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Contribution to the Theme Section 'Range-wide status and conservation of the goliath grouper'

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Mercury concentrations in the goliath grouper of Belize: an anthropogenic stressor of concern

David C. Evers[1,*], Rachel T. Graham[2], Christopher R. Perkins[3], Robert Michener[4], Tim Divoll[1]

[1]BioDiversity Research Institute, 19 Flaggy Meadow Road, Gorham, Maine 04038, USA
[2]Ocean Giants, Marine Program, Wildlife Conservation Society, PO Box 76, Punta Gorda, Belize
[3]Center for Environmental Sciences and Engineering, University of Connecticut, Storrs, Connecticut 06269-5210, USA
[4]IRMS Laboratory, Boston University Stable Isotope Laboratory, Department of Biology, 5 Cummington St., Boston, Massachusetts 02215, USA

ABSTRACT: Global levels of available methylmercury (MeHg) in aquatic ecosystems have increased dramatically over the past century. Recent findings in temperate North America have shown that biological mercury (Hg) hotspots exist, and these hotspots can be related to local emission and effluent sources. Life history traits of the goliath grouper *Epinephelus itajara* place it as a species at high risk of adverse effects from environmental Hg loads. Muscle Hg analyses for 57 goliath groupers sampled in southern Belize reveal that 40% exceed United States governmental advisory criteria for human health; all individual grouper exceeding these criteria were >55 cm total length. People, particularly from coastal areas in southern Belize, commonly consume goliath grouper. The regular consumption of goliath grouper by sensitive groups of people, such as pregnant women, should be closely monitored, particularly in biological Hg hotspots. Stable isotope analysis for $\delta^{13}C$ and $\delta^{15}N$ in goliath grouper indicates a broad prey base with a relatively high trophic status. Through biomagnification and bioaccumulation of MeHg, older individuals are therefore at greatest risk of physiological impairment, particularly when performing complex and coordinated behaviors, such as those associated with spawning aggregations. Potential adverse effects of MeHg loads on goliath grouper, including predator avoidance, impaired growth rates, and lowered reproductive success, warrant investigation. This is particularly urgent for the critically endangered goliath grouper because of recent range-wide population declines, loss of spawning aggregations, and our findings, which present compelling evidence that tropical marine ecosystems are sensitive to Hg inputs.

KEY WORDS: Goliath grouper · Mercury · Belize · Stable isotopes · Human health · Conservation

INTRODUCTION

Mercury (Hg) is a natural element which is redistributed at levels greater than historical patterns through the burning of fossil fuels and Hg-laden waste, industrial use, and activities such as artisanal gold mining. A principal concern with such elevated Hg in the environment is the potential for adverse effects on organisms. The toxic form, methylmercury (MeHg), is of particular concern because of its ability to readily biomagnify in food webs. Humans and certain fish and wildlife species may be at great risk from the effects of environmental MeHg loads through exposure at higher trophic levels of the food web (Mahaffey et al. 2004, Harris et al. 2007). Although the specific concentration of Hg in fish, and, ultimately, people, that poses a risk is not universally accepted, well established guidelines are in place; for example, the United States Environmental Protection Agency (US EPA) has published a human health advisory criterion for fish with muscle Hg concentrations of 0.30 µg g^{-1} wet weight (WW). Scientific evidence of harmful cardiovascular

*Email: david.evers@briloon.org

(Stern 2005), neurobehavioral (Weil et al. 2005), and neurodevelopmental (Mahaffey et al. 2004) effects from the consumption of fish with elevated MeHg concentrations is growing (Anderson 2008). In response to current environmental Hg loads in North America, Mergler et al. (2007, p. 8) concluded that to 'preserve human health, all efforts need to be made to reduce and eliminate sources of exposure.' A similar case can now be made for the health of fish based on an emerging list of recent laboratory and field studies demonstrating adverse effects from elevated MeHg body burdens (Hammerschmidt et al. 2002, Drevnick et al. 2006, 2008, Moran et al. 2007, Larose et al. 2008).

The Mesoamerican Reef is the largest barrier reef system in the western hemisphere and one of the top marine biodiversity sites in the world (Roberts et al. 2002). It is well known for its biologically diverse communities of corals, mangroves, and seagrasses that serve as rich nursery, breeding, and feeding grounds for many commercially and recreationally significant species. In Belize alone, at least 37 species of sharks and 13 species of grouper, including the Caribbean's endemic Nassau grouper *Epinephelus striatus* and critically endangered goliath grouper *E. itajara*, are found along this reef. However, many shark, grouper, and other fish species are rapidly declining in the Mesoamerican Reef because of unsustainable fishing pressures, habitat degradation, and other anthropogenic stressors (Sala et al. 2001, Mumby et al. 2004, Graham et al. 2009, this Theme Section).

We chose this area as a case study because of its global biological importance, conservation needs from increasing recreational and development pressures, and existing scientific infrastructure. Because little is known about MeHg cycling in marine tropical ecosystems in Central America, the template we create here for understanding the (1) distribution of Hg in estuarine and marine systems, (2) sources of anthropogenic Hg, (3) availability to biota, (4) food web dynamics based on stable isotope analyses, and (5) impacts on human and ecological health, can be applied to other areas of the Caribbean. In the present study, we establish a preliminary baseline for Hg exposure in goliath grouper *Epinephelus itajara* in southern Belize to help characterize potential human and ecological health concerns in coastal marine ecosystems.

MATERIALS AND METHODS

Study area. The study area is associated with the Mesoamerican Reef in the Gulf of Honduras of southern Belize (Fig. 1), an embayment bordered by Guatemala and Honduras. Southern Belize encompasses coastal fluvial, estuarine, and mangrove ecosystems favoring young-of-the-year to subadults (up to Age 6), as well as nearshore and offshore seagrass, patch reef, and barrier reef sites that are the preferred habitat of adult goliath grouper (Bullock et al. 1992, Sadovy & Eklund 1999). The present study focused on an embayment adjacent to Guatemala with over 4665 km^2 of coastal grouper habitat delineated by the Sarstoon River, which separates Belize from Guatemala, east to the Sapodilla Cayes on the Belize Barrier Reef and north of Tobacco Caye on the barrier reef to the mouth of the Sittee River located near the coastal village of Riversdale.

Field sample collection. Goliath groupers were sampled and measured at the Punta Gorda fish market in Belize from January 2006 to December 2007 (Graham et al. 2009). Biopsies of muscle tissue from the dorsal and pectoral musculature were removed from goliath grouper between March 2006 and November 2007. Although specific locations within our study were not identified for individuals, we are confident that fish sampled at the Punta Gorda market represent our defined study area based on fisher interviews confirming fishing locations. Samples of 10×40 mm of muscle tissue were cataloged, stored, and frozen in 10 cc

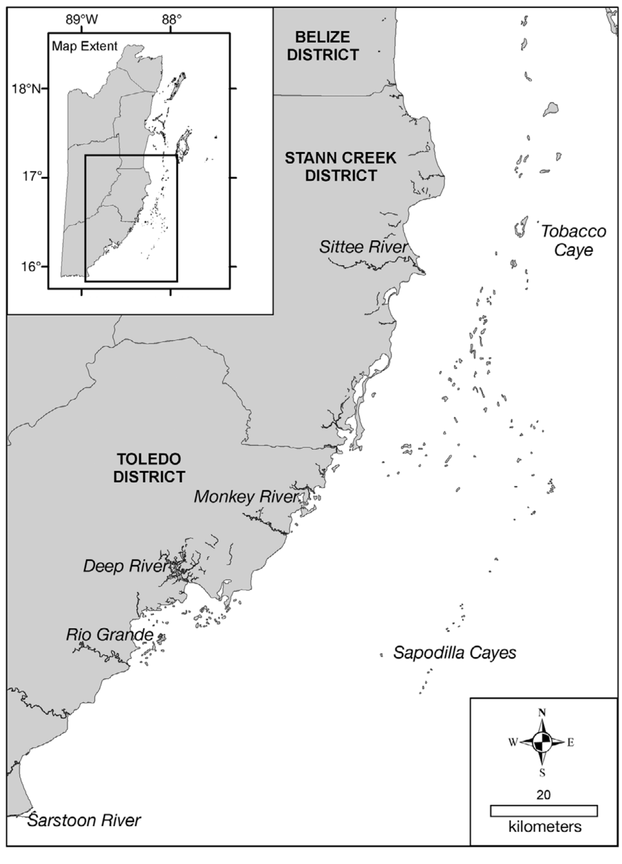

Fig. 1. Study area (rectangle in inset), showing 3 coastal districts of Belize

transport tubes and shipped to the USA. Biopsies of muscle tissue from the base of a fin (Gremillion et al. 2005) and other areas of the body (Peterson et al. 2004) strongly correlate with muscle tissue most commonly consumed by people and are not significantly different from traditional methods of sampling and analysis (Baker et al. 2004). Total length (TL) and weight of freshly caught individuals were collected. Length–weight relationships were determined for goliath grouper landed in Punta Gorda, southern Belize from December 2005 to December 2007 (n = 890) (Graham et al. 2009).

Mercury analysis. All samples were submitted to the Center for Environmental Sciences and Engineering at the University of Connecticut. Each grouper muscle sample was thawed, a 5.0 g piece was removed and homogenized, and a 0.5 g subsample was placed into a 50 ml hot block tube. Four ml of concentrated sulfuric acid and 1 ml of concentrated nitric acid were added to each tube and placed in the hot block at 58°C for 60 min. The tubes were removed from the hot block, cooled to room temperature and 5 ml of potassium permanganate ($KMnO_4$) solution was added. If the sample did not maintain a standard color, more $KMnO_4$ was added in increments of 2 ml until a standard color was maintained. Eight ml of potassium persulfate ($K_2S_2O_8$) was added to each tube and the samples were allowed to stand overnight at room temperature. Two ml of hydroxylamine hydrochloride (NH_2OH-HCl) was added to each tube and then brought up to a final volume of 50 ml using deionized water. All grouper samples were subsequently analyzed on a Perkin Elmer flow injection mercury system (FIMS) cold vapor atomic absorption spectrometer using standard protocols at the University of Connecticut. Quality assurance procedures were employed, including analysis of duplicate samples, method blanks, post-digestion spiked samples, laboratory control samples, and standard reference materials (DOLT-3, National Research Council Canada). Instrument response was evaluated initially, every 20 samples, and at the end of an analytical run using a calibration verification standard and blank. Percent moisture averaged 82 ± 4 % on a subset of 15 samples; this is indicative of typical moisture content for fish muscle tissue and does not confound interpretation of Hg concentrations.

Mercury concentrations of concern. Human health advisory levels for the consumption of fish vary according to different governmental bodies. The US EPA uses a criterion of 0.30 µg g^{-1} WW , while the World Health Organization (WHO) uses an advisory threshold of 0.50 µg g^{-1} WW, except for predatory fish, which have a 1.00 µg g^{-1} WW threshold (WHO 2007). We used the latter WHO threshold for the predatory goliath grouper.

Stable isotope analysis. Samples were also shipped to the Boston University Stable Isotope Laboratory for analysis of δ^{13}C and δ^{15}N. Samples were analyzed using automated continuous-flow isotope ratio mass spectrometry (Michener & Lajtha 2007). All specimens were subsampled and oven-dried at 60°C for 24 h. They were then powdered using a mortar and pestle. The samples were combusted in a EuroVector Euro EA elemental analyzer to provide C:N ratios. The combustion gases (N_2 and CO_2) were separated on a chromatographic column, passed through a reference gas box, and introduced into the GV Instruments IsoPrime isotope ratio mass spectrometer; water was removed using a magnesium perchlorate water trap. Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as the relative per mil (‰) difference between the samples and international standards (Vienna PeeDee Belemnite [VPDB] carbonate and N_2 in air):

$$‰X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \text{ (‰)}$$

where $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The sample isotope ratio was compared to a secondary gas standard, whose isotope ratio was calibrated to international standards. All international standards were obtained from the National Bureau of Standards (NBS) in Gaithersburg, Maryland. For $^{13}C_{VPDB}$ the gas was calibrated against NBS 20 (Solenhofen limestone). For $^{15}N_{air}$ the gas was calibrated against atmospheric N_2 and International Atomic Energy Agency (IAEA) standards N-1, N-2, and N-3 (all are ammonium sulfate standards). In addition to carbon and nitrogen isotopes from the same sample, continuous flow also reported % C and % N data.

Statistical analysis. Mercury concentrations, length–weight relationships, and stable isotope ratios were normally distributed and met homoscedasticity assumptions. An unequal variance *t*-test was used to determine significant differences at $\alpha = 0.05$; this test is considered equivalent to a Student's *t*-test in its ability to control for Type 1 and Type 2 errors, and superior to the Mann-Whitney *U*-test assuming the underlying sample size exceeds 30 and is normally distributed (Ruxton 2006). In order to compare Hg concentrations in a standardized way for individual fish representing a range of sizes, length adjustments were simply made by dividing Hg concentration by TL × 100.

RESULTS

A total of 57 goliath groupers from southern Belize were sampled for Hg concentrations and δ^{13}C and δ^{15}N isotope values in 2006 and 2007. Of these, 40% exceeded the US EPA criterion (Fig. 2).

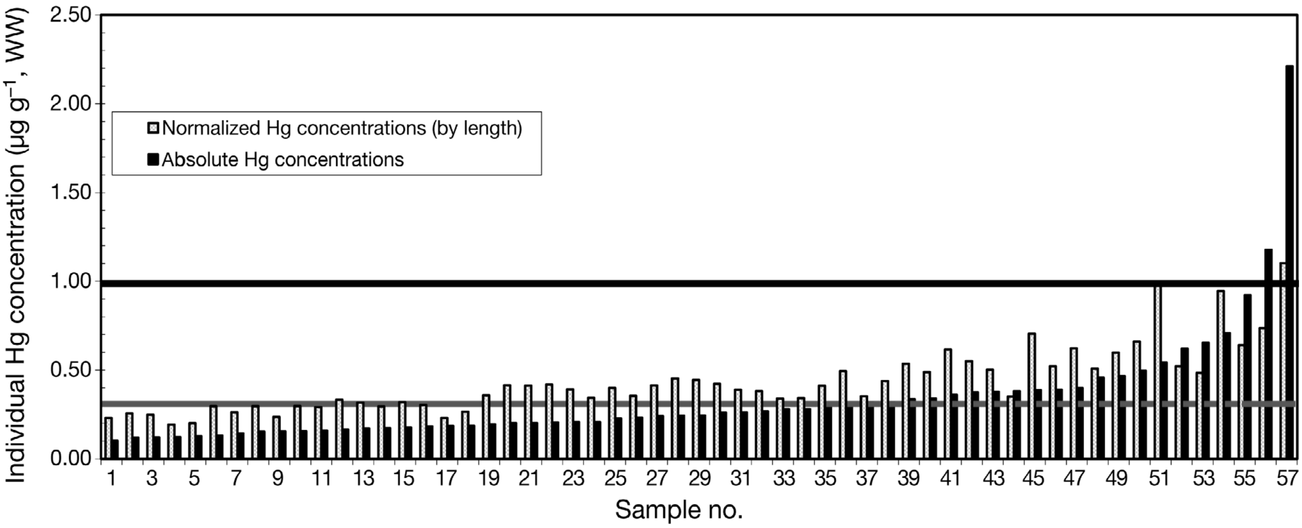

Fig. 2. *Epinephelus itajara*. Distribution of muscle Hg concentrations (µg g^{-1} wet weight [WW]) for goliath grouper (n = 57) in Belize. A total of 40 % of the groupers sampled were above the US Environmental Protection Agency human health standard for Hg of 0.30 µg g^{-1} WW (gray line). The World Health Organization human consumption advisory level is 1.0 µg g^{-1} WW (black line)

The size distribution of groupers was skewed to smaller and younger individuals. There was a highly significant length–weight relationship (n = 890, r^2 = 0.98, p < 0.001). Comparison of length-normalized and absolute Hg concentrations between smaller mangrove-dependent individuals (TL < 110 cm) and larger offshore dwelling individuals (TL > 110 cm) indicated significantly higher Hg concentrations in the offshore individuals for normalized (t = 2.77, df = 4, p = 0.05) and absolute (t = 2.92, df = 4, p = 0.04) concentrations (Fig. 3).

Unexpectedly, the $\delta^{15}N$ values for large, reef-dependent grouper were not significantly different from those for smaller, mangrove-dependent individuals (t = 0.71, df = 7, p = 0.50); however, these same groups differed significantly in $\delta^{13}C$ values (Fig. 4).

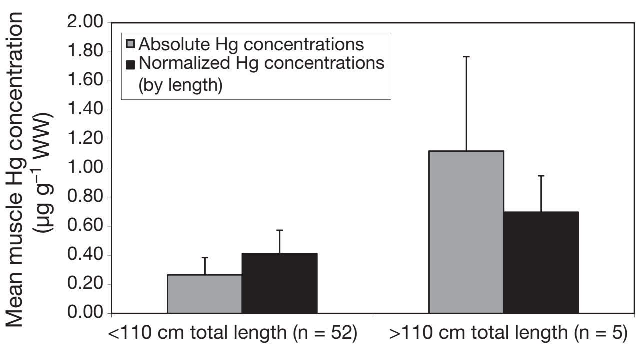

Fig. 3. *Epinephelus itajara*. Comparison of subadult and breeding adult goliath grouper Hg concentrations. Age class is designated by total length (TL), where individuals of TL < 110 cm are subadults and those of TL > 110 cm are adults

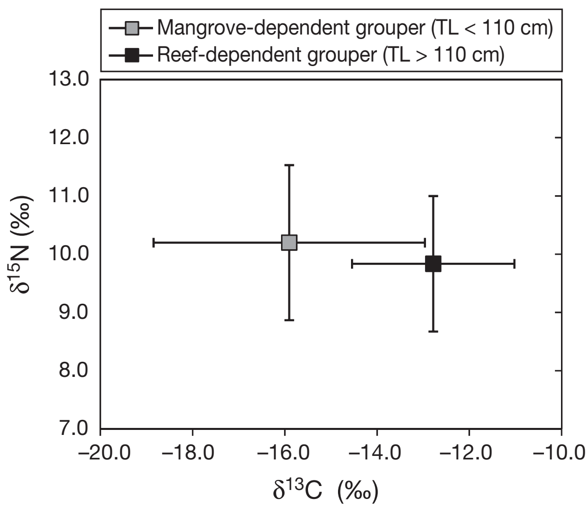

Fig. 4. *Epinephelus itajara*. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ and their relationship with 2 size classes. Grouper inhabit mangrove habitats up to Age 6 or approximately <110 cm in total length (TL) and move to reef areas thereafter (TL > 110 cm)

DISCUSSION

Although global atmospheric deposition models indicate relatively low rates of Hg input into Central America (Mason et al. 1994), there is evidence that tropical marine ecosystems are sensitive to Hg input, suggesting more efficient bioaccumulation than in temperate marine ecosystems. Guzman & Garcia (2002) characterized Hg concentrations in coral from reefs in Panama and Costa Rica for areas without known, elevated point sources. They suggested deleterious Hg effects with land use origins that included legacy gold mining, erosion following deforestation, and recent shoreline development. While fish Hg concentrations for nearshore waters of Belize are not known, recent studies in the northern Gulf of Mexico provide a context suitable for comparison of marine fish Hg concentrations within a similar trophic level (Bank et al. 2007, Lewis & Chancy 2008).

The goliath grouper is a large, long-lived marine fish. Individuals may reach 2.55 m and 308 kg, and are known to live up to 37 yr (Heemstra 1991, Sadovy & Eklund 1999). Although marine fish with these life history characteristics are well recognized for an ability to bioaccumulate MeHg, and include sharks (Garcia-Hernandez et al. 2007), tuna (Storelli et al. 2002), and swordfish (Storelli & Marcotrigiano 2000), Hg concentrations in goliath grouper are poorly documented (Sadovy & Eklund 1999). Our analysis provides the first evaluation of Hg for the goliath grouper and highlights potential concerns regarding Hg and human and ecological health along the Mesoamerican Barrier Reef.

Human health concerns

In the present study, we demonstrate that Hg concentrations in goliath groupers regularly exceed those reported for the Gulf of Mexico as well as the US EPA human health criterion. Mercury concentrations (absolute and normalized values) are highest for the adult grouper inhabiting offshore cayes and banks. Smaller, coastal, and estuarine mangrove-dependent individuals begin exceeding the US EPA criterion of 0.30 µg g^{-1} WW at 55 cm in length. All individuals over 83 cm in length had Hg concentrations greater than the US EPA criterion. Comparisons of length-normalized Hg concentrations in red snappers *Lutjanus campechanus* from within the Gulf of Mexico (Bank et al. 2007) indicate that mean Hg concentrations in similar-sized goliath grouper inhabiting southern Belize mangroves are approximately 40% higher. While red snappers may occupy, on average, a higher trophic position than groupers (δ^{15}N of 14.7 vs. 10.2, respectively), specific comparisons are difficult because we lack isotopic baselines needed for appropriate interpretation (Post 2002).

The amount and frequency of fish consumed, their corresponding Hg body burdens, and the sensitivity of a person to MeHg toxicity are factors that determine human health risk (Mahaffey et al. 2004, Mergler et al. 2007). Marine fish and shellfish Hg concentrations are poorly documented for coastal areas of Central America; therefore, characterizing Hg exposure risk to Belizeans is difficult. Mercury concentrations in the blood of American women are well described by the US National Health and Nutrition Examination Survey (NHANES) (Mahaffey et al. 2004). Mean Hg concentrations of goliath grouper (0.34 µg g^{-1}, range = 0.10 to 2.21 µg g^{-1}) exceed 80% of the mean Hg concentrations of marine fish and shellfish species that form the basis of NHANES studies (Mahaffey et al. 2004). In 2000, Belizean fish consumption was estimated annually at 15 kg per person (WRI 2003). These consumption rates exceed the annual 4.3 kg per person consumption used by the US EPA (for women between the ages of 15 and 44). Assuming that Belizeans consume fish with average Hg concentrations greater than those consumed in the USA, we speculate that at least one-third of Belizean women could be ingesting harmful Hg concentrations from diets of local fish.

The Global Mercury Assessment Program of UNEP recently convened Caribbean countries to collectively evaluate environmental Hg loads for the region (UNEP 2005). Their findings highlight the paucity of available data, yet strong recommendations were prepared to better understand potential threats from environmental Hg loads on ecological and human health. Our analysis strengthens the need for a more thorough evaluation of the risk that marine fish and shellfish Hg concentrations pose to people in coastal communities in Belize and neighboring countries.

Ecological health concerns

Environmental Hg loads and their potential effects on fish are poorly known in tropical marine ecosystems of the Caribbean, including the coastline of Belize (Lacher & Goldstein 1997, UNEP 2005). The few Caribbean studies describing Hg concentrations in marine fish were generally triggered by known point sources such as chlor-alkali plants in Columbia (Olivero-Verbel et al. 2008) and gold mining activities (Olivero et al. 1997, Mol et al. 2001). One coastal study, near Cumaná in Venezuela, documented mean Hg concentrations for 17 species of fish at 0.10 ± 0.04 µg g^{-1} WW (SD), with a maximum of 0.33 µg g^{-1} WW for a 2000 g tajali *Trichiurus lepturus*, in an area without known local anthropogenic Hg sources (Shrestha et al. 1988).

The highly elevated concentrations of Hg found in goliath grouper from the present study are unexpected, given the lack of known proximal point sources. While the northern lagoons of Belize appear to have relatively low MeHg availability to high tropic level predators such as the Morelet's crocodile *Crocodylus moreletii* (Rainwater et al. 2002), southern Belize mangrove and coral reef habitats appear to be either sensitive to Hg input or affected by large undescribed point sources.

Spatial heterogeneity of MeHg availability or biological Hg hotspots is an established phenomenon in temperate freshwater aquatic ecosystems (Evers et al. 2007). Mechanisms for such spatial gradients in tropical marine ecosystems remain relatively undefined, although marine-oriented biogeochemical cycling models for MeHg are being developed (Fitzgerald et al. 2007) and could contribute to greater understanding.

Considering the life history traits of groupers, the general lack of scientific investigation into the ability of goliath grouper and other grouper species to biomagnify and bioaccumulate MeHg is surprising. For example, recent governmental evaluations of continental US goliath grouper populations did not identify environmental Hg loads as a known or potential threat (NMFS 2006), even though the core of the grouper's range in Florida has well described environmental Hg burdens in estuarine and marine fish (Kannan et al. 1998, Adams & McMichael 1999, Strom & Graves 2001)

Mercury concentrations in some goliath grouper individuals are consistent with documented adverse effects in other fish species, including predator evasion, spawning success, and body weight (Friedmann et al. 1996). Little is known about sublethal effects of MeHg on marine fish. However, investigations involving a marine species, the Atlantic croaker *Micropogonias undulatus*, demonstrate that consumption of MeHg-contaminated food of 0.10 µg g^{-1} WW d^{-1} by adults resulted in maternal body burdens of MeHg transferred to larvae as well as to negatively impacted survival skills such as predator evasion behavior (Alvarez et al. 2006), an effect from MeHg that is also found in freshwater fish (Webber & Haines 2003). Such sublethal effects can have significant impacts on predation rates of larvae (Murphy et al. 2008). In neighboring Guatemala, mean Hg concentrations in various relatively small fish species and even saltwater shrimp *Carcinus moenas* were equal to or exceeded 0.10 µg g^{-1} WW in the Gulf of Honduras community of Puerto Barrios, Guatemala (Ramos et al. 1979).

The effects of MeHg on spawning success have been demonstrated in laboratory-dosing studies of freshwater fish (Hammerschmidt et al. 2002, Drevnick et al. 2008) at Hg concentrations regularly found in goliath grouper of southern Belize. Goliath grouper reproduce in spawning aggregations that may include 10s to over 100 individuals (Sadovy & Eklund 1999). The potential negative effects of environmental pollutants on complex fish behaviors (Scott & Sloman 2004), coupled with our findings of a strong relationship between increasing muscle Hg and size, suggest that the reproductive potential for large groupers may be compromised.

Ecological redundancy and associated resistance to ecological changes from human disturbances in marine ecosystems varies geographically, and potential recovery to former levels of ecosystem functioning is complex and not well understood, particularly for tropical nearshore areas (Palumbi et al. 2008). The goliath grouper was formerly common in coastal Belize until the 1980s (Graham et al. 2009). The loss of this apex predator could create changes in community function and structure similar to the dramatic changes to fisheries which occurred when shark populations were severely reduced (Myers et al. 2007). In response, active investigations to determine the source of coastal environmental Hg loads in Belize and patterns of spatial Hg gradients are necessary. In the continental US (primarily Florida), goliath grouper populations have benefited from protective measures including a complete ban on fishing (NMFS 2006). Conservation and, ultimately, recovery of Belizean goliath grouper populations are more tenuous than in Florida, especially if anthropogenic stressors such as greater environmental Hg loads should impede growth rate, reproductive success, and overall fitness.

We recommend identification of point sources and standardized Hg monitoring programs to evaluate spatiotemporal trends and potential risk to human and ecological health. Descriptions of Hg monitoring programs developed for temperate North American landscapes (Harris et al. 2007) including estuarine and marine ecosystems (Evers et al. in press) are available and transferable to tropical ecosystems. Such standardized monitoring efforts will serve as important benchmarks for evaluating adverse effects of Hg in other grouper species and help establish best management practices for declining grouper populations.

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Editorial responsibility: Kevin Rhodes, Hilo, Hawaii, USA

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