BIOACCUMULATION OF MERCURY IN PELAGIC FISHES IN NW GULF OF MEXICO

A Thesis

by

YAN CAI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Wildlife and Fisheries Sciences

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Approved as to style and content by:

Jay R. Rooker (Chair of Committee)	Gary Gill (Member)
Delbert M.Gatlin III (Member)	Robert D. Brown (Head of Department)

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ABSTRACT

Bioaccumulation of Mercury in Pelagic Fishes in NW Gulf of Mexico.

(May 2005)

Yan Cai, B.S., Xiamen University

Chair of Advisory Committee: Dr. Jay R. Rooker

Total mercury (Hg) levels were determined in the tissues of ten taxa of pelagic fishes, with a special emphasis on apex predators (large vertebrates). Highest Hg levels were observed in blue marlin (*Makaira nigricans*), carcharhinid sharks (Genus *Carcharhinus*) and little tunny (*Euthynnus alletteratus*), ranging from 1.08 to 10.52 ppm. Moderate to low concentrations (<1.0 ppm) were observed in blackfin tuna (*Thunnus atlanticus*), cobia (*Rachycentron canadum*), dolphinfish (*Coryphaena hippurus*), greater amberjack (*Seriola dumerili*), king mackerel (*Scomberomorus cavalla*), wahoo (*Acanthocybium solandri*) and yellowfin tuna (*Thunnus albacares*). For the majority of species examined, Hg level did not vary significantly between locations (Texas and Louisiana) and years (2002 and 2003). The relationship between Hg level and fish size/weight was also explored and six taxa (blackfin tuna, carcharhinid sharks, dolphinfish, king mackerel, wahoo, yellowfin tuna) showed significant positive relationships between Hg level and body size and/or weight. Natural dietary tracers, stable isotopes ($\delta^{15}N$, $\delta^{13}C$) and fatty acids were used to evaluate the relationship between Hg and trophic position and the relationship between Hg and dietary history. Stable nitrogen isotope analysis showed that Hg levels in fish tissues were positively associated with trophic position. Based on the $\delta^{13}C$ and $\delta^{15}N$ values of pelagic

consumers examined in this study, three natural groups were identified with cluster analysis, and the same groupings were detected based on fatty acid profiles. This not only confirmed the existence of these natural groupings, but also indicated that the distinguishing factors for the grouping was likely connected with the dietary history of these fishes. The classification tree based on the fatty acid profiles of pelagic fishes readily separated fishes from different regions, suggesting that diets of pelagic taxa within the same region are similar or these consumers share a common source of organic matter in their food web. Findings from this study complement other Hg investigations conducted in the Gulf and also furthered our understanding of the link between feeding ecology and Hg accumulation. Moreover, the combined use of stable isotope and fatty acid techniques provided new insights on the dietary history of pelagic fishes in the Gulf of Mexico.

DEDICATION

To

My parents

Shanquan Cai and Yvqin Wang

ACKNOWLEDGEMENTS

On the top of the list of all those I need to thank, is my major advisor: Dr.Jay R.Rooker. From day one, and all through these three years, he has been such a great advisor/professor as well as a good friend. Not only that he constantly guided me through the maze of graduate study, but also he always made sure that I have a comfortable, enjoyable time during my stay in US. Jay, you are an exceptional professor as well as an exceptional person. As your graduate student, I admire all your academic achievements as well as your personality. You have set a great example for all of us to follow. Also, I thank Richard Kraus for your valuable help, suggestion and advises received from you. I can not tell you how much I have benefited from your funny, intelligent and honest talks. A special thanks to Jason Turner for his help and scientific contribution to this study. I appreciate all the field assistance provided by Mike Lower. I also need to thank all the fellows in the Rooker's Lab (Jessica Beck, Joseph J.Mikulas and Lindsay Glass) and LOER lab (Ron Lehman, Key-Young Choe and Seunghee Han) for their everyday assistance. I'm especially grateful for Dr.Gary Gill and Ron Lehman's help in my mercury research. Last, but not the least, I thank my parents, for teaching me how to be an open-minded, happy and loving person all through these years. Their unconditional love and support provided the ultimate strength for me to step forward, in good times and bad times.

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INTRODUCTION

Mercury accumulation in pelagic fishes

Mercury (Hg) is a heavy metallic or silver colored secondary mineral liquid at room temperature. Due to its extreme volatility, Hg enters the atmosphere very easily. The two main sources of Hg release are natural processes (e.g. volcanic eruption) and human-related activities (e.g. fossil fuel combustion, gold mining) (SEMARNAP 1996, Malm et al 1995). It is estimated that about two-thirds of the total world's yield of Hg has been produced during the twentieth century, and human related inputs of Hg to the environment have increased about 3-fold since 1900 (Andren & Nriagu 1979). There are three forms of Hg (elemental, inorganic and organic), and all three forms are harmful to humans and wildlife. However, the organic form, mono methylHg (MeHg), is the major exposure pathway to humans (Fitzgerald & Clarkson 1991, Wiener et al. 2003). Once Hg falls to earth and becomes dispersed in water, rain, snow, and dry particles, it can be converted by microorganisms to MeHg (Gilmour et al. 1998, Gill et al. 1999, Benoit et al. 2003). MeHg is a neurotoxin that can cause nervous system disorders by crossing the blood brain barrier, and prenatal life is more susceptible to brain damage from MeHg since it inhibits cellular processes basic to cell division and neuronal migration (Choi et al. 1978, Clarkson 1987, 1992).

MeHg is easily absorbed in the body tissue of aquatic plant and animals, and can be passed by consumption to higher trophic levels in aquatic food chains (Lawson &

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Mason 1998, Simon & Boudou 2001). Once MeHg is inside the flesh, it is not readily eliminated and can accumulate from one trophic level to the next such that levels in higher order marine consumers (e.g., tunas, sharks, billfishes) may be more than a million times higher than the surrounding water (U.S.EPA 1997). Because MeHg comprises an average of more than 95% of the total Hg in fish, total Hg is often measured to represent the MeHg level in fish (Bloom 1998). Since many of the species targeted by recreational and commercial anglers have been shown to contain elevated levels of MeHg (Ache et al. 2000), public concern for human health problems associated with Hg contamination has increased substantially. Currently, fish consumption advisories for Hg exist in 45 states, with Alabama, Florida, Georgia, Hawaii, Louisiana, Maine, Mississippi, North Carolina, South Carolina, and Texas having coastal fish consumption advisories (FDA 2001, U.S. EPA 2004a, 2004b).

Many biotic, ecological, and environmental factors contribute to the uptake of MeHg in fishes (Wiener et al. 2003), with dietary uptake accounting for more than 90% of the total uptake of MeHg in wild fishes (Hall et al. 1997). When the rate of elimination of Hg is lower than the uptake rate, concentration of MeHg increases with increasing age or body size of the fish (Walker 1976, Wiener & Spry 1996). The uptake of MeHg is also influenced by diet history and trophic position, with rates of Hg accumulation higher for fishes feeding at a higher trophic position (Freeman et al. 1978, Lyle 1984, Cabana & Rasmussen 1994, Cabana et al. 1994, Wiener et al. 2003). Moreover, other studies have shown the spatial variation in fish Hg levels was attributed to environmental factors like pH, water temperature, lake size, and dissolved organic carbon (DOC) concentrations that control the biogeochemical processes and transformation of MeHg in the ecosystem (Bodaly et al.1984, Häkanson et al.1988, Bodaly et al. 1993).

Using stable isotope and fatty acid analysis

Since most MeHg is transferred up the food chain, information on the feeding ecology of marine consumers is needed to determine the source(s) of MeHg and examine the bioaccumulation of this stressor in marine consumers. Since conventional gut analysis has a number of inherent constraints (e.g. only reflects short-term information on diet, provides limited information on source of organic matter and intermediate links in the food web), stable isotope analysis and fatty acids analysis are increasingly used to reconstruct feeding histories (Iverson 1993, 1997a, 1997b, Kirsch et al.1998, Brown et al. 1999, Kaehler et al. 2000, Kharlamenko et al. 2001, Kurle & Worthy 2001, Hatase et al. 2002, Adams et al. 2003a). To date, stable isotopes and fatty acids have been used extensively to improve our understanding of trophic relationship within aquatic systems. Recently, dietary tracer analysis has been coupled with contaminant analysis to determine the pattern and extent of biomagnification of Hg and other contaminants in aquatic food webs (Kidd et al. 1995, Cook et al. 2004).

Stable isotope analysis is particularly well suited to identify dominant pathways of carbon transfer in food webs because this method can estimate assimilation of food resources over time (Peterson & Fry 1987). Studies employing stable isotope analysis can provide information on the origins and pathways of organic matter because the isotopic composition of consumer tissue is closely related to that of their diet (de Niro & Epstein 1976, Wada et al. 1991). Carbon stable isotope values ($\delta^{13}C$) in the muscle tissue of marine consumers are typically enriched by ~1‰ per trophic level (Fry & Sherr 1984, France & Peters 1997), and thus it is possible to use this natural marker to estimate the

source(s) and contribution of organic matter to each consumer (Thayer et al. 1978, McConnaughey & McRoy 1979, Fry et al. 1987, McClelland & Valiela 1998). In contrast, fractionation of nitrogen stable isotopes ($\delta^{15}N$) is approximately 3 to 4‰ per trophic level and thus $\delta^{15}N$ values have been used to delineate the trophic position of consumers (Owens 1987, Wada et al. 1991). Therefore, the different fractionation rates of $\delta^{13}C$ and $\delta^{15}N$ allows a cross-referenced analysis to determine both the consumer's diet and its trophic position within the food web (Fry 1983, Peterson et al. 1985, Jepsen & Winemiller 2002, Rooker et al. 2004).

Although coarse in taxonomic resolution, stable isotope data can quickly reveal important feeding links among consumers, and the approach can overcome some of the methodological limitations associated with stomach contents analysis. Nevertheless, when producer signatures are similar it is difficult to assess the contribution of different food sources and feeding linkages using only stable isotopes. Consequently, alternative approaches have been sought to indirectly quantify trophic interactions and overcome the deficiencies of past studies. It has long been recognized that storage lipids, particularly fatty acids, are influenced by diet (e.g. Cowey et al. 1976). Fatty acids are the largest constituent of lipids and those of carbon chain length 14 or greater are often deposited in animal tissue via their diet (Sargent & Whittle, 1981). Diet type has been found to influence fatty acid composition of consumer tissue and it has been suggested that certain fatty acids, or their ratios, can be used to provide a more precise indication of an organism's diet than conventional approaches (McGee 1996). In fact, fatty acids are increasingly being used as chemical markers of biogeochemical processes and trophic

relationships (Clarke et al. 1987, Edrington et al. 1995, Napolitano 1998). To be useful as a trophic marker, a fatty acid must be synthesized at low trophic levels and then transferred unchanged (or in a recognizable form) to upper levels of the food web. Many novel fatty acids exist in the marine environment can only be biosynthesized by certain primary producers. Such fatty acids are often essential dietary components of higher order consumers and useful natural biomarkers for reconstructing trophic relationships (Ackman 1980, Sargent et al. 1988). Long-chain polyunsaturated fatty acids (PUFA) are primary examples of such essential dietary components, and many studies have successfully used these fatty acids to reconstruct feeding histories (Graeve et al. 1994a, 1994b, Iverson et al. 1997a, 1997b, Brown et al. 1999, Turner & Rooker 2005a, 2005b).

Here, I examine the trophic structure and contaminant bioaccumulation of Hg in pelagic fishes in the Gulf of Mexico. Efforts were focused on determining contaminant loads of Hg across several pelagic taxa present in the Gulf. Stable isotopes and fatty acids were also used as indicators of trophic position and feeding history of consumers, and related to observed patterns of MeHg in the tissue of top-level predators.

Objectives

1. Measure Hg level in the tissue of pelagic fishes from the NW Gulf of Mexico.
2. Assess spatial and annual variation in Hg level in the tissue of pelagic fishes.
3. Determine the relationship between fish size (length, weight) and Hg level in the tissue of pelagic fishes.

4. Explore the relationship between trophic position (based on analysis of stable nitrogen isotopes) and Hg in the tissue of pelagic fishes.
5. Relate feeding histories to Hg levels in pelagic fishes.

METHODS

Sample collection

Pelagic fishes were sampled at docks from two regions of the NW Gulf: Galveston/Freeport, Texas and Venice, Louisiana (Fig 1). In addition, samples were collected with hook-and-line to complement dock sampling efforts. To assess annual variation, samples were collected in two years (2002, 2003). For each fish sample, approximately 20g of muscle tissue were removed from the dorsal region behind the head. All tissue samples were placed into sample bags individually, labeled with collection date, species name, and fish length (total length in cm). Samples were stored on ice in a cooler before being transported back to laboratory and put into a freezer (-20 ℃). Ten taxa/species were targeted.

Determination of total Hg in fish tissues

Measurements of total Hg in fish tissue were conducted using a Milestone DMA-80 Direct Hg Analyzer (Cizdziel et al. 2002). Fish muscle samples were taken directly from the freezer, cut into small pieces (ca. 0.1-0.3 g), weighed and placed directly into sample boats for analysis. The instrument was calibrated each day before Hg analysis. Calibration was conducted using standard reference materials (SRM) prepared by the National Research Council of Canada (TORT-2 and DORM-2). Blanks and separate standard reference materials (DORM-1 or 1566a) were always analyzed at the beginning of every batch of ten samples to assess accuracy. Blanks consisted of an empty boat. Three replicates were conducted for the first three samples of each batch. If the relative percent difference was within 10%, the rest of the batch was analyzed once

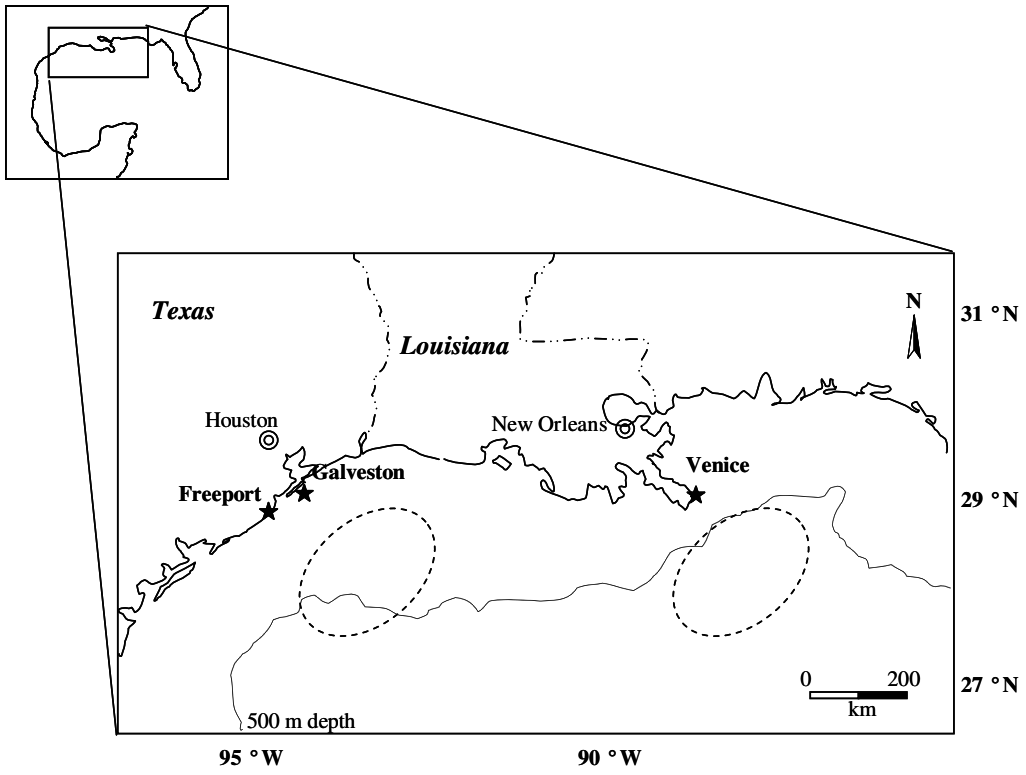

Fig 1. Map of sampling locations: offshore area near Galveston/Freeport in Texas and Venice in Louisiana in NW Gulf of Mexico.

only. Otherwise, beginning samples of the batch were run again. Fish samples that did not fall within the concentration range of the standards were re-analyzed with a more appropriate amount of wet tissue. To evaluate the dehydration effect on samples with different storage times, a second piece of each sample was weighed when the first piece was introduced into the instrument for Hg analysis. After drying in the oven at 50 ℃ for 48 hrs, they were weighed again to determine the water concentration of the tissue at the time of analysis and then introduced into the instrument for Hg analysis. We observed no significant dehydration effect (constant dry Hg concentration/wet Hg concentration ratio, $R^2 \approx 1$, Fig 2); therefore, only wet weight Hg concentrations were reported.

Calibration curves were highly linear (mean: $R^2 = 1.00$; range: $R^2 = 0.97 – 1.00$; n = 68). Regression equations from the above analyses were used to correct the wet-weight Hg concentration data directly measured by the DMA-80 Hg analyzer. SRM (DORM-2 or DOLT-1) was analyzed every 10 samples and the recovery from these samples ranged from 90.6 % to 112.2 % and averaged 97.8 % (SD = 5.2 %) (Table 1). Detection limits was 0.2 ng/g. The coefficient of variation of Hg concentration in triplicate samples ranged from 0.3 to 7.1 %, and averaged 2.9 %. Because MeHg comprises an average of more than 95% of the total Hg in fish (Bloom 1998), total Hg was measured to represent the MeHg level in fish in the present study. To simplify the presentation, the term “Hg level” is used from this point on to represent total Hg level (wet wt. concentration) in muscle tissue.

Table 1. Total Hg level in Standard Reference Materials (SRM) as tested through the whole study period (2004), the accuracy of the determination was also included.

	[Hg]total (ppm) wet wt.		
Date	Determined	Certificated	Recovery
Jan 27	4.39	4.64 ± 0.26	94.5%
Jan 27	4.39	4.64 ± 0.26	94.7%
Jan 31	4.37	4.64 ± 0.26	94.1%
Feb 3	4.73	4.64 ± 0.26	101.9%
Feb 17	4.49	4.64 ± 0.26	96.7%
Feb 20	0.25	0.225 ± 0.037	112.2%
Feb 24	4.32	4.64 ± 0.26	93.1%
Feb 24	0.06	0.0642 ± 0.0067	90.6%
Mar 5	4.86	4.64 ± 0.26	104.8%
Mar 9	4.40	4.64 ± 0.26	94.7%
Mar 9	0.28	0.27 ± 0.059	104.0%
Mar 22	4.42	4.64 ± 0.26	95.2%
Mar 22	4.41	4.64 ± 0.26	95.1%
Mar 29	4.51	4.64±0.26	97.3%
Apr 5	4.40	4.64±0.26	94.7%
Apr 20	4.52	4.64±0.26	97.4%
May 10	4.24	4.64±0.26	91.4%
May 25	0.24	0.225±0.037	104.8%
Jun 24	4.46	4.64±0.26	96.0%
Jul 14	4.72	4.64±0.26	101.7%
Jul 28	4.54	4.64±0.26	97.9%
Jul 30	4.63	4.64±0.26	99.7%
Average			97.8%
SD			5.2%

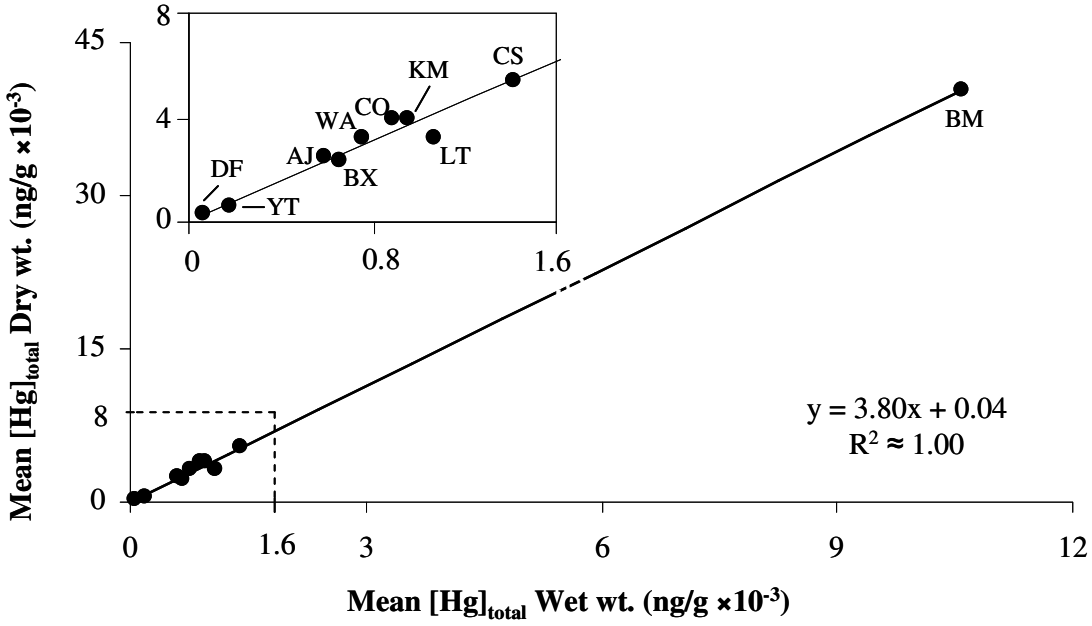

Fig 2. Water content of ten taxa of pelagic fishes (expressed as dry Hg con./wet Hg con. Ratio) was constant ($R^2 \approx 1.00$, range 0.99-1.00). Each solid point represents one species/taxa. Inset graph is the enlarged image of the dashed part of the graph. AJ: greater amberjack, BM: blue marlin, BX: blackfin tuna, CO: cobia, CS: carcharhinid sharks, DF: dolphinfish, KM: king mackerel, LT: little tunny, WA: wahoo, YT: yellowfin tuna.

Stable isotope analysis

Tissue samples from randomly selected individuals (~ 5 per species) of nine taxa were subject to stable isotope analysis (blue marlin *Makaira nigricans,* cobia *Rachycentron canadum,* dolphinfish *Coryphaena hippurus,* greater amberjack *Seriola dumerili*, king mackerel *Scomberomorus cavalla,* little tunny *Euthynnus alletteratus,* wahoo *Acanthocybium solandri,* yellowfin tuna *Thunnus albacares*). For each sample, approximately 0.6 mg of the fish muscle tissue (skinned and boneless) was ground and used for isotopic determination. Isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) and total carbon and nitrogen content were determined using a Finnigan MAT DeltaPlus continuous-flow stable isotope mass spectrometer attached to a Carlo Erba elemental analyzer at the University of Texas at Austin Marine Science Institute. Isotope ratios were reported in parts per thousand (‰) relative to standards (PeeDee Belemnite for carbon, and atmospheric N for nitrogen), and defined in delta (δ) notation as

$$\delta^{15}N \text{ or } \delta^{13}C = (R_{sample}/ R_{standard} -1)\times 10^3$$

where $R=^{15}N/^{14}N$ or $^{13}C/^{12}C$. A secondary standard reference material (chitin of marine origin, Sigma Aldrich Co., USA, No. C-8908) were used to verify the accuracy of isotopic measurements (Herzka & Holt 2000).

Fatty acid analysis

Lipid was be extracted in duplicate aliquots using techniques from Folch et al., (1957) as modified by Iverson et al. (2001). The Hilditch procedure (Iverson et al. 1992) was used to prepare fatty acid methyl esters (FAME). Analysis of FAME was run in duplicate using temperature-programmed gas chromatography on a Perkin Elmer

Autosystem II Capillary FID gas chromatograph. Separation of FAME was performed on a 30 m x 0.25 mm internal diameter column coated with 50% cyanopropyl polysilohexane (0.25 μm film thickness; J&W DB-23; Folsom, CA, USA). Helium was used as a carrier gas. Individual peaks of FAME were identified by comparing retention times with known composition and commercially available standards (Nu Check Prep., Elysian, MN, U.S.A.). A computerized integration system (Turbochrome 4 software, PE Nelson) was used to calculate chromatographic data (Iverson et al. 1997a, 1997b). Individual fatty acids were converted to mass percent of total fatty acids using conversion factors from Ackman (1972, 1991). The fatty acid nomenclature used here is of the form 18:2(n-3), where "18" designates the total number of carbon atoms, "2" the number of double bonds, and "3" the position of the first double bond (n) from the methyl end of the fatty acid. Average data of the individual fatty acids are expressed as a mass percentage of total fatty acids.

Statistical analyses

To assess regional and interannual variability in Hg level, analysis of covariance (ANCOVA) was performed for each species, setting location and year as main factors, with LogHg as the dependent variable. The covariate (size) was used to compensate for size-related differences between regions or years. To explore the relationship between Hg level and fish body size, linear regression analysis was conducted for each species, setting size as the independent variable, Hg level as the dependent variable. Linear regression analysis was also conducted to test for the effect of trophic position (expressed as $\delta^{15}N$ value) on Hg levels in the tissue of pelagic fishes, setting $\delta^{15}N$ value

as the independent variable, Hg level as the dependent variable. Hierarchical cluster analysis was used to identify natural associations or groupings with similar dietary histories using fatty acid signatures. Euclidean distance and the average linkage joining algorithm were used to produce hierarchical trees for fatty acid data and intervals of squared Euclidean distance were used. After observing that fishes from the same location, rather than fishes of the same species, were grouped together, T-tests were performed for each taxa to test if there was a significant difference in fatty acid profile between Texas and Louisiana. Prior to all parametric testing, assumptions of normality (error terms) and homogeneity of variances were examined using K-S test and Levene test. Distributions of Hg were not always normal between years/locations for each taxa due to the lack of samples for those taxa in certain year/location. To better normality and homogeneity of variance, all Hg levels were Log_{10} transformed. Alpha value was set as 0.05. All the statistic analyses were performed using SPSS software.

RESULTS

Hg survey of pelagic fishes

Hg levels in a total of 387 samples from ten species of pelagic fishes were quantified (Table 2), and mean Hg level per species ranged from 0.07 to 10.52 ppm. Mean Hg level of three taxa were higher than the FDA 2001 criterion value of 1.0 ppm wet wt: blue marlin (*Makaira nigricans*), carcharhinid sharks (Genus *Carcharhinus*), and little tunny (*Euthynnus alletteratus*) (10.52, 1.61 and1.08 ppm, respectively). In fact, blue marlin Hg level was ten times the FDA level. In addition to these taxa, five others were above a reduced advisory level of 0.3 ppm wet wt. set by EPA (U.S.EPA 2002): king mackerel (*Scomberomorus cavalla*), mean=0.96 ppm; cobia (*Rachycentron canadum*), mean=0.89 ppm; wahoo (*Acanthocybium solandri*), mean=0.78 ppm; blackfin tuna (*Thunnus atlanticus*), mean=0.64 ppm; greater amberjack (*Seriola dumerili*), mean=0.60 ppm. Mean Hg levels of yellowfin tuna (*Thunnus albacares*) and dolphinfish (*Coryphaena hippurus*) were below all consumption advisory levels (0.18 ppm and 0.07 ppm, respectively).

Interannual and regional variation in Hg level was investigated and the majority of taxa examined showed no effect for either factor (Table 3). Hg levels of only one species, yellowfin tuna, varied significantly between years ($P = 0.041$), with lower levels in 2003 (0.10 ppm wet wt.) compared to 2002 (0.25 ppm wet wt.); no interannual effect was observed for blackfin tuna ($P = 0.267$), dolphinfish ($P = 0.574$), wahoo ($P = 0.296$), king mackerel ($P = 0.506$), or cobia ($P= 0.230$). Differences in Hg level between Texas and Louisiana were not statistically significant for blackfin tuna ($P = 0.111$), dolphinfish

Table 2. Hg level of ten taxa of pelagic fishes from NW Gulf of Mexico.

Common name	N	[Hg]total ppm wet wt.		Total length (cm)	
		mean	range	mean	range
blackfin tuna*	48	0.64	0.004 ~ 1.41	73.2	22.2 ~ 87.6
blue marlin**	9	10.52	4.95 ~ 18.68	285.1	256.5 ~ 311.1
carcharhinid sharks **	9	1.61	0.46 ~ 4.08	69.6	14.7 ~ 95.6
cobia*	17	0.89	0.20 ~ 2.40	97	76.0 ~ 142.1
dolphinfish	57	0.07	0.008 ~ 0.49	78.6	38.0 ~ 135.5
greater amberjack*	44	0.6	0.24 ~ 1.07	84.1	68.6 ~ 111.8
king mackerel*	39	0.96	0.37 ~ 1.46	84.1	63.5 ~ 104.1
little tunny**	9	1.08	0.24 ~ 2.52	56.3	51.5 ~ 66.2
wahoo*	52	0.78	0.013 ~ 3.31	133.1	102.9 ~ 175.3
yellowfin tuna	103	0.18	0.072 ~ 0.87	112.1	54.0 ~ 158.8

N = Number of individuals.

** > FDA 2001 recommended criteria level (1.0 μg/g wet wt.).

* > EPA 2002 recommended criteria level (0.3 μg/g wet wt.).

Table 3. Species-specific ANCOVA results examining year and location effect on the Hg level of pelagic fishes from NW Gulf of Mexico.

species	P-value	
	year	location
	2002 vs. 2003	TX vs. LA
blackfin tuna	0.267	0.111
cobia	0.230	N/A
dolphinfish	0.574	0.457
king mackerel	0.506	N/A
wahoo	0.296	0.234
yellowfin tuna	0.041*	0.009*

* $P < 0.05$

N/A : insufficient data for statistical testing

(P = 0.457) and wahoo (P= 0.234); however, Hg level in yellowfin tuna was significantly higher (P =0.009) in Texas (0.20 ppm) than Louisiana (0.18 ppm). Samples size limitations did not warrant interannaul and/or regional comparisons for blue marlin, greater amberjack, little tunny, or carcharhinid sharks.

Relationships between size and Hg level as well as body weight and Hg level were examined for six taxa and all regressions were significant with positive slopes, indicating that Hg level increases with increasing length or weight (Figs 3a, 3b, 4a, 4b). Blackfin tuna, carcharhinid sharks and yellowfin tuna showed highest R-square values for the Hg level and size: blackfin tuna (R^2 = 0.73, n= 48, p<0.001), carcharhinid sharks (R^2 = 0.69, n= 9, p<0.001), yellowfin tuna (R^2 = 0.64, n= 103, p <0.001). Blackfin tuna and wahoo showed the highest slope values (b=0.034, 0.02 respectively) (Table 4). Carcharhinid sharks, yellowfin tuna, dolphinfish and king mackerel showed similar slope values (b = 0.011, 0.01, 0.01 and 0.01, respectively). Linear regression analysis of Hg level by weight indicated that relationships were significant for the same six taxa (Table 5). Highest R^2 values were observed in blackfin tuna (R^2 = 0.79, n = 48, p < 0.001), dolphinfish (R^2 = 0.56, n = 57, p < 0.001), and yellowfin tuna (R^2 = 0.55, n = 103, p < 0.001). Blackfin tuna and wahoo also showed the highest slope values (b = 1.36 and 1.91, respectively). Carcharhinid sharks, yellowfin tuna, dolphinfish and king mackerel showed similar slope values from the linear regression analysis on Hg and body weight (b = 0.84, 0.79, 0.72 and 0.69, respectively, Fig 5).

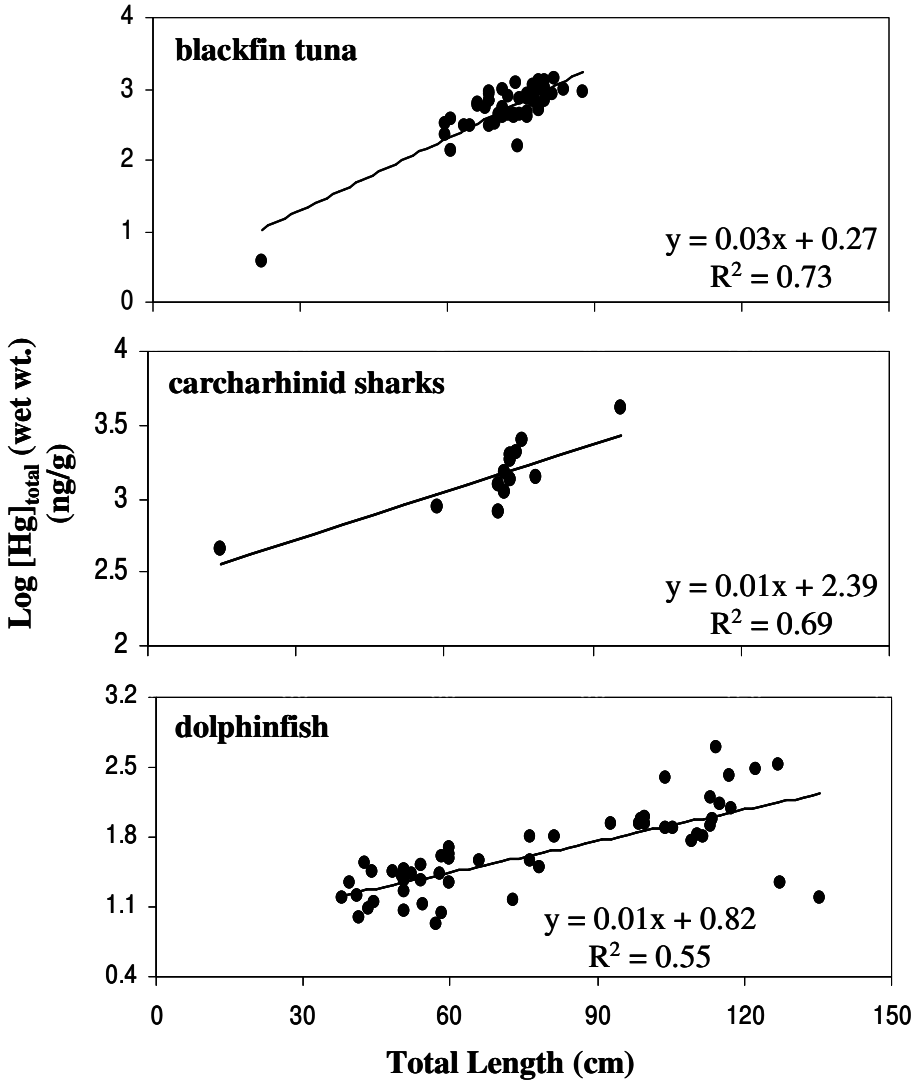

Fig 3a. Relationships between Hg levels (ng/g) and total body length (cm) in blackfin tuna, carcharhinid sharks and dolphinfish from NW Gulf of Mexico.

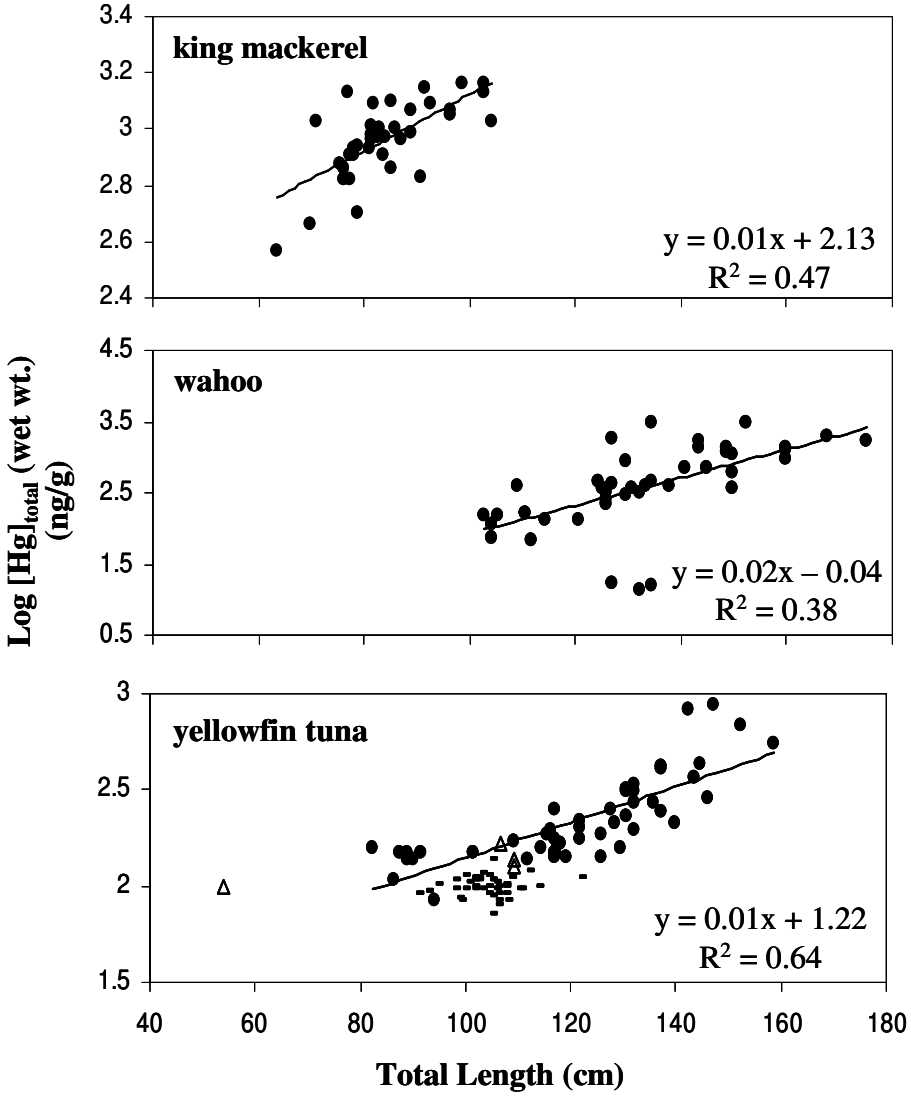

Fig 3b. Relationships between Hg levels (ng/g) and total body length (cm) in king mackerel, wahoo and yellowfin tuna from NW Gulf of Mexico. In yellowfin tuna figure, (-) are samples from Louisiana in 2003, (Δ) are samples from Texas in 2002, (●) are samples from Louisiana in 2002. Hg levels were significantly different among these groups in yellowfin tuna (ANOVA, $p<0.05$). For yellowfin tuna, only samples from Louisiana in 2002 showed a significant linear relationship between Hg level and total body length.

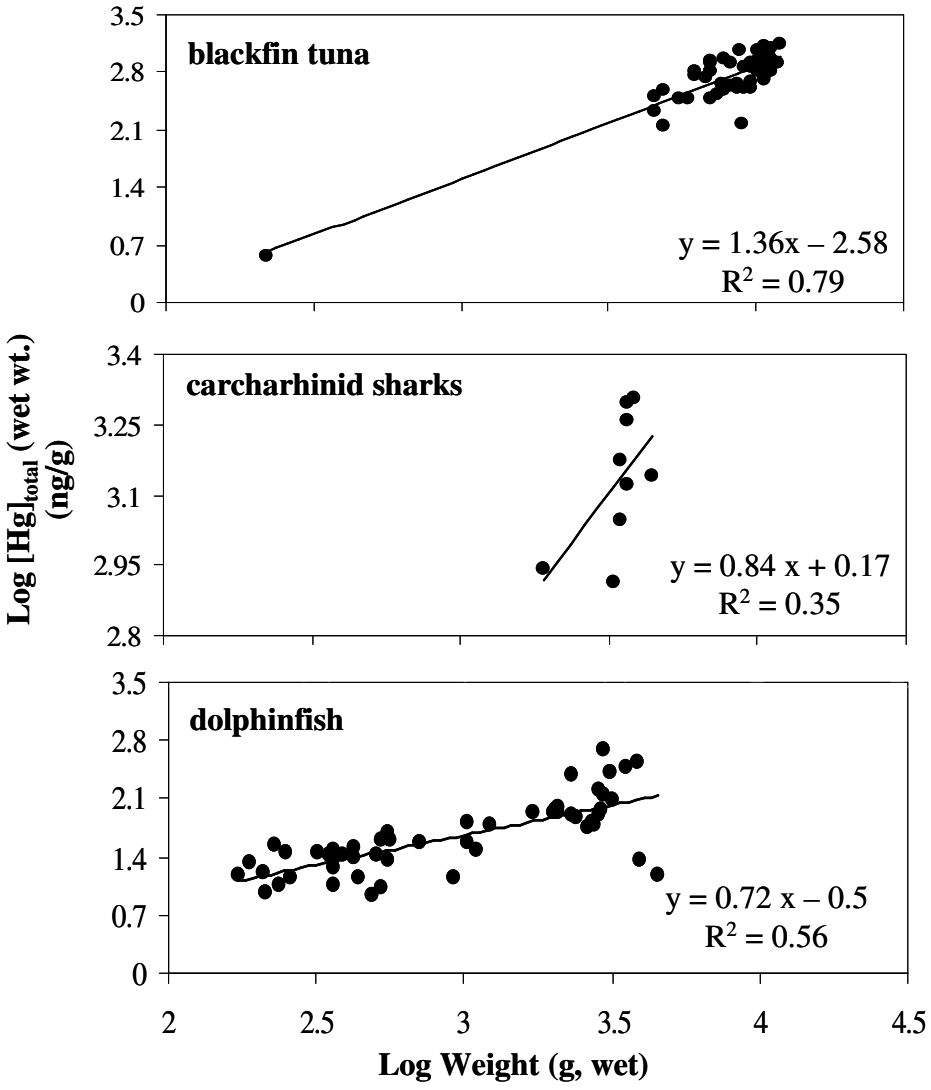

Fig 4a. Relationship between Hg levels (ng/g) and total fish body weight (g) in blackfin tuna, carcharhinid sharks and dolphinfih from NW Gulf of Mexico.

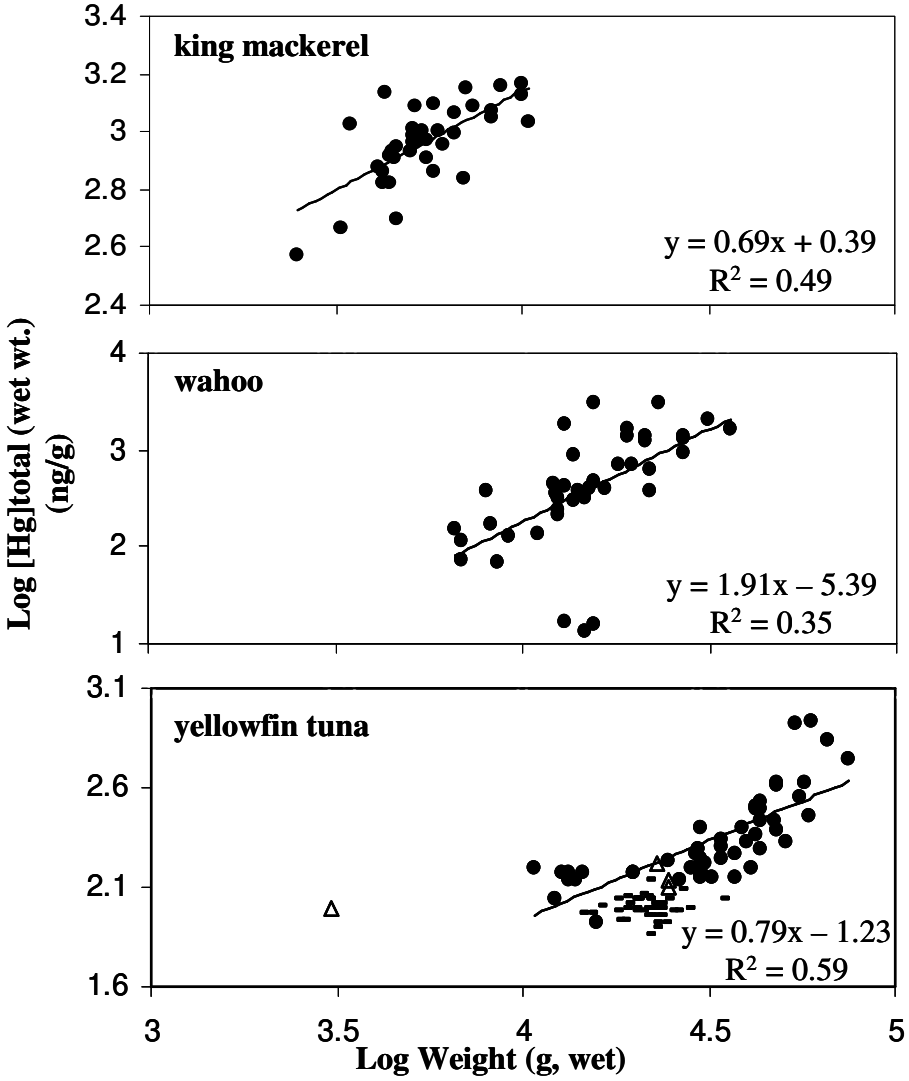

Fig 4b. Relationships between Hg levels (ng/g) and total body weight (g) in king mackerel, wahoo and yellowfin tuna from NW Gulf of Mexico. In yellowfin tuna figure, (-) are samples from Louisiana in 2003, (Δ) are samples from Texas in 2002, (●) are samples from Louisiana in 2002. Hg levels were significantly different among these groups in yellowfin tuna (ANOVA, $p<0.05$). For yellowfin tuna, only samples from Louisiana in 2002 showed a significant linear relationship between Hg level and total body weight.

Table 4. Regression analysis of Hg level as a function of size for apex predator species in NW Gulf of Mexico. Significant linear functions (Y = a + bX) were fitted. Y = Log_{10}[Hg], which is log transformed Hg level (ng/g), x is total length (cm). The estimated intercept (a), slope (b), R^2 value and P-value are listed by taxa/species.

species	a	b	R^2	P-value
blackfin tuna	0.27	0.034	0.73	0.000
carcharhinid sharks	2.40	0.011	0.69	0.000
dolphinfish	0.82	0.01	0.55	0.000
king mackerel	2.13	0.01	0.47	0.000
wahoo	-0.04	0.02	0.38	0.000
yellowfin tuna*	1.22	0.01	0.64	0.000

* Equation based on samples from Louisiana, 2002 only.

Table 5. Regression analysis of Hg level as a function of body weight for apex predator species in NW Gulf of Mexico. Significant linear functions (Y = a + bX) were fitted. Y = Log_{10}[Hg], which is log transformed total mercury concentration (ng/g wet weight), X=Log_{10}Weight, which is log transformed total body weight (g, wet). The estimated intercept (a), slope (b), R^2 value and P-value are listed by taxa/species.

species	a	b	R^2	P-value
blackfin tuna	-2.60	1.36	0.79	0.000
carcharhinid sharks	0.17	0.84	0.35	0.000
dolphinfish	-0.50	0.72	0.56	0.000
king mackerel	0.39	0.69	0.49	0.000
wahoo	-5.40	1.91	0.35	0.000
yellowfin tuna*	-1.23	0.79	0.59	0.000

* Equation based on samples from Louisiana, 2002 only.

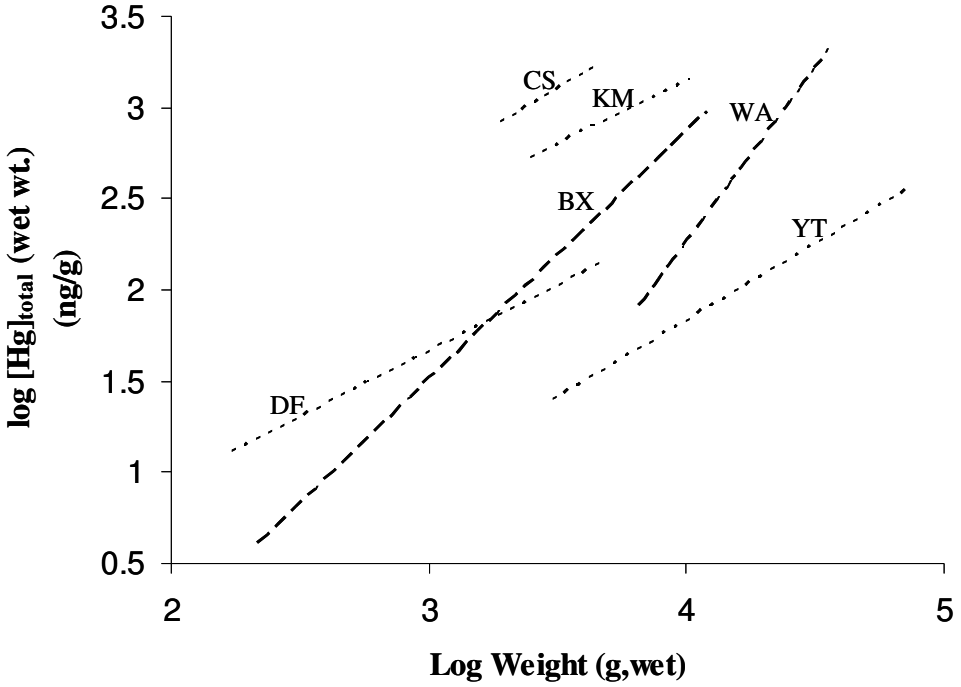

Fig 5. Comparison of regression of Hg level as a function of body weight for six pelagic taxa. BX: blackfin tuna, y=1.36x-2.6, R^2=0.79; CS: carcharhinid sharks, y=0.84x+0.17, R^2=0.35; DF: dolphinfish, y=0.72x-0.5, R^2=0.56; KM: king mackerel, y=0.69x+0.39, R^2=0.49; WA: wahoo, y=1.91x-5.4, R^2=0.35; YT: yellowfin tuna, y=0.79x-1.23, R^2=0.59.

Relationship between Hg level and trophic position

Nitrogen stable isotope values ($\delta^{15}N$) of nine pelagic species ranged from 6.7 to 16.2 ‰ (Fig 6). Based upon $\delta^{15}N$ values, little tunny had the highest trophic position ($\delta^{15}N$ = 13.9 to 16.2 ‰), followed by king mackerel ($\delta^{15}N$ = 12.9 to15.5 ‰). Although blue marlin had the highest Hg level (mean = 8.37 ppm), its trophic position was intermediate ($\delta^{15}N$ = 10.0 to 11.2 ‰). The lowest $\delta^{15}N$ values were observed for dolphinfish (6.7 to 9.3 ‰), indicating this species fed at the lowest trophic position of all taxa examined. Excluding blue marlin, there was a significant positive relationship between Hg level and $\delta^{15}N$: $y = 0.004e^{0.38x}$, where $x = \delta^{15}N$ and y = Hg level ($R^2 = 0.63$, $P = 0.011$).

Carbon stable isotope values of the nine pelagic fish species examined varied within a small range (-17.43 to -15.00‰, Fig 7). $\delta^{13}C$ values of consumers were most depleted for greater amberjack, yellowfin tuna and blackfin tuna (-17.43, -17.18 and -17.08‰ respectively), and most enriched for cobia (-15.00‰). King mackerel, little tunny, blue marlin and dolphinfish showed intermediate $\delta^{13}C$ values (-16.59, -16.41, -16.12 and -16.00‰, respectively). No significant relationship was detected between total Hg and $\delta^{13}C$ values.

Relationship between Hg level and diet history

The fatty acid composition of consumer pelagic fish tissue was analyzed, and approximately 70 fatty acids and their isomers were routinely identified. Only those found at levels ≥ 0.5% were presented here to simplify the presentation (Table 6). Fatty acid signatures of pelagic fishes were characterized by high levels of polyunsaturated

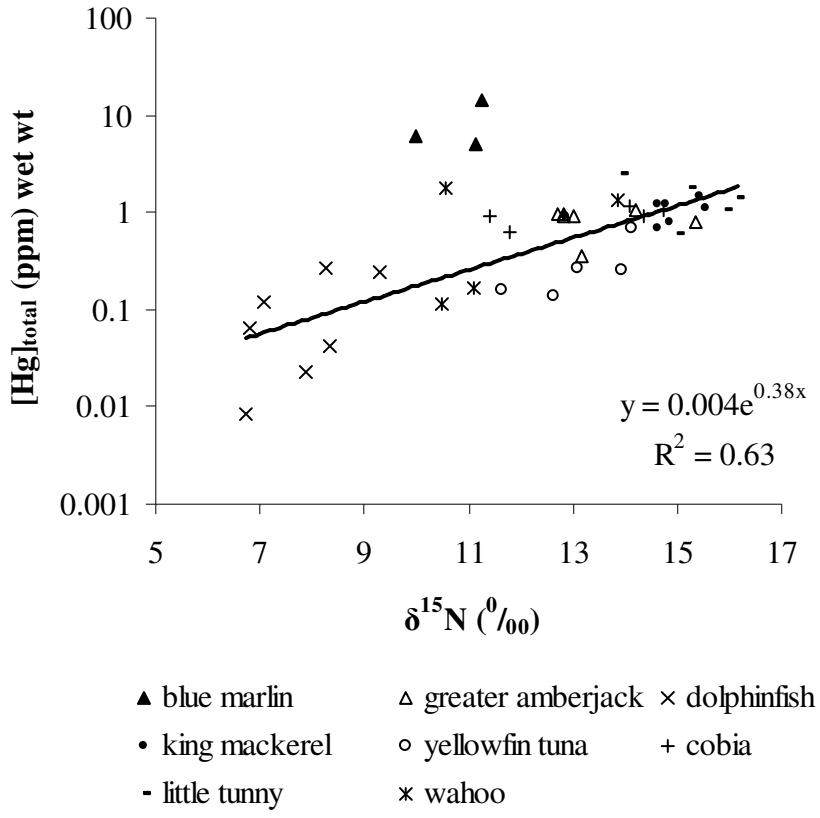

Fig 6. Linear regression of Hg level as a function of trophic position for pelagic fishes collected in the Gulf of Mexico. An exponential equation was fitted to data from eight species. Blue marlin was not used in the regression.

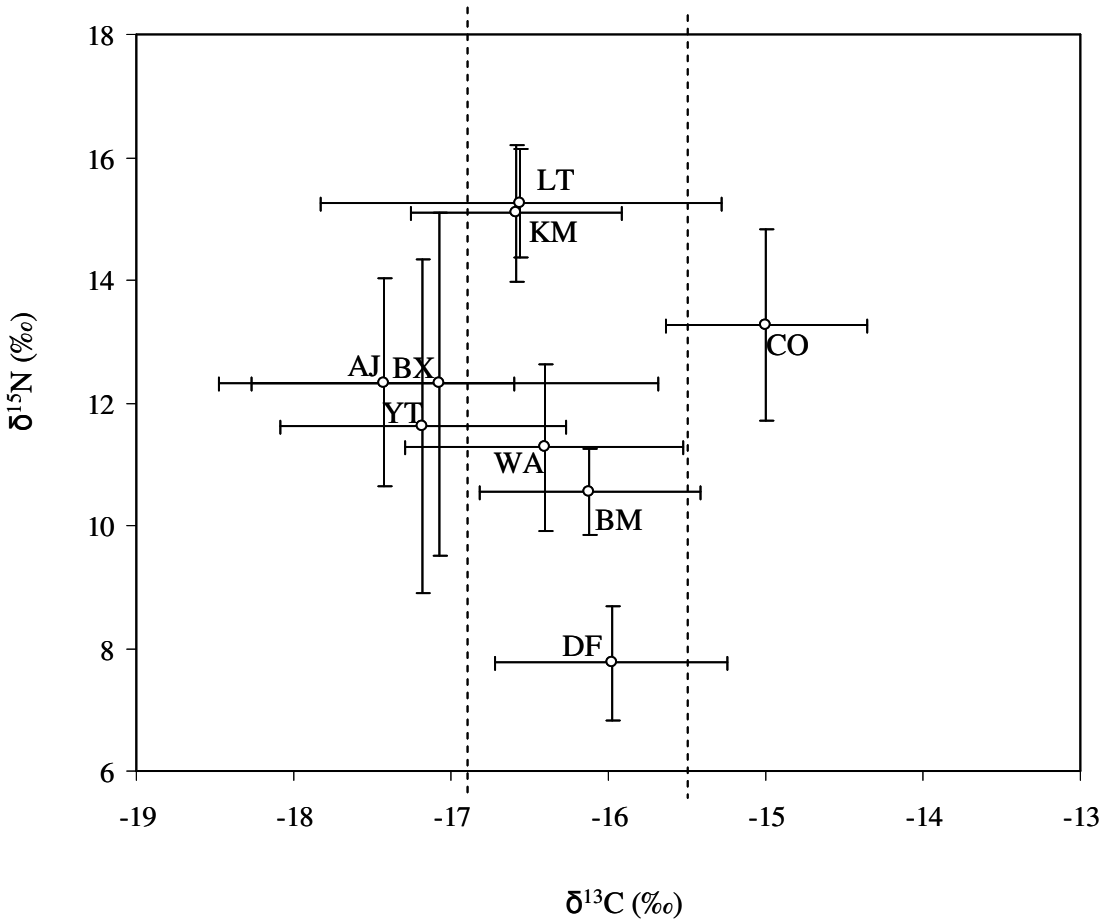

Fig 7. Stable-carbon and stable-nitrogen isotope values (mean±SD) of nine pelagic fishes from NW Gulf of Mexico. AJ:greater amberjack; BX:blackfin tuna; BM:blue marlin; CO:cobia; DF:dolphinfish; KM:king mackerel; LT:little tunny; WA:wahoo; YT:yellowfin tuna. Dashed lines indicated groupings following fatty acid classification tree results (Fig 11, 12).

Table 6. Fatty acid composition (wt.% total fatty acids) of pelagic fish from NW Gulf of Mexico. AJ:greater amberjack; BM:blue marlin; BX:blackfin tuna; CO:cobia; CS:carcharhinid sharks DF:dolphinfish; KM:king mackerel; LT:little tunny;; WA:wahoo; YT:yellowfin tuna. SAT:saturated fatty acid; MONO:monounsaturated fatty acid; and PUFA:polyunsaturated fatty acid.

fatty aicd	AJ n=18	BM n=4	BX n=12	CO n=6	DF n=17	KM n=11	LT n=3	CS n=4	WA n=1	YT n=7
14:0	1.10	1.58	2.58	1.15	0.36	0.43	3.03	0.44	4.27	1.23
15:0	0.39	0.48	0.48	0.33	0.36	0.24	0.67	0.20	0.67	0.43
ISO16	0.70	1.88	1.16	3.54	4.62	0.85	0.34	3.22	0.19	1.59
16:0	21.14	18.30	19.31	15.25	15.76	19.74	22.75	18.89	21.45	18.36
16:1(n-9)	0.56	0.37	0.29	0.42	0.45	0.34	0.33	0.22	0.67	0.27
16:1(n-7)	2.22	2.26	4.55	2.14	0.89	1.09	5.07	1.54	6.76	2.30
16:2 (n-4)	0.96	1.03	0.87	0.53	1.25	0.67	0.72	0.18	0.63	0.96
16:4(n-1)	1.09	1.99	1.19	1.62	1.12	1.59	1.33	1.70	1.73	1.10
17:0	0.68	0.59	0.75	0.95	0.88	0.73	1.12	0.38	1.38	0.98
17:1	0.32	0.28	0.34	0.49	0.29	0.30	0.25	0.26	0.12	0.28
18:0	7.93	9.27	7.72	10.45	9.53	9.45	8.57	10.77	5.72	8.16
18:1(n-9)	8.41	9.51	10.11	10.96	7.74	7.68	7.44	4.99	10.13	8.31
18:1(n-7)	2.04	1.80	2.51	2.13	1.37	2.00	7.82	9.03	2.90	1.83
18:2(n-6)	0.53	0.87	0.97	0.83	0.43	0.58	0.88	0.51	0.96	0.89
20:1(n-7)	0.52	0.50	0.83	0.38	0.18	0.47	0.87	1.04	0.92	0.49
20:4(n-6)	3.49	4.70	2.71	9.49	7.09	5.50	2.19	5.16	2.41	3.44
20:5(n-3)	3.25	2.99	6.35	4.24	3.49	4.43	4.50	1.55	7.40	5.02
22:5(n-6)	2.92	3.51	2.00	3.33	3.76	3.69	1.93	7.23	1.55	2.95
22:5(n-3)	2.99	2.71	2.63	3.43	2.18	2.12	2.29	4.56	3.31	2.05
22:6(n-3)	33.57	29.36	23.63	22.70	34.26	32.27	20.02	22.80	20.38	33.45
SAT	31.94	32.10	32.00	31.67	31.50	31.44	36.48	33.89	33.68	30.76
MONO	13.76	14.43	18.28	16.02	10.62	11.58	21.53	16.82	21.38	13.19
PUFA	54.31	53.47	49.71	52.31	57.87	56.98	41.99	49.29	44.94	56.05

fatty acids (PUFAs), which constituted between 41.99 (little tunny) and 57.87% (dolphinfish) of the total fatty acid content. Among the five major PUFAs (22:6(n-3) [docosahexaenoic acid, DHA], 22:5(n-3)[docosapentaenoic acid, DPA] , 20:5(n-3) [eicosapentaenoic acid, EPA], 20:4(n-6) [arachidonic acid, AA], and 18:2(n-6) [linoleic acid], 22:6(n-3) was the dominant PUFA, comprising more than 50% of all PUFAs combined. 22:6(n-3) is also the most abundant single fatty acid among all fatty acids detected, accounting an average of 27.25% of total fatty acids across species. Saturated fatty acids (SAT) were the second largest fatty acid group present in the muscle tissue of pelagic fishes, comprising an average of 32.55% of the total fatty acid content. The 16:0 fatty acid was the major fatty acid in the SAT group, it was also the second most abundant fatty acid among all fatty acids, comprising 19.10% of the total fatty acid content.

Hierarchical cluster analysis based on fatty acid profiles of ten pelagic taxa showed that most species were grouped together by location first, then by species (Fig 8).Year and size effect were not observed to have an impact on the grouping, and measures of dissimilarity were highest between taxa from Texas and Louisiana (Figs 9, 10). Fishes from Texas showed significantly higher proportion of PUFAs than those from Louisiana ($F = 10.4$, $P = 0.002<0.05$). A classification tree based on fatty acid components was built for pelagic fishes from each region (Figs 11, 12), and in Texas four groups were detected basing on a rescaled squared Euclidean distance value of 4: cobia as group #1, blue marlin, king mackerel and dolphinfish were identified as group

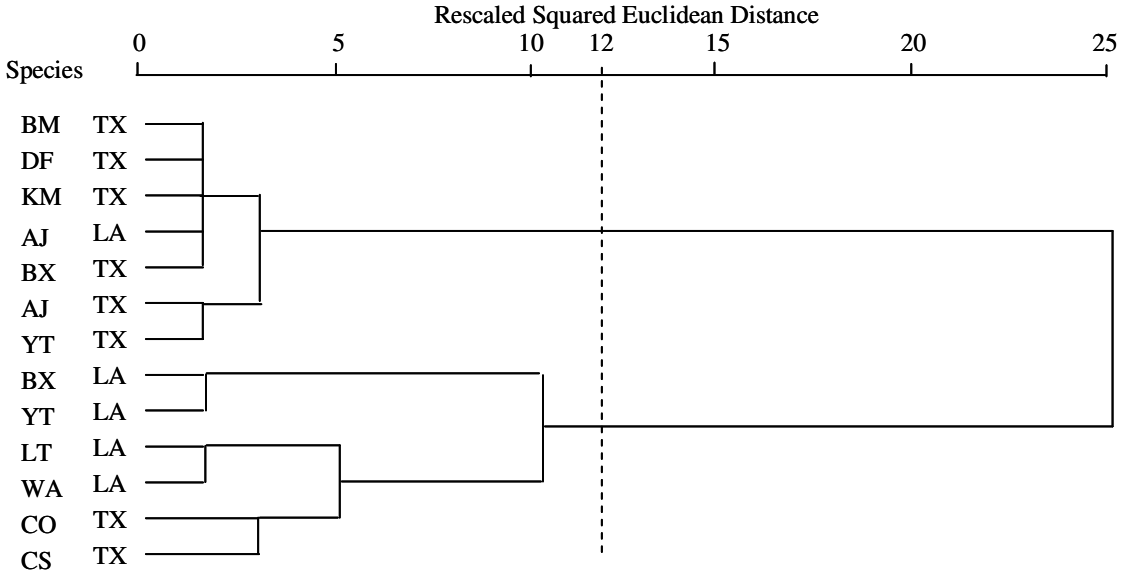

Fig 8. Results of hierarchical cluster analysis on fatty acid profiles of pelagic fishes from Texas and Louisiana. AJ: greater amberjack; BX:blackfin tuna; BM:blue marlin; CO:cobia; CS: carcharhinid sharks; DF:dophinfish; KM: king mackerel; LT: little tunny; WA: wahoo; YT:yellowfin tuna. TX: Texas; LA: Louisiana.

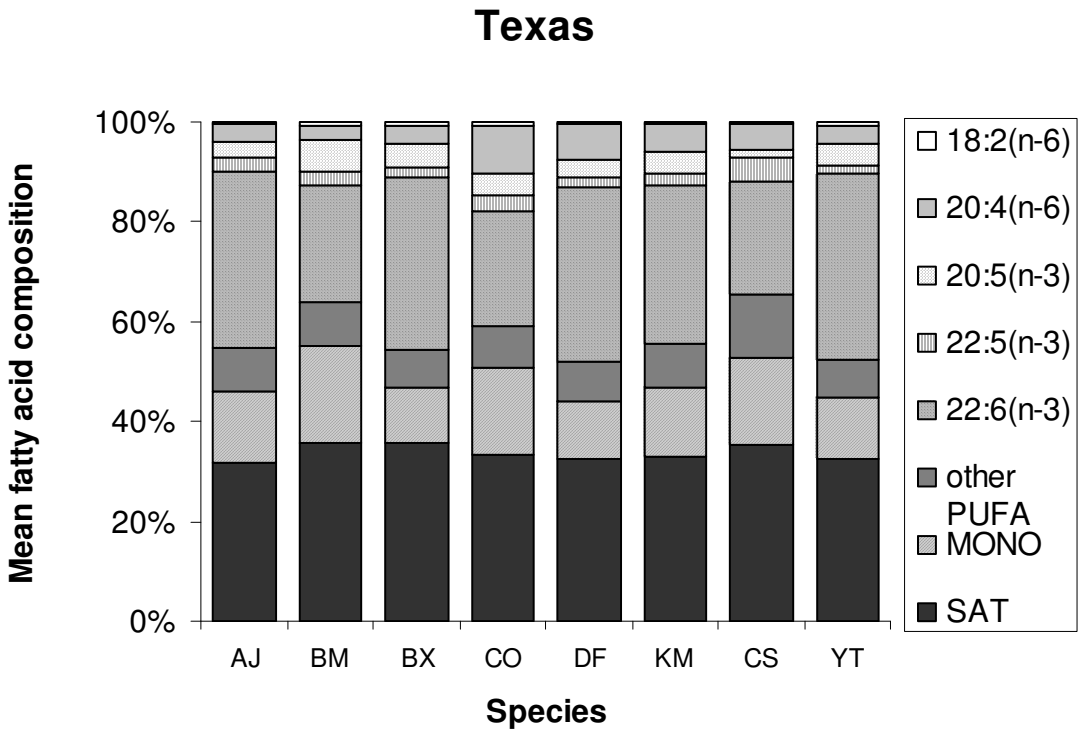

Fig 9. Mean % fatty acid composition of eight pelagic fishes from Texas. AJ:greater amberjack; BM: blue marlin; BX:blackfin tuna; CO:cobia; CS:carcharhinid sharks; DF:dolphinfish; KM:king mackerel; YT:yellowfin tuna. SAT:saturated fatty acid; MONO:monounsaturated fatty acid; and PUFA:polyunsaturated fatty acid.

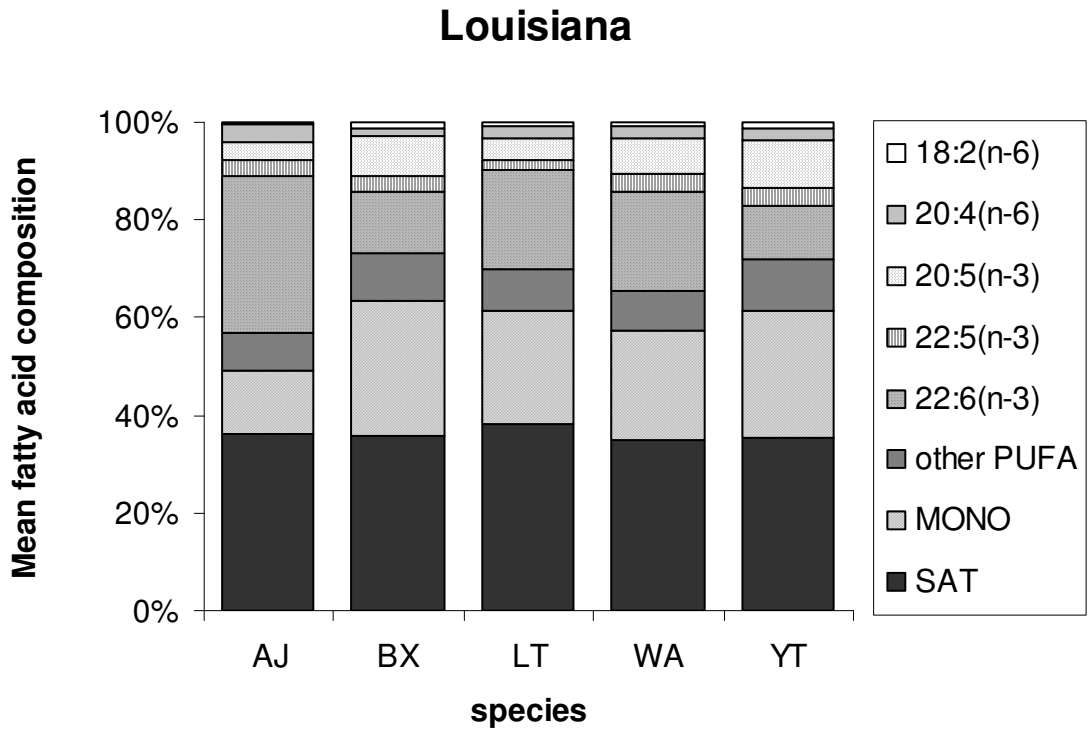

Fig 10. Mean % fatty acid composition of five pelagic fishes from Louisiana. AJ:greater amberjack; BX:blackfin tuna; LT:little tunny; WA:wahoo; YT:yellowfin tuna. SAT:saturated fatty acid; MONO:monounsaturated fatty acid; and PUFA:polyunsaturated fatty acid.

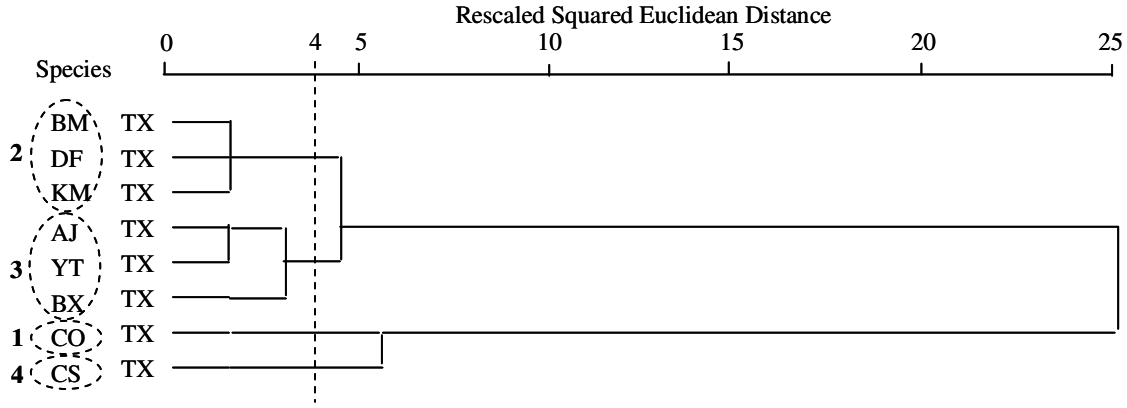

Fig 11. Results of hierarchical cluster analysis on fatty acid profiles of pelagic fishes from Texas. AJ:greater amberjack; BM:blue marlin; BX:blackfin tuna; CO:cobia; CS:carcharhinid sharks; DF:dophinfish; KM:king mackerel; YT:yellowfin tuna. TX:Texas. Dashed circles indicated groups which were clustered according to a squared Euclidean distance value of 4.

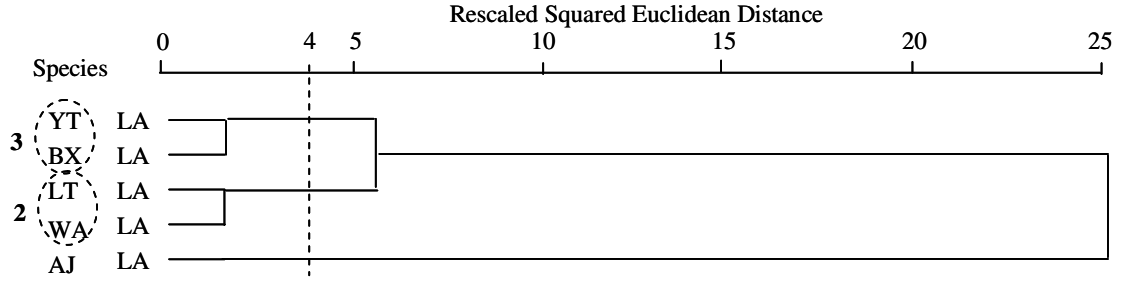

Fig 12. Results of hierarchical cluster analysis on fatty acid profiles of pelagic fishes from Louisiana. AJ:greater amberjack; BX:blackfin tuna; LT:little tunny; WA: wahoo; YT:yellowfin tuna. LA:Louisiana. Dashed circles indicated groups which were clustered according to a squared Euclidean distance value of 4.

#2, greater amberjack, yellowfin tuna and blackfin tuna as group #3, carcharhinid sharks as group #4 (Figure 11). In Louisiana two similar groups were detected basing on a rescaled squared Euclidean distance value of 4: little tunny and wahoo as group #2, yellowfin tuna and blackfin tuna as group #3 (Fig 12). Greater amberjack in Louisiana was an exception from the groupings. Also, group #1 species were not included in Louisiana cluster analysis due to the lack of samples in the region.

DISCUSSION

Species-specific pattern in Hg level was observed in the present study and based on Hg levels the 10 taxa grouped into three general categories: high range (> 1.0 ppm), middle range (between 0.3 and 1.0 ppm) and low range (< 0.3 ppm). High range taxa included blue marlin, carcharhinid sharks and little tunny; middle range included blackfin tuna, cobia, greater amberjack, king mackerel and wahoo; low range included dolphinfish and yellowfin tuna. My classification and observed Hg levels were similar in many respects to results from other studies conducted in the Gulf of Mexico (Adams et al. 2003b, FDA 2004, U.S.EPA 2004a). For example, blue marlin and little tunny were two of the three species observed in my high range category and these two species had the highest Hg levels of pelagic fishes collected from a recent Hg survey completed in Florida (Adams et al. 2003b). Moreover, carcharhinid sharks, the second highest Hg level taxa in this study, were also the second highest Hg fish on the EPA not-to-eat fish list (U.S.EPA 2004b). While my high category taxa were similar to other studies, both blue marlin and carcharhinid sharks showed considerably higher Hg levels (up to 2-3 times higher) than previous studies (blue marlin: mean 10.52 ppm vs. 3.08 ppm; carcharhinid sharks: mean 1.61 ppm vs. 0.78 ppm; Adams et al. 2003b, FDA 2004). Similarly, except for blackfin tuna, observed Hg levels of all taxa in the middle range (king mackerel, wahoo, greater amberjack, cobia) were 0.3-0.5 ppm higher than previous reports (Adams et al. 2003b, Watanabe et al. 2003). In contrast, both low range taxa (dolphinfish and yellowfin tuna) showed lower Hg levels (0.04-0.12 ppm lower) than the other studies (Adams et al. 2003b, FDA 2004, U.S.EPA 2004a). Observed patterns

suggest that regional variation in fish tissue Hg occurs within the Gulf of Mexico; however, differences in sample size and fish length/weight preclude further interpretation of these data.

MeHg accumulates from one trophic level to the next, and the highest natural Hg levels are often detected in long-lived predators (Andersen & Dephledge 1997). Compared to estuarine fishes, pelagic fishes usually have a much longer life span and larger body size, thus the extended bioaccumulation process may result in higher Hg levels. Sager (2004) measured the total Hg levels of three estuarine fishes (flounder, *Paralichthys lethostigma*; spotted seatrout, Cynoscion nebulosus; and red drum, Sciaenops ocellatus) from the same area (NW Gulf of Mexico), and all three species showed lower Hg levels (range: 0.06-0.10) than the EPA 0.3ppm threshold. Similarly, a study in Galveston Bay, Texas examined the same species and indicated that Hg levels of these species were normally below 0.25 ppm, with an average near 0.10 ppm (GBNEP, 1992). In contrast, eight out of ten pelagic taxa examined in the present study had higher Hg levels than the EPA 0.3 ppm threshold, with three species above the FDA 1.0 ppm level. Considering that all the pelagic fishes in this study were apex predators with either long life spans (>10 years) or large body size (>100 cm) or both (Collette & Nauen 1983, Smith-Vaniz 1984, Robins & Ray 1986, Bauchot 1987, Allen & Steene 1988, Quéro 1990, Claro 1994, Lieske & Mayers 1994, Randall 1995, Sommer et al. 1996, Collette 1999, Oxenford 1999, IGFA 2001), elevated Hg level detected in these fish were not surprising. Moreover, the species with the highest Hg level in this study, blue marlin, was largest species examined (mean = 285 cm lower jaw fork length), and

very long-lived (up to 28 years) (Kailola et al. 1993). In contrast, several of the other taxa examined (e.g. dolphinfish, cobia, greater amberjack, yellowfin tuna) typically live 4-10 years (Le Guen & Sagakawa 1973, Manooch & Potts 1997, Franks et al. 1999, Massuti et al. 1999), and thus even with a relatively large body size they do not live long enough to accumulate large amounts of Hg. The life span effect seems to apply particularly well to dolphinfish since this species, which has the shortest lifespan of any of the taxa examined (3-5 years) also had the lowest Hg level in its tissue.

Regional differences in Hg level were not detected for the majority of taxa examined. The lack of an apparent east-west pattern could be due to several reasons, including the fact that oceanographic processes (current patterns, eddy movement) link the two regions (Cochrane & Kelly 1986, Oey 1995). Moreover, many of the pelagic fishes surveyed here (e.g. yellowfin tuna, little tunny, blue marlin) are known to be highly migratory species that move distances far beyond the coast line of Texas and Louisiana (Blackburn 1965, Leggett 1977, Holland et al. 1990, Deriso et al. 1991, FAO 1994, Kerstetter et al. 2003). For example, blue marlin was recorded to move up to 1200 km over 30 days (Kerstetter et al. 2003). As a result, individuals caught in Texas or Louisiana waters may have spent a portion of their life in the other parts of the Gulf, including the other sampling location used in this study. In fact, Francesconi and Lenanton (1992) found that a fish's site fidelity (length of exposure to the source) was an important factor in determining Hg levels in the tissues of consumers. Finally, the environmental chemistry of Hg is very complex, and subtle changes in chemical, physical, biological, and hydrologic conditions can cause substantial shifts in its physical

form and valence state over time scales ranging from hourly to seasonal (Amyot et al. 1994, Krabbenhoft et al.1998, Lalonde et al. 2002). The entry of MeHg into the base of the food web and its subsequent trophic transfer in the lowest levels are still poorly understood (Wiener et al. 2003). Therefore, even if there is significant spatial differences of Hg levels occur in the environment (including water, seston, plants and invertebrates), it cannot guarantee significant Hg level differences in fishes (Riisgårdand & Famme 1986, Francesconi & Lenanton 1992, Watras & Bloom 1992).

Annual differences (2002 vs. 2003) were not detected for the majority of the taxa examined, and this was expected due to two reasons. First, the 2 year period was relatively a short time interval with no dramatic natural or anthropological evens that could induce huge environmental changes (e.g. huge storms could redistribute buried Hg and reintroduce Hg into a food web). Second, predators targeted in this study were at the top level of the food web, and it takes much longer time for their Hg levels to react to the environmental change. For example, Sager (2002) found elevated Hg levels in predatory fishes 30 years after a local Hg-contaminated water release was ended even though the Hg level of some fishes at lower trophic levels dropped below safety level the next year.

Six species in the present study exhibited a significant positive relationship between Hg level and size, and similar relationships have been documented previously (e.g. Huckabee et al.1979, Grieb et al. 1990, Monteiro & Lopes 1990, Wiener & Spry 1996, Sager 2002). Moreover, recent work by Adams (2004) on pelagic species (yellowfin tuna and little tunny) showed positive relationships between Hg level and fish length/weight. In the present study, slope values from linear regressions of Hg level and

size were similar (0.010-0.011) for several species (carcharhinid sharks, yellowfin tuna, dolphinfish, and king mackerel), suggesting that the rate of Hg accumulation may be closely tied to growth. To verify this theory, Hg was plotted against body weight, and all six species not only showed significant positive relationships between their Hg level and body weight (Fig 3), but exactly the same four species (Fig 4) showed similar Hg gain rates (slope values). The positive relationship between Hg level and size likely results from a slow rate of elimination of MeHg relative to its rapid rate of uptake (Huckabee et al. 1979, Trudel & Rasmussen 1997). Given the fact that MeHg forms covalent bonds with proteins in muscle after it is transported through the blood (Carty & Malone 1979), and in carnivorous fish, protein assimilation is generally equal to 80% of total assimilation from food consumption (Brett & Groves 1979), the positive relationship between Hg accumulation and body mass accumulation observed here is in accord with expected patterns of accumulation.

Across species there was a positive exponential relationship between Hg level and trophic position (expressed as $\delta^{15}N$). The positive effect of trophic position on Hg level has been observed in other studies (Walker 1976, Freeman et al. 1978, Lyle 1984, Cabana et al. 1994). Kidd et al. (1995) found significant relationships between Hg level and $\delta^{15}N$ for yellowperch (*Perca flavescens*), northern pick (*Esox lucius*), and lake cisco (*Coregonus artedii*). Besides Hg, concentrations of other contaminants in the tissue of fishes also increase with increasing trophic position. For example, Hobson et al. (2002) reported a linear relationship between PCB concentration and $\delta^{15}N$ values for organisms in the North Water food web. The positive relationships between Hg level (and other

contaminants) and trophic position in fishes confirmed the existence of biomagnification in aquatic ecosystems, which occurs because consumers feeding at a higher trophic position will consume larger prey with higher contaminant loads than smaller prey (Muir et al. 1988, Watras et al. 1998, Bowles et al. 2001). Overlap of $\delta^{15}N$ values was observed for many pelagic fish species in this study due to the relatively large intraspecific variation of $\delta^{15}N$ values (Fig 6). The broad range of trophic levels of several pelagic fishes indicated these consumers may be feeding on prey from different trophic levels and this may be a function of a generalist feeding strategy or ontogenetic shifts in diet (Kidd et al. 1995, Herzka & Holt 2000).

Although stable carbon isotope analysis may not always allow precise reconstruction of an animal's diet, the approach provides a means of discrimination among animals belonging to particular dietary guilds (Gannes et al. 1998). For example, Ambrose and DeNiro (1986) used $\delta^{13}C$ values to separate 43 species of east African mammals into different feeding categories (e.g. forest floor feeders, browsers and grazers). Other studies have also used $\delta^{13}C$ values to distinguish pelagic animals (lower range) from benthic animals (higher range) (Hatase et al. 2002, Hobson et al. 2002). Similarly, based on the $\delta^{13}C$ values of pelagic consumers examined in this study, three natural groups were identified following the same groupings obtained by using fatty acid composition. $\delta^{13}C$ values of cobia were the highest and did not group with other taxa. $\delta^{13}C$ values of the remaining two groups were enriched by ~2 ‰ (blackfin tuna, greater amberjack yellowfin tuna), and ~1‰ (blue marlin, dolphinfish, king mackerel, little tunny and wahoo) relative to cobia. Natural associations based on $\delta^{13}C$ values were not

specifically linked to Hg levels of consumers. For example, blue marlin and little tunny were in the high Hg group and dolphinfish was in the low Hg group; however, all three species had similar $\delta^{13}C$ values. This indicated that Hg level was not directly connected with the potential distinguishing factors that divided these fishes into those natural groupings. The fact that these natural groupings were also obtained from fatty acid analysis not only confirmed the existence of these natural groupings, but also indicated that the distinguishing factors for the grouping was somewhat connected with the dietary history of these fishes. One possible explanation for observed patterns could be linked to pelagic or benthic foraging strategies of taxa examined. In a marine ecosystem, $\delta^{13}C$ values of benthic and pelagic prey differ, thus consumers feeding on or near the bottom can be distinguished from consumers feeding in the water column (Hatase et al. 2002, Hobson et al. 2002). Moreover, in the northwestern Gulf, $\delta^{13}C$ values of benthic prey of large consumers are more enriched compared to their pelagic counterparts (Rooker, unpublished data), indicating the approach may be useful for identifying benthic and pelagic foraging patterns of large consumers. In the present study, cobia had the most enriched $\delta^{13}C$ value and thus appeared to feed on more benthic prey than all the other pelagic fishes in this study. Thus, observed groupings based on $\delta^{13}C$ values were likely related in part to the feeding strategies of the predator (e.g. benthic versus pelagic consumer), and appeared unrelated to Hg loads of consumers .

In this study, the classification tree based on the fatty acid profiles of pelagic fishes readily separated fishes from different regions (Texas vs. Louisiana). Because the fatty acid composition of the diet influences the lipid composition of tissues in fishes

(Cowey & Sargent 1972, Yu et al. 1977, Nelson 1992, Dos Santos et al. 1993, Xu et al. 1993), it appears that pelagic taxa within the same region share similar prey resources or a common source of organic matter in their food web. Since many of the pelagic taxa examined here are opportunistic feeders (Beaumariage 1973, Manooch et al. 1984, Snelson et al. 1984, Meyer & Franks 1996, Abitia-Cardenas et al. 1999), their diets may be comprised of a few dominant prey species, which vary from one region to another. Also, the primary source of carbon supplied to primary, secondary, and tertiary consumers may vary between regions (Melville & Connolly 2003, Lepoint et al. 2004), leading to regional differences in fatty acid signatures. Although regional variation in fatty acid signatures or diets were present, species-specific variation in Hg levels between the two regions was negligible. The lack of accordance between natural groupings based on fatty acids and Hg levels (e.g. fatty acid group contained both high and low Hg taxa) appears to indicate that Hg accumulation is not strongly tied to prey selection patterns. The lack of agreement between the two measures does not indicate prey selection patterns are unrelated to Hg bioaccumulation but rather that other factors (trophic position, age, Hg levels of entire prey community) may be the primary determinants of Hg loading. Also, because Hg level of consumers is the result of a life-long Hg accumulation process, my shorter-term dietary indicators do not represent the cumulative lifetime feeding patterns of the consumers.

SUMMARY AND CONCLUSION

Species-specific patterns in fish tissue Hg were observed among the ten taxa of pelagic fishes from NW Gulf of Mexico. Based on observed Hg level and regulatory standards set by FDA and EPA, three natural groups were identified. High Hg group (>1.0 ppm), middle Hg group (range from 0.3 ppm to 1.0 ppm) and low Hg group (< 0.3 ppm). Three taxa (blue marlin, carcharhinid sharks, little tunny) showed Hg levels higher than the FDA 2001 criterion value of 1.0 ppm wet wt.; five taxa (blackfin tuna, cobia, greater amberjack, king mackerel and wahoo) were above a reduced advisory level of 0.3 ppm wet wt. set by EPA; and, two taxa (dolphinfish and yellowfin tuna) were below both FDA and EPA levels. Compared to findings from other studies of pelagic fishes in the Gulf of Mexico, most of the species examined here showed higher Hg levels. Moreover, the majority of the pelagic fishes investigated in this study showed elevated Hg levels compared to estuarine fishes from the same area, which indicated that higher Hg levels occurred with top-predator fishes with longer life-span and larger body size.

Regional and interannual differences in Hg levels of pelagic taxa examined were neglible. Regional differences (Texas vs. Louisiana) in Hg level were not detected for the majority of taxa examined. It could be due to several reasons including the oceanographic processes (current patterns, eddy movement) linking the two regions, the movement of the migratory species and the complexity of Hg bioaccumulation process. Annual differences (2002 vs. 2003) were not detected for the majority of the taxa examined, and this finding could be explained by the short time interval of the 2 years

and the time consuming process for Hg changes in the lower levels of a food web to reach the top level.

Six taxa (blackfin tuna, carcharhinid sharks, dolphinfish, king mackerel, wahoo and yellowfin tuna) in the present study exhibited a significant positive relationship between Hg level and body length as well as Hg level and body weight. Also, the four taxa that showed similar Hg gain rates against body length also showed similar Hg gain rates against body weight. This suggested that Hg accumulated as fish body size/mass increased.

Natural dietary markers (stable isotopes and fatty acids) indicated that Hg levels of pelagic taxa were linked to trophic position and feeding histories. $\delta^{15}N$ values in the fish muscle tissue showed a positive exponential relationship between Hg concentration and trophic position (expressed as $\delta^{15}N$) across species (blue marlin excluded). This indicated that Hg accumulated from one trophic level to the next, and higher-order consumers usually possessed highest Hg levels. The broad range of trophic levels of most pelagic fishes in the present study indicated possible diet shifts (from lower trophic level prey to higher trophic level prey) that occurred with these fishes as they grew. Groupings based on $\delta^{13}C$ value didn't show any connection with Hg level, indicating that Hg accumulation had little or no connection with the benthic or pelagic feeding habits of the species.

Fatty acid components of the pelagic fishes were investigated. The fact that the classification tree built based on the fatty acid data readily separated fishes from different regions (Texas vs. Louisiana) suggested that there were significant spatial

difference in the prey items of these pelagic fishes. In addition, in both Texas and Louisiana, pelagic fishes within the same region were divided into same nature groups by the classification tree built based on their fatty acid profiles. Moreover, I could divide these pelagic fishes into the same groups based on stable carbon isotope data ($\delta^{13}C$ values), further confirming that the grouping was based on certain kinds of dietary guilds whose exact nature was still not clear. However, Hg pattern of these pelagic fishes did not show any connection with their fatty acid pattern or the following groupings. This indicated that Hg level changes in pelagic fishes take longer time than it takes for the fatty acid profile to change, and trophic position is the major factor that influences the Hg level in pelagic fishes.

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