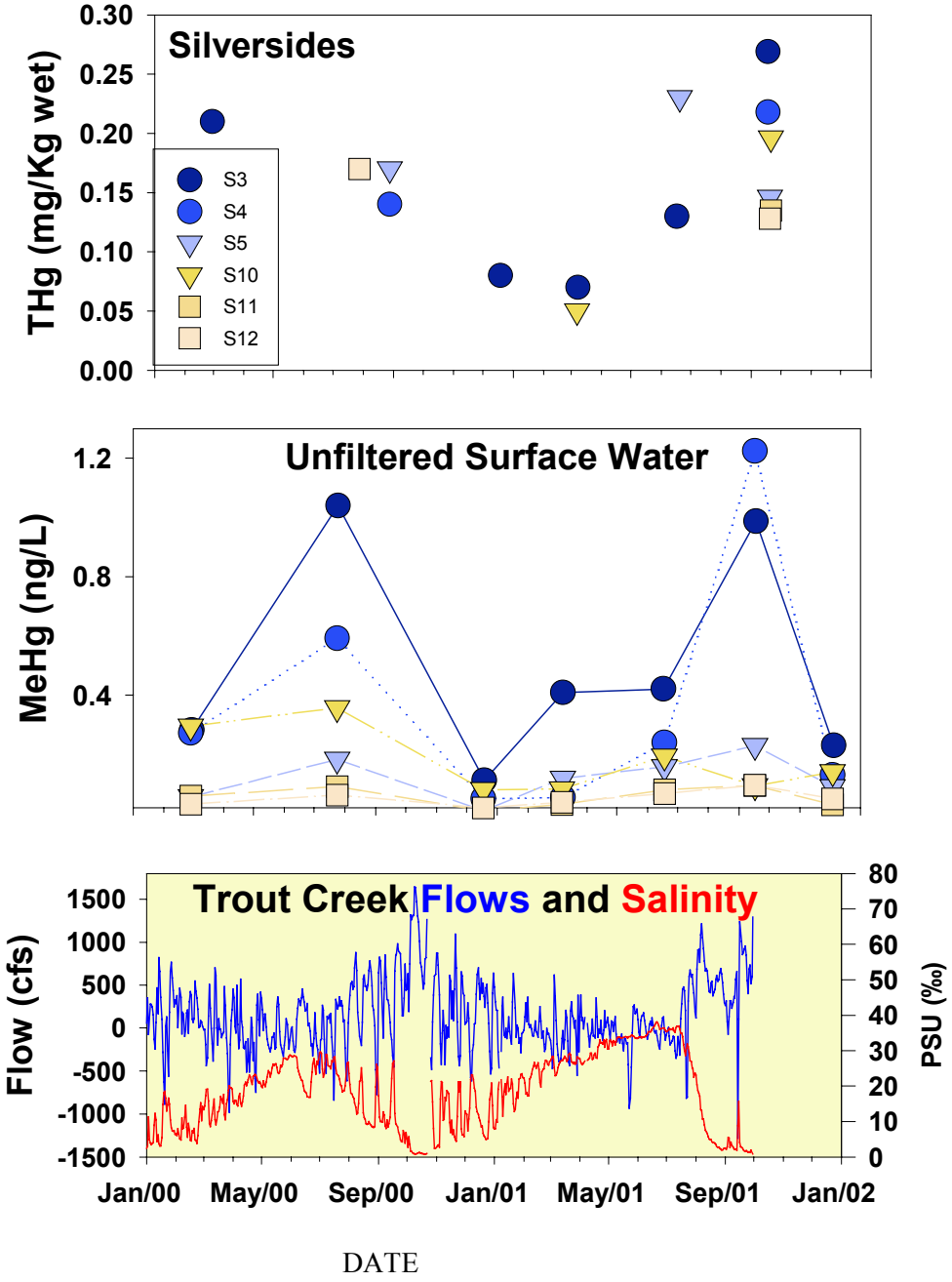

Figure 7. A comparison of levels of THg in forage fish (top panel), MeHg concentration in water (middle panel), and flows at Trout Creek (bottom panel, left axis)

Silversides are but a portion of the 324 fish samples analyzed for mercury to date. These fish include three other forage fish species (bay anchovy (*Anchoa mitchilli*), rainwater killifish (*Lucania parva*), and mojarra, (*Eucinostomus gula*) for which bay-wide mercury data exist. Three gamefish species: spotted seatrout (*Cynoscion nebulosus,* **Figure 8**), red drum (*Sciaenops ocellatus*), and gray snapper (*Lutjanus griseus*)) included in that earlier study have also been analyzed for the period 2000 to 2002. In addition, crevalle jack (*Caranx hippos*) have also been sampled and were found to have high mercury concentrations generally exceeding 1 µg/g. Freshwater species have been added to infer MeHg exposure in upstream sites. These include mosquitofish (*Gambusia* spp.), Mayan cichlid (*Cichlasoma urpophthalmus*), largemouth bass (*Micropterus salmoides*), and peacock bass (*Cichla ocellaris*). Other species have been collected opportunistically.

Silversides are the only fish found across a wide salinity gradient that seem to have higher mercury concentrations at sites of lower salinity (**Figure 9**) at the sites of highest MeHg concentrations in sediment and water in the mangrove transition zone (sites 3, 4, 9, and 10). Other species show either no relation of mercury concentrations with salinity (crevalle jack, gray snapper, and anchovy) or an increase in mercury with increasing salinity (killifish and mojarra).

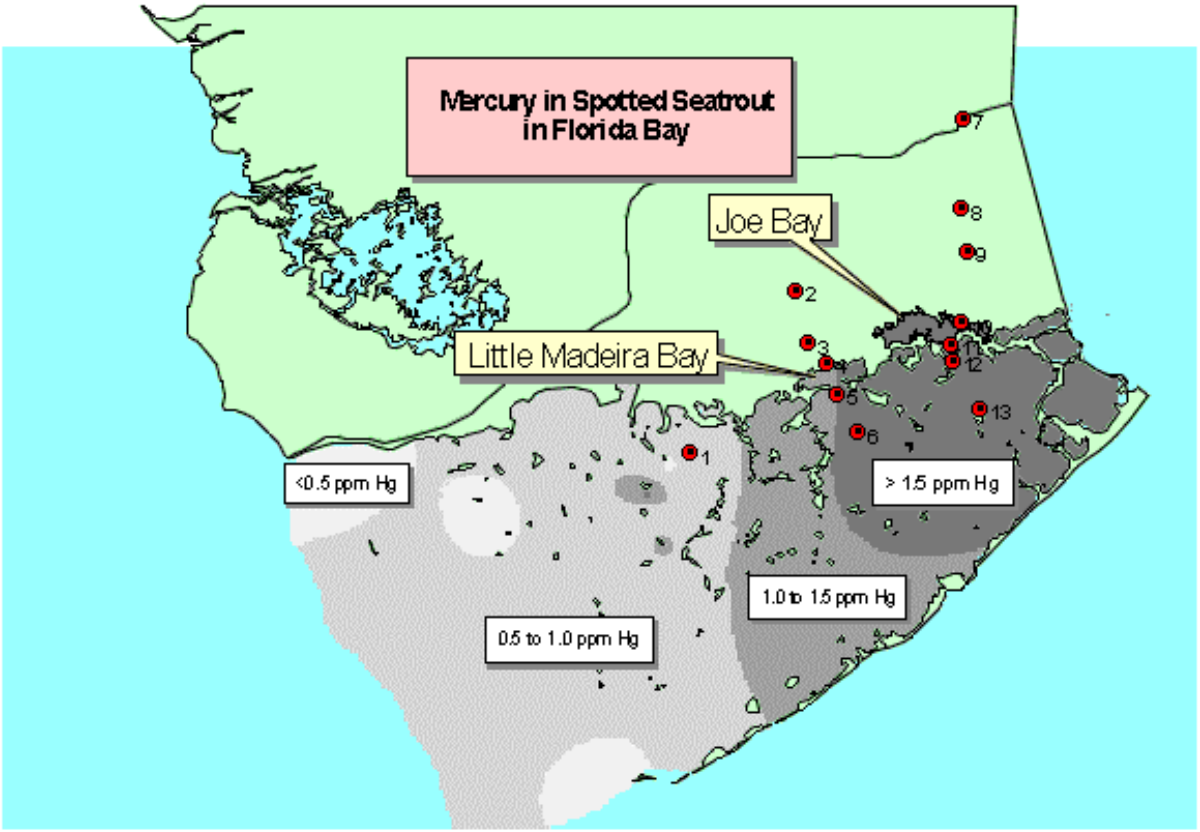

Figure 8. Concentration of THg in spotted sea trout from Florida Bay

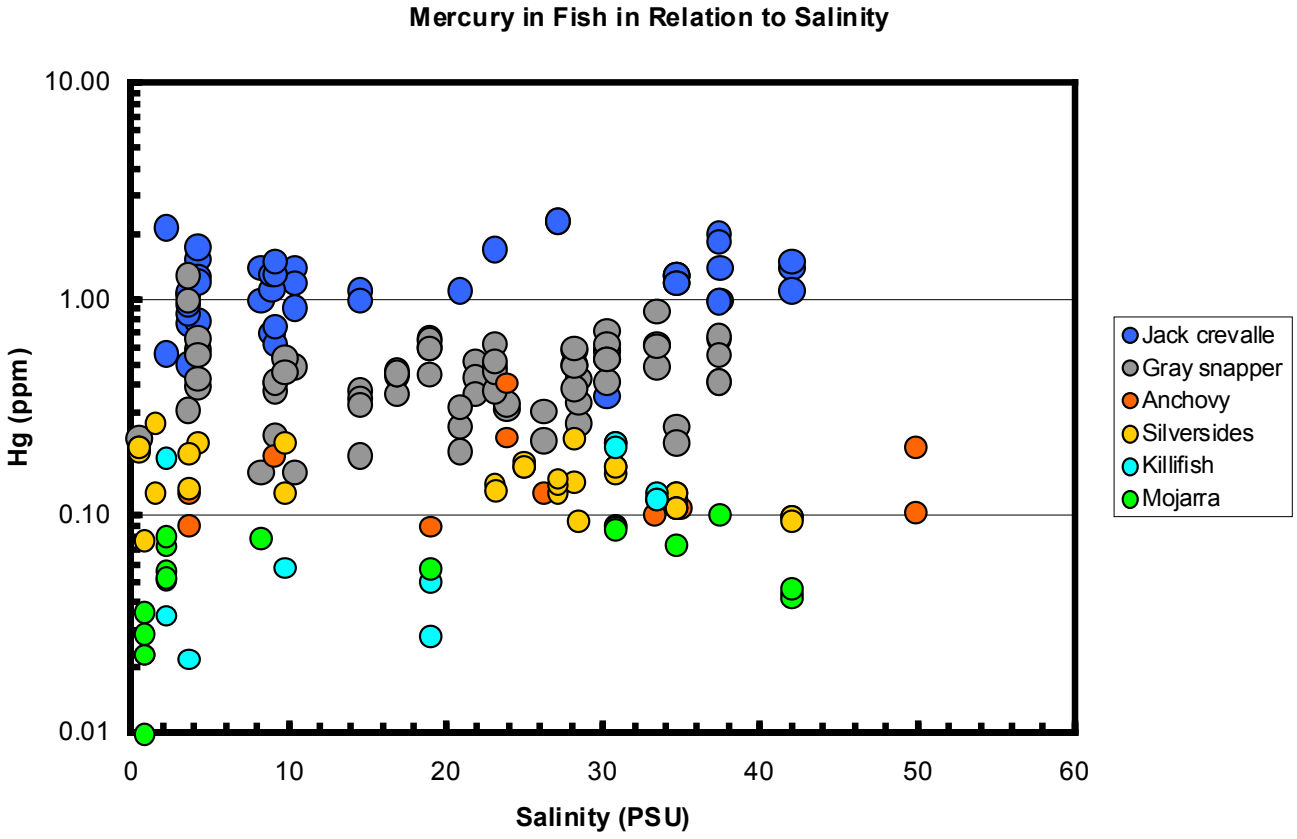

Figure 9. Relationship between THg levels in fish and salinity of water at collection site

Analysis of the stable carbon and nitrogen isotope concentration of these fish should allow characterization of their position in the food web, the relative importance of terrestrial/freshwater and estuarine sources of their food and, by inference, their mercury content. A plot of mercury concentrations in select fish species against the stable carbon isotope signature indicates these estuarine fish derive nutrition from both the mangrove and terrestrial food web (d13C ca -30) and from the seagrass or microalgal-dominated food web (d13C ca -14) of the open bay (**Figure 10**). Surprisingly, higher mercury concentrations in fish with a strong mangrove or terrestrial stable carbon isotope signature in their diet have not been observed. Because fish acquire MeHg through feeding, this suggests that MeHg has entered the lower trophic levels of the food web throughout eastern Florida Bay, not just in the mangrove transition zone, where the highest MeHg concentrations in water and sediments are found.

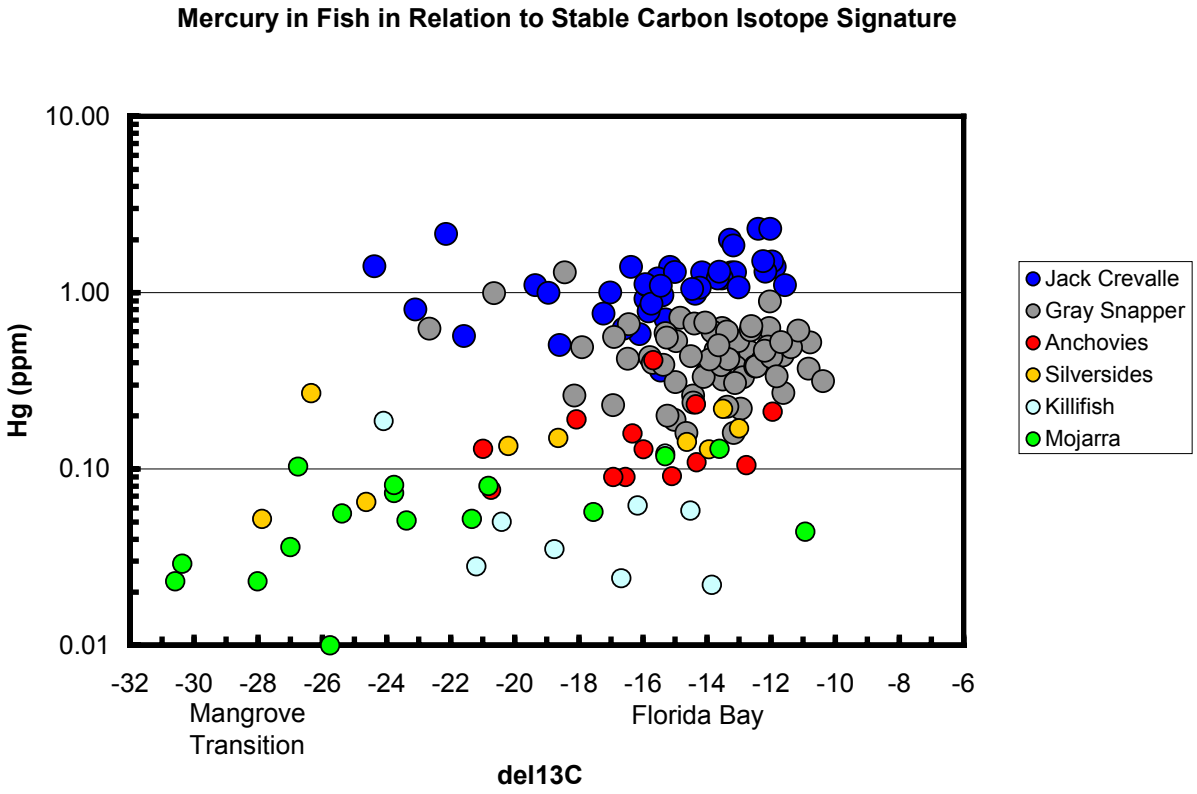

Figure 10. Relationship between THg in fish and stable carbon isotope signature

Stable nitrogen isotope signatures serve as a measure of relative trophic level, as well as a potential indicator of the trophic base of the food web. Higher d15N values are found in the gamefish (gray snapper and crevalle jack) than in the four forage fish, which is consistent with their higher trophic position (**Figure 11**). Higher mercury concentrations in these gamefish are consistent with MeHg biomagnification up food chains. The two pelagic-feeding forage fish: anchovies and silversides, have higher mercury concentrations than the two more benthic-feeding forage fish: killifish and mojarra, despite similar d15N values.

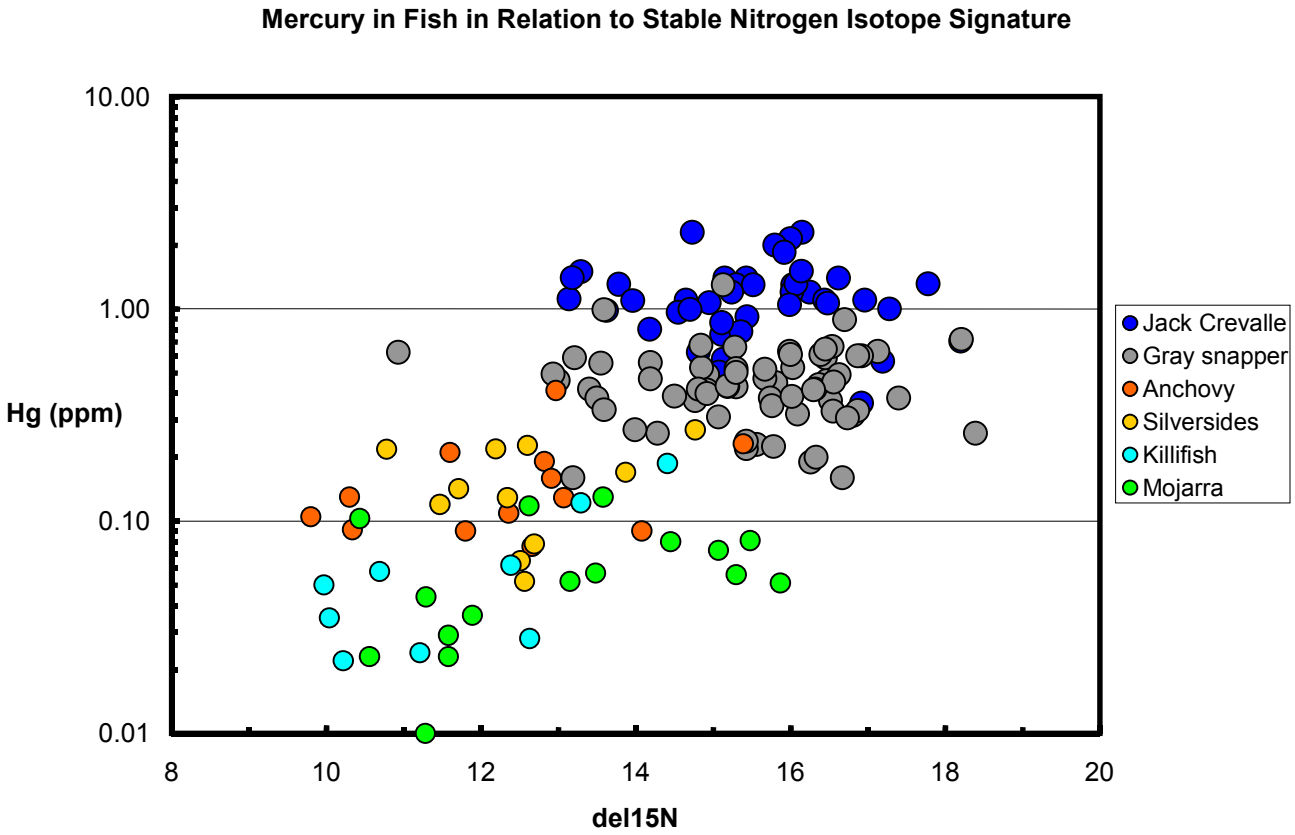

Figure 11. Relationship between THg in fish and stable nitrogen isotope signature

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