VARIATIONS IN MERCURY BIOACCUMULATION IN FISH ALONG THE RIVER CONTINUUM OF FOUR ARIZONA FRESHWATER ECOSYSTEMS

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ABSTRACT

VARIATIONS IN MERCURY BIOACCUMULATION IN FISH ALONG THE RIVER CONTINUUM OF FOUR ARIZONA AQUATIC ECOSYSTEMS

EDYTH HERMOSILLO

Mercury (Hg) is released to the environment from natural sources such as volcanic activities and by anthropogenic activities such as fossil fuel combustion, industrial processes, gold mining and waste incineration. Inorganic mercury in transported and deposited in the atmosphere bound to particulates. Upon reaching the aquatic ecosystems, inorganic mercury is converted to methylmercury (MeHg) by sulfate-reducing bacteria in the sediment. MeHg is highly toxic and is bioaccumulated by aquatic organisms. Therefore, higher mercury concentrations in fish successively occur because of mercury food chain magnification. This study examines how bioaccumulation of mercury in fish changes along the river continuum as the food-base shifts from leaf litter to algae. We collected non-native fish, leaf litter and algae samples along the river continuum from headwater to the confluence of four freshwater systems: Oak Creek, Wet Beaver Creek, West Clear Creek and the Verde River. To estimate trophic-level and food-web patterns in these study sites, nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope ratios were measured and stable isotope ratios of hydrogen (δD) were used to distinguish allochthonous (leaf-litter) and autochthonous (algae) energy sources in these four different aquatic ecosystems.

This study was designed to test mercury concentrations in fish as it increases with trophic level and correlate positively with $\delta^{15}N$ values. As well as mercury concentrations in fish increasing along the RCC as streams shift from being a detrial to algal based, observing significant changes in mercury concentrations in fish based on leaf-litter or algal as the energy source.

A total of 159 individuals of non-endangered fish species across all sites were captured using hoop nets, trammel nets and electro-fishing. Fin clips per fish were analyzed for $\delta^{15}N$, $\delta^{13}C$, δD, while dorsal muscle tissue samples were analyzed for Hg concentration. Stable-isotope measurements are a common tool for identifying food web relationships; nitrogen isotopes tell us “how high” an animal feeds in a food web, carbon and hydrogen isotopes tell us what food base (i.e. algae or detritus) an animal utilizes. Algal samples were collected from rock cobbles by scraping the algal growth with a stiff-bristled toothbrush as well as different species of algae were collected and identified. At the same time, four random leaf specimens were collected from the surface of the water at

each site which were used for isotope analysis and Hg concentration. To estimate algae growth, three sets of six clay tiles were placed in the water at each site and removed every three weeks, scraped off and calculated for ash-free dry mass (AFDM). To measure leaf-litter inputs, a stream segment reach of 50m was measured at both sides of the stream and three 5-gallon buckets were placed on both sides of the banks. Material in litter-fall buckets was collected every other week and weighed for total mass. Benthic organic matter (BOM) storage was also estimated by choosing an 50m stream reach and establishing three random transects across the stream from bank to bank of 1m wide intervals collecting leaves by hand. Materials collected were calculated for AFDM.

Results showed Hg concentrations in fish increased along the RCC as streams shift from being a leaf-litter to algal based. Natural abundance of nitrogen ($\delta^{15}N$) stable isotope ratios indicated an increase in mercury concentrations with an increase in trophic level. The results of this study will guide future work in determining if long-term monitoring of tissue concentrations of mercury in aquatic biota is needed to assess remedial effectiveness that will be protective of both human health and the environment.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

ACRONYM	DEFINITION
AMRU	*Ambloplites rupestris* – Rock bass
ANOVA	ANALYSIS OF VARIANCE
ICPU	*Ictalurus punctatus* – Channel catfish
LECY	*Lepomis cyanellus* – Green sunfish
LEMA	*Lepomis macrochirus* – Bluegill sunfish
MIDO	*Micropterus dolomieui* – Smallmouth bass
MISA	*Micropterus salmoides* – Largemouth bass
OCDN	Oak Creek downstream
OCMD	Oak Creek middle ridge
OCUP	Oak Creek upstream
ONMY	*Oncorhynchus mykiss* – Rainbow trout
POAN	*Pomoxis annularis* – Crappie spp.
PYOL	*Pylodictis olivaris* – Flathead catfish
SATR	*Salmo trutta* – Brown trout
VROC	Verde River at Oak Creek confluence
VRWB	Verde River at Wet Beaver Creek confluence
VRWC	Verde River at West Clear Creek confluence
WBDN	Wet Beaver Creek downstream
WBUP	Wet Beaver Creek upstream
WCDN	West Clear Creek downstream
WCUP	West Clear Creek upstream

CHAPTER 1.0 INTRODUCTION

Mercury (Hg) is transported in the atmosphere as inorganic mercury, deposited over land and ultimately finding its way into freshwater ecosystems. Mercury enters the environment as a result of natural events such as erosion of soils, volcanoes and fires, surface degassing and from anthropogenic sources such as industrial processes, commercial products and the combustion of fossil fuels (Fitzgerald et al., 1998). Upon reaching the aquatic ecosystems, biogeochemical processes convert inorganic Hg to methylmercury (MeHg), which is highly toxic. It is well documented that methylmercury biomagnifies at higher levels in aquatic ecosystems and that consumption of mercury contaminated fish can be toxic to both humans and wildlife (Cizdziel et al., 2003). The bioaccumulation of mercury depends on the activity of sulfate-reducing bacteria, mainly in anoxic sediments (Bank et al., 2007). The accumulation of MeHg in higher organisms results primarily from the ingestion of food containing MeHg rather than direct uptake of MeHg from the water. The structure of a food-web determines the effectiveness of MeHg transfer from algae to top predators and studies that correlate $\delta^{15}N$ and Hg bioaccumulation show that the number of trophic levels between predators and prey is important given that increases of trophic levels in an aquatic ecosystem leads to higher mercury concentrations in top predators (Morel et al., 1998). Therefore, higher mercury concentrations in fish successively occur because of mercury magnification in the food-chain.

Fish populations are mainly affected by mercury within their tissues (Cizdziel et al., 2003) which is the reason why the study of the distribution and retention of mercury in fish tissue is necessary and important, also because fish muscle is the main route of human exposure to MeHg. Because streams are often more receptive to seasonal and local physical disturbances, mercury in streams is strongly controlled by runoff from the watershed. Elevated runoff after rain and snowmelt tends to carry higher concentrations of mercury, along with higher concentrations of dissolved organic carbon (DOC) and suspended sediments (Brigham, 2009). Mercury successfully binds to both DOC and suspended sediments; processes that enhance transport of these elements also enhance the transport of mercury. Mercury is transported by streams in particulate and dissolved forms changing concentrations as the stream increases with distance. During the transport, some of the mercury is removed by settling of particles, some of the inorganic mercury is methylated, and methylmercury present in the flowing water may be lost through removal mechanisms, including biological uptake. Preliminary data indicate that the behavior of streams during wet seasons is very different from that in the dry season. In the wet season, streams act as transporters of sediment-bound and dissolved mercury from upper reaches to lower reaches, and mercury methylation processes are thought to be relatively insignificant due to the higher flows and lower temperatures since circulating the water also circulates oxygen and heat into lower layers of the stream limiting the biochemical process of transforming inorganic Hg into toxic methylmercury and preventing working its way up into the food chain. In the dry season, sediment deposits in some stream reaches downstream and serve as mercury sources to the flowing water, and mercury concentrations increase with distance downstream (Becker et al., 1995).

Stable Isotopes

Stable isotopes are used as indicators of the origins of materials in the environment as these materials are transported and transformed (Lajtha, 1994). In order to understand stream food-webs, distinguishing the energy derived from internal (autochthonous) primary producers from that of external (allochthonous) primary producers is important. Because freshwater food-webs rely on allochthonous primary producers transferred to the aquatic ecosystems as leaf-litter and autochthonous primary producers such as algae, stable isotope ratios of hydrogen (δD) potentially distinguish allochthonous inputs because it differs between terrestrial and aquatic primary producers (Solomon et al., 2009). Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide great tools for estimating the trophic positions and carbon flow to consumers in food webs (Post, 2002).

River Continuum Concept

The relative importance of allochthonous and autochthonous food sources to stream food webs are explained in the River Continuum Concept (RCC). The RCC states that contributions from allochthonous and autochthonous materials and the subsequent structures of macroinvertebrate functional feeding groups will vary along the stream gradient in accordance with the physical and chemical environment (Solomon et al., 2009). Food energy in narrow and forested headwater streams comes mainly from surrounding terrestrial sources such as leaves and dissolved organics that fall into, since the thick shore vegetation prevents the penetration of sunlight, in turn decreasing the production of organic material through photosynthesis in the water. In mid-sized streams the forest canopy opens up to allow instream organic materials such as algae to become the main energy source. In large rivers the biological communities depend on the transport of organic materials from upstream (leaf-litter) as well as instream organic material production (algae) (Vannote et al., 1980).

Study Objectives

In order to address the potential effects of mercury contamination we need to know the concentrations of mercury in muscle tissue of fish that can be harmful to aquatic resources and humans and the types of sampling and analysis that are necessary to define potential risks to these organisms (Bank et al., 2007). The objectives of this study were designed to test the hypotheses that (1) Mercury concentrations in fish will increase with trophic level and will correlate positively with $\delta^{15}N$ values and (2) Mercury concentrations in fish will increase along the RCC as streams shift from being a detrial to algal based. Significant changes in mercury concentrations in fish will be seen based on leaf-litter or algal food-base. Therefore, this study examines how bioaccumulation of mercury in fish changes along the river continuum as the food-base shifts from leaf litter to algae by collecting non-native fish, leaf litter and algae samples along the river continuum from headwater to the confluence of four freshwater systems: Oak Creek, Wet Beaver Creek, West Clear Creek and the Verde River. Examining samples collected at three sites in Oak Creek (the headwater, *middle and confluence), two sites in Wet Beaver and West Clear Creeks (the headwater and confluence) and at three sites in the Verde River, each site near the confluence of each creek. And to estimate trophic-level and food-web patterns in these study sites, nitrogen and carbon stable isotope ratios were measured and stable isotope ratios of hydrogen were used to distinguish allochthonous (leaf-litter) and autochthonous (algae) energy sources in these four different aquatic

ecosystems. The results of this study will guide future work in determining if long-term monitoring of tissue concentrations of mercury in aquatic biota is needed to assess remedial effectiveness that will be protective of both human health and the environment. This expanded collection will provide us with the broader view necessary to identify mercury accumulation due to leaf or algal food-base, as well as helping to define potential risks of mercury concentrations in fish that can be harmful to aquatic resources and humans. (*Note: Site was excluded for comparison of mercury concentration along the river continuum, though biota sampled was used for statistical analysis).

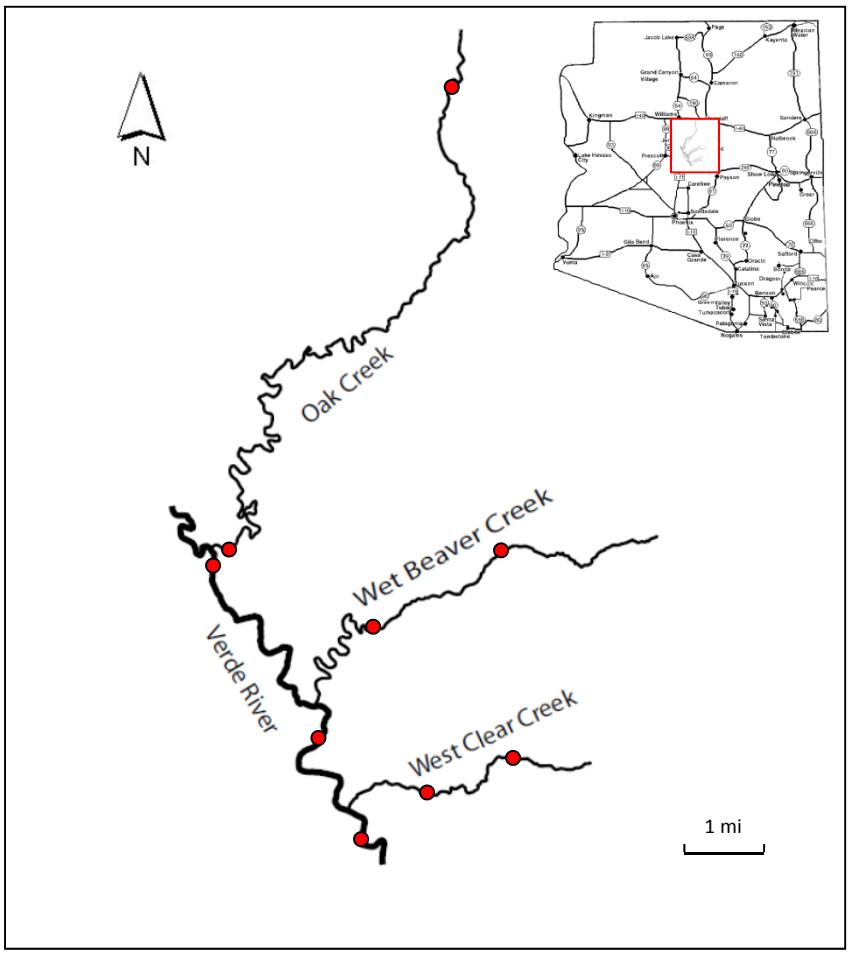
CHAPTER 2.0 MATERIALS AND METHODS

2.1 Sampling Strategies

Site Selection

The consideration for selecting these sites is that there was no historical data on contaminants in fish tissue, lack of information about mercury levels in fish and because most of these places are highly harvested for fish. All three creeks connect to the Verde River which is a key component of the Colorado River watershed and it provides important habitat for a variety of native and non-native species. Sites were chosen to be as close as possible to upstream, downstream and confluences depending on accessibility (FIG. 1).

FIG. 1. Locations of sampling sites in Arizona.



Fish processing

The species studied were bluegill sunfish (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), channel catfish (*Ictalurus punctatus*), flathead catfish (*Pylodictis olivaris*), largemouth bass (*Micropterus salmoides*), white crappie (*Pomoxis annularis*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and smallmouth bass (*Micropterus dolomieui*), and rock bass (*Ambloplites rupestris*). In all, 159 fish were captured and analyzed for mercury (Table 1). Muscle tissue was selected for analysis because it is the tissue consumed by humans. Permits for the capture of fish collected for this study were authorized by the Arizona Game & Fish Department (AZGFD). These permits gave us the ability to capture some individuals of each exotic fish species if observed during collection, providing us with a better sampling of food-web members as well as a better understanding on the bioaccumulation of mercury within these food-web members and a the broader view necessary to identify mercury accumulation due to leaf or algal food-base.

Table 1. Fish average sample information, Hg concentrations, and isotope data by site.

Site	Taxa	*N*	Hg Conc [ng/g]			$\delta^{13}C$ [‰]			$\delta^{15}N$ [‰]			δD [‰]			Total Length (mm)	Weight (g)
			Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean	Mean
OCDN	CRAPPIE	2	213.96	±	29.91	-25.18	±	0.50	11.37	±	0.15	-178.75	±	4.75	64.50	4.80
OCDN	LEMA	1	653.74	±	N/A	-22.08	±	N/A	12.28	±	N/A	-122.30	±	N/A	124.00	32.80
OCDN	MIDO	11	293.64	±	33.18	-25.02	±	0.41	13.42	±	0.42	-144.31	±	4.62	146.00	66.72
OCDN	PYOL	3	352.31	±	27.68	-23.96	±	0.23	13.76	±	0.27	-141.44	±	7.32	178.00	69.20
OCMD	LEMA	3	423.82	±	23.88	-21.85	±	0.37	8.30	±	0.53	-141.61	±	4.78	110.25	22.75
OCMD	MIDO	4	361.06	±	98.48	-24.07	±	0.78	8.59	±	0.72	-148.37	±	10.77	138.00	39.53
OCUP	SATR	12	137.96	±	16.70	-25.40	±	1.02	10.94	±	0.46	-149.79	±	3.38	235.33	147.58
OCUP	ONMY	12	77.98	±	3.77	-18.27	±	0.37	11.03	±	0.12	-121.47	±	4.60	237.50	141.36
VROC	AMRU	5	494.50	±	76.82	-23.93	±	0.51	14.91	±	0.53	-141.24	±	6.00	176.00	113.68
VROC	ICUP	1	420.49	±	N/A	-23.34	±	N/A	12.68	±	N/A	-135.40	±	N/A	334.00	291.80
VROC	MIDO	1	265.91	±	N/A	-26.13	±	N/A	12.30	±	N/A	-164.60	±	N/A	91.00	8.30
VROC	MISA	3	874.87	±	140.08	-23.47	±	0.85	12.53	±	0.59	-115.63	±	11.67	324.00	530.83
VRWB	LEMA	2	318.73	±	2.32	-23.75	±	0.42	12.02	±	0.32	-133.65	±	11.55	103.50	18.35
VRWB	MIDO	11	374.80	±	77.23	-23.67	±	0.20	13.30	±	0.41	-149.51	±	4.05	146.91	59.66
VRWB	MISA	2	531.03	±	170.83	-23.75	±	0.45	13.58	±	1.01	-146.20	±	15.60	182.00	82.05
VRWC	AMRU	1	424.88	±	N/A	-24.56	±	N/A	13.43	±	N/A	-145.64	±	N/A	150.00	60.50
VRWC	LEMA	1	375.51	±	N/A	-24.52	±	N/A	14.65	±	N/A	-145.80	±	N/A	112.00	23.30
VRWC	MIDO	2	1069.71	±	873.85	-23.06	±	0.27	14.06	±	1.23	-127.95	±	29.25	236.00	464.50
VRWC	PYOL	4	555.49	±	92.10	-24.52	±	0.34	14.34	±	0.30	-158.75	±	3.29	203.50	109.78
WBDN	LECY	10	413.91	±	26.98	-22.66	±	0.15	9.60	±	0.23	-117.79	±	2.89	120.80	33.36
WBDN	LEMA	12	944.31	±	40.11	-22.29	±	0.32	10.31	±	0.56	-111.13	±	5.49	126.00	31.60
WBDN	MIDO	6	590.49	±	47.14	-21.84	±	0.41	9.66	±	0.86	-129.63	±	2.53	155.17	48.78
WBDN	PYOL	9	834.79	±	163.39	-23.95	±	0.30	10.27	±	0.30	-159.66	±	6.67	183.22	90.22
WBUP	MIDO	18	332.65	±	43.83	-21.88	±	0.18	10.40	±	0.25	-137.92	±	3.06	167.06	64.90
WCDN	LECY	2	236.42	±	34.78	-22.61	±	0.07	8.57	±	0.33	-129.22	±	4.39	122.50	26.60
WCDN	LEMA	1	211.08	±	N/A	-24.70	±	N/A	9.60	±	N/A	-149.40	±	N/A	101.00	16.20
WCDN	MIDO	10	384.29	±	43.57	-23.36	±	0.32	9.15	±	0.38	-142.77	±	3.66	179.00	69.23
WCDN	PYOL	2	216.55	±	24.07	-25.56	±	0.42	8.00	±	0.25	-207.45	±	11.65	148.00	36.70
WCUP	MIDO	6	256.44	±	41.30	-24.86	±	0.57	8.12	±	0.42	-159.47	±	7.21	142.83	40.83
WCUP	ONMY	1	64.75	±	N/A	-15.93	±	N/A	11.55	±	N/A	-105.10	±	N/A	217.00	100.10
WCUP	PYOL	1	294.93	±	N/A	-24.52	±	N/A	8.69	±	N/A	-138.34	±	N/A	127.00	22.50

Fish were captured using hoop nets, trammel nets and electro-fishing. Hoop netting is the capture of fish by entrapment in an enclosed mesh trap, and is most effective in deep, slow water. A trammel net consists of three layers of net, a loose, small inner mesh

panel of netting is in between two outer layers of netting, which have a larger mesh size; it is set vertically in the water in order that fish attempting to pass through the net will become entangled in one or more of the meshes. Electro-fishing captures fish by stunning them with electric current, and is most effective in less than one meter of water. Captured individuals were measured to the nearest millimeter. Then fin clips from the caudal (tail) fin were obtained using stainless steel scissors and stainless steel tweezers to handle fin clips, wrapped individually in aluminum foil and placed in labeled coin envelopes. Fin clips were used to analyze for stable isotopes. To euthanize fish in the field, we used an overdose (0.5 g/L water) of tricane methanesulfate (MS-222) followed by dislocation of the cervical cord for death assurance. They were rinsed with ambient water, wrapped individually in aluminum foil, placed in polyethylene Ziploc© bags and placed on ice for delivery to the laboratory within 24 hours of collection to be frozen until analysis. Fish must be euthanized because of the size of the sample required for mercury analysis. In the laboratory, frozen fish were partially thawed during processing to preserve the integrity of the tissue and the cells, then fish were weighed and fillets were removed. Prior to use, all fish processing equipment was washed with detergent and rinsed with distilled water as well as in between samples. Fish were placed on a dissection tray and fillets were removed with stainless steel scalpel. The skin was removed from the underlying muscle tissue after filleting. Skin was removed from the fillets to provide the most conservative (highest concentrations) assessment of mercury. Sufficient mass of tissue was removed to meet the analytical detection requirements and the remainder was saved as archived material. Fish tissue was then placed on drying oven under 60^{o}C for at least 24 hours. Once dried, samples were crushed into powder using a mortar and pestle, which was cleaned between samples. Material was then transferred to 50ml glass vials, which were individually labeled.

Fin clips, algae and leaves were analyzed for δD, δ^{15}N and δ^{13}C (stable isotopes of hydrogen, nitrogen and carbon). Stable-isotope measurements are a common tool for identifying food web relationships; nitrogen isotopes indicate "how high" an animal feeds in a food web, carbon and hydrogen isotopes indicate the food base (i.e. algae or detritus) an animal utilizes. Muscle tissue was used to analyze for mercury concentrations. Fin clip, algae and leaf samples were oven-dried at 60^{o}C for 24 hours. Subsamples of about 0.350mg for biota sampled were weighed into silver cups for δD isotopic analysis. Three standards (with known δD values for non-exchangeable H), Chicken Feather (δD = -147‰), Cow Hoof (δD = -187‰), and Bowhead Whale Baleen (δD = -108‰), were used as calibration standards obtained from L. Wassenaar. As much as 12-22% of the hydrogen in complex organic molecules is freely exchangeable with ambient water vapor (Solomon et al., 2009; Wassenaar and Hobson, 2000). For this reason, accurate organic δD measurements require controlling for hydrogen isotope exchange (Bowen et al. 2005), especially when samples are analyzed from different geographical locations. To negate the effect of exchangeable hydrogen on bulk-tissue δD values, all samples and calibration standards were equilibrated with local water vapor according to Wassenaar and Hobson (2003). Biota sampled and standards were pyrolyzed at 1400^{o}C, producing H_2 and CO gases that are separated chromatographically, and the H_2 was analyzed for stable isotope composition using an isotope-ratio mass spectrometer Thermo Electron TC/EA (Thermo-Chemical Elemental Analyzer) and Delta Plus-XL. Based on results indicating very little

variation in the proportion of exchangeable H among samples of very different chemical compositions (Solomon et al., 2009; Wassenaar and Hobson, 2000), it is assumed that our organic samples possessed similar amounts of exchangeable and non-exchangeable H as these three standards. Repeated analyses of several internal organic standards showed that organic δD values were precise to within ±3.0‰ (SD), on average. Subsamples of fin clips of about1.000mg were weighed into tin cups and subsamples of leaves and algae material were weighed between 4.000 and 6.000mg to be analyzed for carbon and nitrogen isotope ratios. Data was normalized using four internationally-accepted isotope standards (IAEA CH_6, CH_7, N_1, and N_2). The Colorado Plateau Stable Isotope Lab main working standard is peach leaves (NIST 1547). External precision on these standards is ± 0.10‰ for $\delta^{13}C$ and ± 0.20‰ for $\delta^{15}N$. Standard materials are Vienna Pee Dee belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. All $\delta^{13}C$ and $\delta^{15}N$ values were normalized on the VPDB and AIR scales with IAEA CH6 (–10.4‰), CH_7 (–31.8‰), N_1 (0.4‰) and N_2 (20.3‰). Thermo-Finnigan Delta^{plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer was used for C/N analyses. All isotope analyses were performed at the Colorado Plateau Stable Isotope Laboratory at NAU.

Algae Processing

Algae samples were collected in two different forms. First, samples were collected from rocks by scraping the algal growth with a stiff-bristled toothbrush into a plastic tray and transferring the slurry into a labeled 100-mL plastic container. One rock scrape was collected at each site, naming it biofilm (Table 2). Second, different observed species of algae were collected by hand in all sites and placed in a 1abeled 100-mL plastic container (Note: Not all same species of algae were found at every site). Each algae specimen was then taken to the laboratory and frozen to be later identified under a microscope. Once samples were ready for identification, algae were partly thawed. Once identified, algae were looked under a light microscope to remove any micro-invertebrate. This was done to assure only algae were being analyzed and isotope results were not affected. All samples were dried at 60^{o}C for at least 24 hours and grounded into powder using a mortar and pestle to be analyzed for stable isotopes and mercury concentrations. Equipment was cleaned between samples using ethanol and distilled water.

Table 2. Algae sample information, Hg concentration and stable isotope data for each species per site.

Site	Algae Species	*N*	Hg Conc [ng/g]			$\delta^{15}N$ [‰]			$\delta^{13}C$ [‰]			δD [‰]		
			Mean		SE	Mean		SE	Mean		SE	Mean		SE
OCDN	Biofilm	1	22.57	±	N/A	0.14	±	N/A	2.65	±	N/A	-109.15	±	N/A
OCDN	Cladophora	1	33.24	±	N/A	10.18	±	N/A	-30.43	±	N/A	-231.09	±	N/A
OCDN	Spyrogyra	2	35.95	±	1.62	6.72	±	1.50	-27.12	±	3.21	-244.01	±	1.07
OCMD	Biofilm	1	22.99	±	N/A	0.69	±	N/A	7.53	±	N/A	-184.80	±	N/A
OCMD	Cladophora	2	44.53	±	0.46	-0.15	±	0.65	-35.94	±	0.12	-260.97	±	2.16
OCMD	Nostoc	1	22.35	±	N/A	-0.93	±	N/A	-18.49	±	N/A	-259.15	±	N/A
OCUP	Biofilm	1	22.82	±	N/A	1.83	±	N/A	16.80	±	N/A	-170.25	±	N/A
OCUP	Cyanobacteria	1	27.41	±	N/A	5.74	±	N/A	-27.46	±	N/A	-154.70	±	N/A
OCUP	Diatom mix	1	38.86	±	N/A	4.54	±	N/A	-33.56	±	N/A	-224.20	±	N/A
OCUP	Spyrogyra	2	33.14	±	9.65	7.02	±	0.22	-27.55	±	0.09	-225.36	±	3.97
VROC	Biofilm	1	21.65	±	N/A	0.14	±	N/A	3.95	±	N/A	-119.75	±	N/A
VROC	Cladophora	3	37.41	±	2.22	7.15	±	N/A	-40.20	±	N/A	-210.91	±	N/A
VROC	Spyrogyra	1	29.11	±	N/A	7.24	±	N/A	-30.14	±	N/A	-254.01	±	N/A

VRWB	Biofilm	1	24.26	±	N/A	0.19	±	N/A	4.13	±	N/A	-115.12	±	N/A
VRWB	Cladophora	1	37.30	±	N/A	6.90	±	N/A	-30.21	±	N/A	-244.78	±	N/A
VRWB	Spyrogyra	2	38.93	±	1.49	5.17	±	0.38	-27.44	±	0.33	-231.11	±	4.28
VRWC	Biofilm	1	19.70	±	N/A	0.24	±	N/A	4.68	±	N/A	-125.17	±	N/A
VRWC	Cladophora	3	36.59	±	3.28	5.58	±	1.71	-29.75	±	1.57	-237.05	±	11.51
WBDN	Biofilm	1	15.85	±	N/A	0.17	±	N/A	5.47	±	N/A	-115.91	±	N/A
WBDN	Cladophora	1	18.21	±	N/A	7.43	±	N/A	-28.47	±	N/A	-169.20	±	N/A
WBDN	mougeotia	2	17.14	±	0.92	7.34	±	3.20	-24.04	±	0.83	-210.07	±	4.66
WBUP	Biofilm	1	23.80	±	N/A	0.69	±	N/A	7.72	±	N/A	-144.03	±	N/A
WBUP	Cladophora	1	17.54	±	N/A	1.64	±	N/A	-34.80	±	N/A	-245.45	±	N/A
WBUP	Nostoc	1	29.40	±	N/A	-0.60	±	N/A	-28.55	±	N/A	-247.07	±	N/A
WCDN	Biofilm	1	19.75	±	N/A	0.40	±	N/A	7.06	±	N/A	-138.70	±	N/A
WCDN	Cyanobacteria filament	4	20.48	±	1.63	-0.01	±	0.25	-27.27	±	0.71	-209.13	±	5.27
WCUP	Biofilm	1	32.69	±	N/A	0.91	±	N/A	12.63	±	N/A	-147.77	±	N/A
WCUP	Cladophora	2	27.82	±	5.35	1.88	±	0.11	-33.75	±	1.31	-238.50	±	3.99
WCUP	Nostoc	1	33.50	±	N/A	-0.15	±	N/A	-17.54	±	N/A	-243.40	±	N/A

To estimate algae growth, three sets of six 6x6in clay tiles were placed in the water at each site. Each set was removed every three weeks and placed in labeled plastic zip-lock bags. Samples were taken to the laboratory to be scraped off the same day of collection into a plastic tray using a stiff-bristled toothbrush and then transferred into a labeled 100-mL plastic container. Then a few drops of formaldehyde were added to each container to preserve algae until ready to be dried. Samples were dried at 60^{o}C for at least 24 hours and grounded into power using a mortar and pestle for homogeneity. Total sample weight was obtained and then, if sample was higher than 1 gram, 4 sub-samples were obtained. Material transferred to a pre-weighed and numbered crucible was weighed. Crucibles were ashed at 550 °C for 1 hour, then cooled in a desiccator for ~ 3 hours and weighed again. Forceps were used to handle crucibles. Ash free dry mass was then calculated by the surface area of the tile (Table 3).

Leaf-litter Processing

Our study design closely for leaf-litter collection followed the methods used in *Methods to Study Litter Decomposition* (Graça et al., 2007). An accessible stream segment was chosen as homogenous as possible in terms of riparian vegetation, geomorphology and substrate. A reach of 50 m was measured in both sides of the stream and three 5-gallon buckets were placed on both sides of the banks within the 50m reach fixed by heavy rocks. Buckets were randomly distributed by extending a measuring tape along the study reach. This process was done at each site. Buckets were labeled with site name and meter mark. Ten spaced measurements of the channel width were reported along the study reach. Material in litter-fall buckets were collected every other week and enclosed it in labeled brown paper bags. Any branches larger than 1cm in diameter were discarded. Material collected was carried to the laboratory to be processed. Leaves were dried at 60 °C and then weighed to calculate total weight by surface area of bucket (Table 3).

Benthic leaf storage was also estimated in by choosing an accessible 50m stream reach. Three random points along the selected stream reach was chosen. A transect was established across the stream from bank to bank (including dry parts of the channel) of 1 m wide intervals. The width of the channel in each transect was noted. Leaves were collected by hand. The samples were rinsed with stream water and all inorganic

materials and wood pieces were eliminated. Materials per transect were placed to a labeled zip-lock plastic bag. The materials collected were then carried to the laboratory and frozen until ready to be dried and calculated for ash-free dry mass. Samples were partly thawed and dried 60^{o}C for at least 24 hours and grinded into power using a Wiley mill grinder for homogeneity. Total sample weight was obtained and because all samples were higher than 1 gram 5 sub-samples were obtained. Forceps were used to handle crucibles. Material transferred to a pre-weighed and numbered crucible was weighed. Crucibles were ashed at 550 °C for 1 hour, then cooled in a desiccator for ~ 3 hours and weighed again. Ash free dry mass was then calculated by transects width (Table 3).

Table 3. Leaf-Litter sample information, Hg concentration and stable isotope data for each site.

Site	Leaf- litter Total Mass g/m^2	BOM AFDM g/m^2	Algae AFDM g/m^2
OCDN	6.67	0.60	27.49
OCMD	8.69	2.14	79.82
OCUP	N/A	10.72	8.29
VROC	2.86	0.59	226.58
VRWB	8.62	0.25	20.32
VRWC	3.33	0.92	75.78
WBDN	1.39	0.79	74.21
WBUP	10.69	10.65	3.48
WCDN	9.55	8.37	1.27
WCUP	10.10	7.27	1.12

At the same time that algae samples were collected, four random leaf specimens were collected from the surface of the water at each site (Table 4) and placed in labeled zip-lock bags and taken to the laboratory to be frozen until ready for analysis. Samples were partly thawed, rinsed with DI water, dried 60^{o}C for at least 24 hours and grinded into power using a Wiley mill grinder for homogeneity. These leaf samples were used to analyze for mercury concentration and stable isotopes.

Table 4. Mass information for leaf-litter collected from buckets, benthic organic matter (BOM) collected from transects and algae scrapes from tiles.

Site	*N*	Hg Conc. [ng/g]			δ^{13}C [‰]			δ^{15}N [‰]			δD [‰]		
		Mean		SE	Mean		SE	Mean		SE	Mean		SE
OCDN	4	60.03	±	3.04	-28.15	±	0.45	-0.50	±	0.46	-138.71	±	2.64
OCMD	4												
OCUP	4	51.48	±	1.19	-29.06	±	0.27	1.67	±	0.47	-150.34	±	0.97
VROC	4	56.20	±	2.84	-28.52	±	0.36	0.12	±	0.31	-141.67	±	2.78
VRWB	4	60.57	±	3.35	-28.44	±	0.40	-0.53	±	0.08	-136.53	±	1.94
VRWC	4	55.08	±	3.37	-29.15	±	0.27	0.60	±	0.27	-137.65	±	0.94
WBDN	4	53.02	±	1.65	-28.11	±	0.28	2.34	±	0.25	-126.34	±	2.34
WBUP	4	59.59	±	2.78	-30.06	±	0.21	-1.44	±	0.08	-141.19	±	1.94
WCDN	4	59.75	±	1.66	-29.46	±	0.24	-2.77	±	0.30	-137.79	±	2.53
WCUP	4	56.27	±	2.12	-30.16	±	0.12	-1.62	±	0.14	-138.99	±	3.32

2.2 Data Analysis

Mercury Analysis Process

Hydra-C mercury analyzer was used to determine mercury concentration. A small amount of sample was weighed and deposited into a nickel sample boat. The weighed sample is then placed into the Hydra C where oxygen begins to flow over the sample. The decomposition furnace temperature is then increased in two stages; first to dry the sample, then to decompose it. The evolved gases are carried through a heated catalyst to produce free mercury while removing halogens, nitrogen oxides, and sulfur oxides. The remaining combustion products including elemental mercury (Hg^0) are swept through a gold amalgamation trap where elemental Hg is trapped and concentrated. After the amalgamation step, the trap is heated to release the mercury into a carrier gas which transports it into an atomic absorption spectrometer. The concentration of mercury is expressed in ng/g (dry weight).

Certified Reference Materials (CRMs) included fish protein (DORM-3) provided by the National Research Council of Canada (Ottawa, Ontario, Canada) and peach leaves (SRM 1547) provided by the National Institute of Standards and Technology (NIST). Certified Dereference Materials were analyzed at the beginning and at the end of a set of 10 samples to demonstrate the accuracy of the method. The mass range used for these reference materials were 20-30 mg for DORM-3 and SRM 1547. A blank was analyzed at the beginning to confirm that Hg was not carried over between samples. This was followed by a double of the 10^{th} sample being analyzed. The mass range used for muscle tissue of fish was 20-35 mg. The mass range used for algae was 30-120 mg depending on how much organic matter sample contained. The mass range used for leaves was 25-30 mg. Between analyses, the ash material was removed from the boat using a folded Kim-wipe.

Data Analysis

Data was analyzed using JMP Statistical Discovery Software (version 8.0, SAS Institute Inc., Cary, NC, USA). Maximum Type I error were set at $\alpha = 0.05$. Normality and homogeneity of variance assumptions were checked plots of the residuals. We used $\log_{10}$-transformed Hg concentrations in all analyses as this equalized variance and normalized residuals. Significant ANOVA results were followed by multiple comparisons using Tukey's HSD post-hoc test.

CHAPTER 3.0 RESULTS

3.1 Mercury Concentrations in the Aquatic Food Web

Hydrogen, carbon and nitrogen stable isotope data and the mercury concentrations in the samples collected from the four different aquatic ecosystems are summarized in Tables 1, 2 and 4. The data showed clear evidence of biomagnification of mercury. The highest concentrations were found in fish (64.75-1944 ng/g; dry weight), intermediate concentrations in leaf-litter (51-61 ng/g), and the lowest concentrations in algae specimens (17–39 ng/g). The lowest concentrations in fish were detected in rainbow trout from upstream Oak Creek since it was found that these fish were stocked by the AZGFD before our collection. Our results demonstrate that the low mercury concentrations in this species are due to the fact they were released from a hatchery.

Mercury measurements indicated that the proportion increased with trophic level. This trend is illustrated by a strong exponential relationship between Hg concentration and $\delta^{15}N$ values for the biota sampled in each site (FIG. 2). The overall biomagnification of mercury in the aquatic food web is illustrated by a plot of Hg concentration versus the $\delta^{15}N$ value for all fish sampled (FIG. 3A).

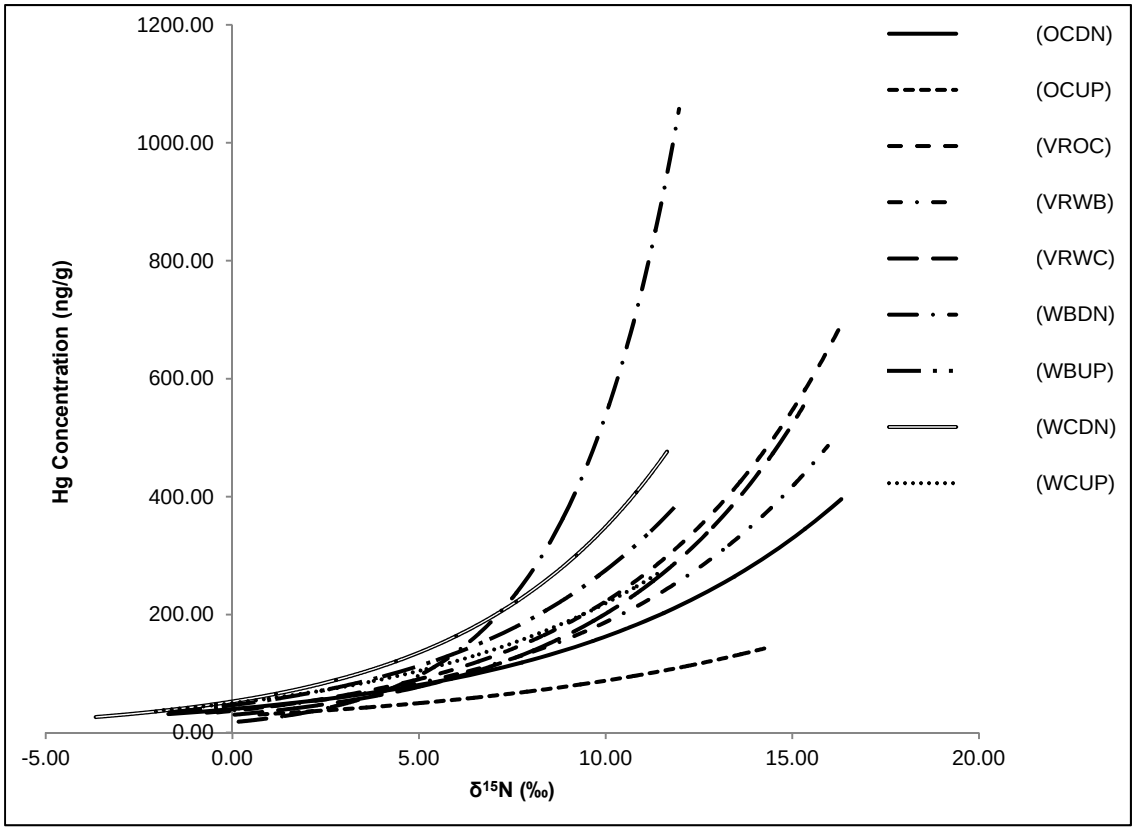


FIG. 2. Log-transformed relationship pooled data of Hg concentration and $\delta^{15}N$ in all study sites for all fish species, algae and leaf-litter. OCUP ($r^2 = 0.6481$, $P < .0001$), OCDN ($r^2 = 0.5277$, $P < .0001$), VROC ($r^2 = 0.7053$, $P < .0001$), VRWB ($r^2 = 0.7467$, $P < .0001$), VRWC ($r^2 = 0.7689$, $P < .0001$), WBUP ($r^2 = 0.5778$, $P < .0001$), WBDN ($r^2 = 0.7943$, $P < .0001$), WCUP ($r^2 = 0.7351$, $P = .0011$) and WCDN ($r^2 = 0.5474$, $P < .0001$).

3.2 Individual Fish Species

For all fish species, apart from *Oncorhynchus mykiss, Ictalurus punctatus, Lepomis cyanellus* and *crappie* spp, Hg concentrations were significantly correlated with fish length (FIG. 4). The lack of any significant relationship between Hg and fish size for *O. mykiss* can be explained by fact that these fish were stocked from a hatchery before our collection. While sample size for *Ictalurus punctatus* and *Crappie* spp. weren't large enough for comparisons; therefore given the limited data collection, mercury bioaccumulation in these species is not discussed further. The lack of a relationship between Hg concentration and fish size for the *Lepomis cyanellus* specimens indicates that these species either have a slow rate of growth with age or a very efficient mercury biomagnification pathway through aquatic insects (Bowles et al., 2001).

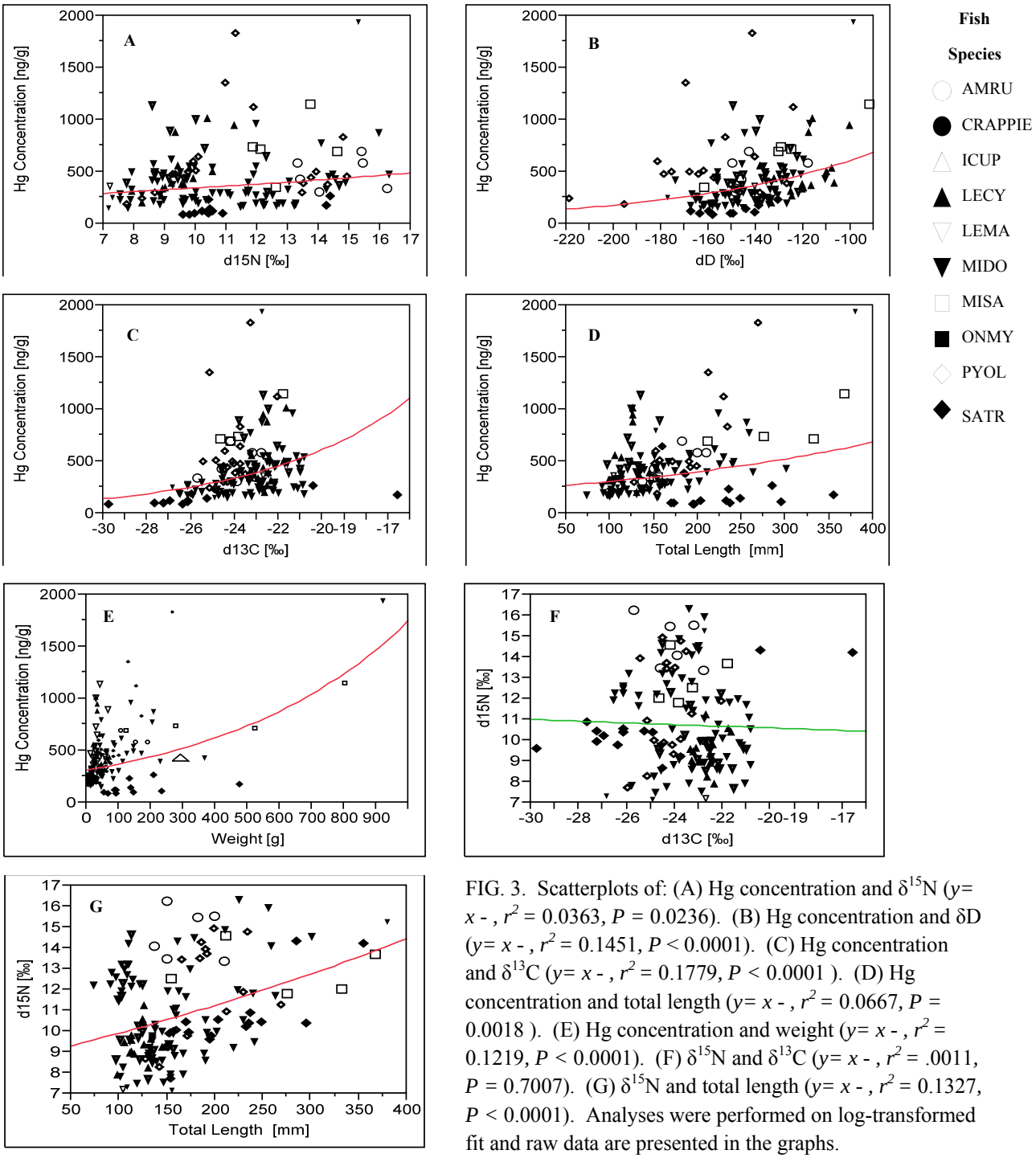


FIG. 3. Scatterplots of: (A) Hg concentration and $\delta^{15}N$ ($y= x - , r^2 = 0.0363, P = 0.0236$). (B) Hg concentration and δD ($y= x - , r^2 = 0.1451, P < 0.0001$). (C) Hg concentration and $\delta^{13}C$ ($y= x - , r^2 = 0.1779, P < 0.0001$). (D) Hg concentration and total length ($y= x - , r^2 = 0.0667, P = 0.0018$). (E) Hg concentration and weight ($y= x - , r^2 = 0.1219, P < 0.0001$). (F) $\delta^{15}N$ and $\delta^{13}C$ ($y= x - , r^2 = .0011, P = 0.7007$). (G) $\delta^{15}N$ and total length ($y= x - , r^2 = 0.1327, P < 0.0001$). Analyses were performed on log-transformed fit and raw data are presented in the graphs.

Differences in Hg bioaccumulation are indicated by the slopes in plots of Hg concentration versus $\delta^{15}N$ for all samples collected for each site (FIG. 5). Only *Lepomis cyanellus, Salmo trutta, Micropterus dolomieui* and *Micropterus salmoide* displayed statistically significant positive correlations between Hg concentration and $\delta^{15}N$ (FIG. 6), which indicate that trophic position increases as mercury concentration increases. The lack of a relationship between $\delta^{15}N$ values and Hg for *O. mykiss, I. punctatus, L. macrochirus, P. olivaris, A. rupestris* and *Crappie* spp. specimens indicates that these

species do not change their trophic position as mercury increase suggesting that some of the variability in Hg concentrations in these fish could be associated to differences in the trophic position of the individual fish.

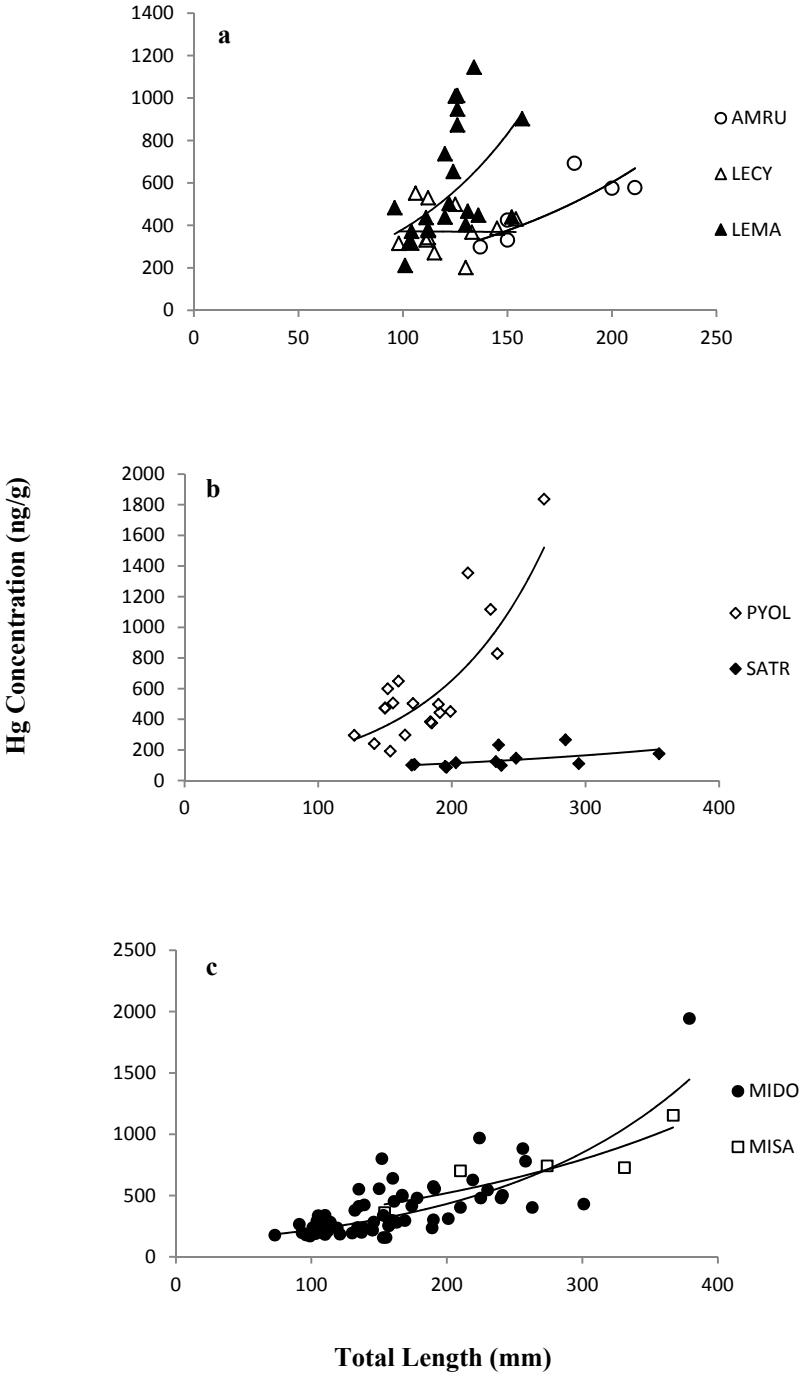


FIG. 4**.** Relationship between Hg concentration and fish length for each fish species, except ONMY, ICUP, and Crappie. (a) *Lepomis macrochirus*, *Lepomis cyanellus.* (b) *Pylodictis olivaris and Salmo trutta.* (c) *Micropterus dolomieui* and *Micropterus salmoide.*

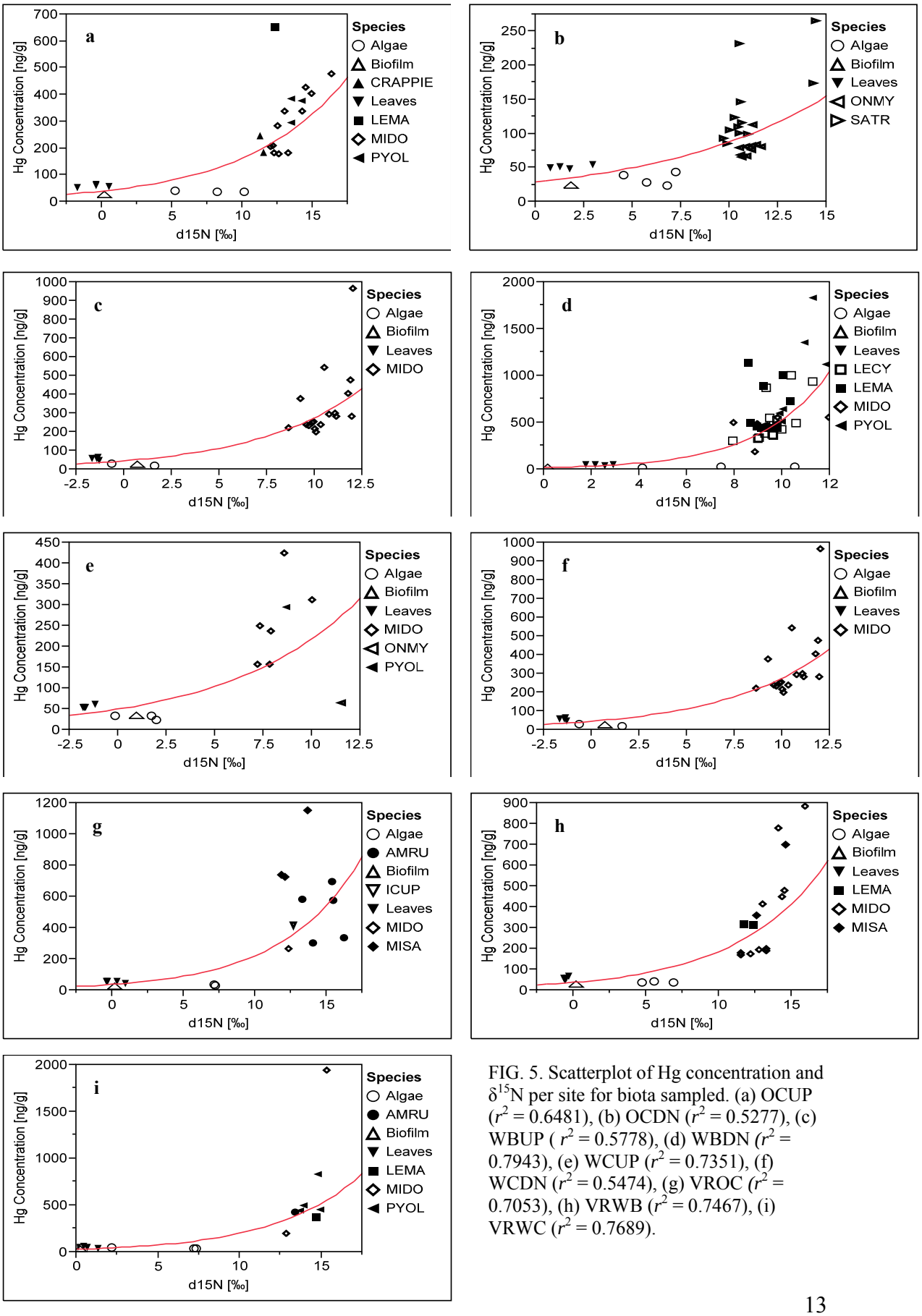


FIG. 5. Scatterplot of Hg concentration and $\delta^{15}N$ per site for biota sampled. (a) OCUP ($r^2 = 0.6481$), (b) OCDN ($r^2 = 0.5277$), (c) WBUP ($r^2 = 0.5778$), (d) WBDN ($r^2 = 0.7943$), (e) WCUP ($r^2 = 0.7351$), (f) WCDN ($r^2 = 0.5474$), (g) VROC ($r^2 = 0.7053$), (h) VRWB ($r^2 = 0.7467$), (i) VRWC ($r^2 = 0.7689$).

CHAPTER 4.0 DISCUSSION

The data presented in this study show differences in Hg concentration, trophic position and fish length among fish species and sites. This study further illustrates the utility of stable isotope measurements in understanding food-web interactions, and their importance in the processes of mercury bioaccumulation. Sampling and monitoring considerations for environmental sampling are discussed in this section as well as regulatory context.

4.1 Species Differences in Hg concentration

Mercury concentrations in rock bass ranged as high as 692 ng/g and averaged 460 ng/g. In green sunfish, mercury concentrations ranged as high as 552 ng/g and averaged 325 ng/g. Bluegill sunfish ranged as high as 1146 ng/g and averaged 488 ng/g. In smallmouth bass ranged as high as 1944 ng/g and averaged 436 ng/g. Mercury concentrations in largemouth bass ranged as high as 1155 ng/g and averaged 702 ng/g. Rainbow trout ranged as high as 114 ng/g and averaged 72 ng/g. Flathead catfish ranged as high as 1836 ng/g and averaged 451 ng/g. In brown trout, mercury concentration ranged as high as 266 ng/g and averaged 138 ng/g (TABLE 1, 10).

Tissue Hg Concentration, Trophic Position and Fish Length Among Fish Species

Mercury concentrations in the fish muscle tissue increased with total length (mm) in AMRU ($P = .0337$), LEMA ($P = .0131$), MIDO ($P < .0001$), MISA ($P = .0475$) and PYOL ($P < .0001$), indicating higher Hg accumulation in larger fish, hence older fish (FIG. 3). In general, there is a statistical significance between Hg concentration and total length among all fish species ($P = .0028$). Mercury concentrations in the fish muscle tissue also increased with weight (g) of the fish in LEMA ($P = .0357$), MIDO ($P < .0001$) and PYOL ($P < .0001$), which it correlates with Hg accumulation in larger fish. These results demonstrate that fish size ($P = .0028$) and weight ($P < .0001$) does affect Hg concentration in fish (FIG. 3D, E). (Note: rainbow trout were excluded from these analyses).

Natural abundance of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope ratios were used to quantitatively test for differences in trophic levels among fish species and the importance of trophic structure on Hg bioaccumulation. The $\delta^{15}N$ was significantly higher in LECY ($P = .0041$), MIDO ($P = .0248$) and SATR ($P = .0114$), indicating the higher the trophic level, the higher the Hg concentration. The difference in carbon signatures and Hg concentration was significant for MIDO ($P = 0.0007$), PYOL ($P = 0.0219$) and SATR ($P = 0.0095$). However, there is a significant statistical difference between Hg concentration and trophic position ($\delta^{15}N$) among all fish species ($P = .0321$) accepting our hypothesis of mercury concentrations in fish will increase with trophic level and will correlate positively with $\delta^{15}N$ values (FIG. 6; FIG. 3A).

The stable nitrogen isotope signature and total fish length are positively correlated for MIDO ($P = .0267$), PYOL ($P = .0098$) and SATR ($P = .0043$), indicating the size-

dependent shifts in $\delta^{15}N$ for these fish species are associated by an increase in Hg and are related to species variation in age-specific selection for higher trophic positioned prey leading to increased rates of MeHg bioaccumulation (FIG. 3G).

The natural abundance stable isotope ratios of hydrogen (δD) were used to compare allochthonous and autochthonous (leaf-litter and algae) energy sources fish utilizes. The δD was significantly higher in MIDO ($P < .0001$), MISA ($P = .0032$) and SATR ($P = .0056$), indicating higher Hg accumulation in larger fish utilize leaf-litter as an energy source and smaller fish utilize algae as an energy source (FIG. 3B).

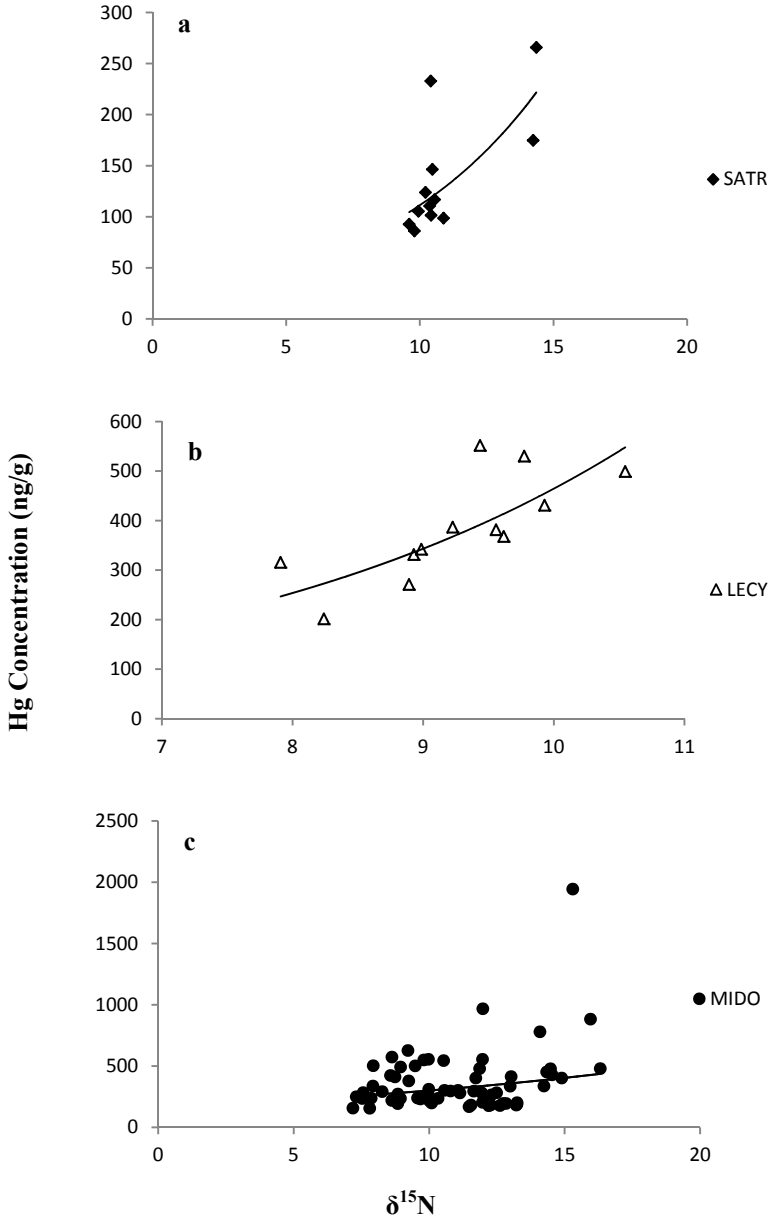


FIG. 6**.** Relationship between Hg concentration and trophic position for each fish species, except ONMY, ICUP, and Crappie, LEMA, PYOL, and AMRU. (a) *Lepomis cyanellus*. (b) *Salmo trutta.* (c) *Micropterus dolomieui*.

Tissue Hg Concentration, Trophic Position and Fish Length Among Sites

An ANOVA model based on mercury concentration and stable nitrogen isotope signature showed a positively correlated for all sites: OCUP ($P < .0001$), OCMD ($P < .0001$), OCDN ($P < .0001$), VROC ($P < .0001$), VRWB ($P < .0001$), VRWC ($P < .0001$), WBUP ($P < .0001$), WBDN ($P < .0001$), WCUP ($P = .0011$) and WCDN ($P < .0001$) demonstrating Hg moves through trophic structure. In addition, and despite the positive correlations among Hg, fish size, and trophic position, inspection of the data and ANOVA also indicated that the relations differed among sites. Figure 2 and Table 5 shows that different sites bioaccumulate Hg differently, meaning a particular site is prone to bioaccumulating Hg by organisms having a higher Hg concentration for a given trophic position as indicated by $\delta^{15}N$.

Site	Vs	Site	p-Value
WBDN		OCUP	0.0000
WBDN		OCDN	0.0000
WCDN		OCUP	0.0000
WBDN		VRWB	<.0001
VROC		OCUP	0.0001
WBUP		OCUP	<.0001
WCUP		OCUP	0.0011
WBDN		VRWC	0.0025
VRWC		OCUP	0.0030
WBDN		WCUP	<.0001
WBDN		WBUP	<.0001
WBDN		VROC	0.0033
WCDN		OCDN	0.0063
VRWB		OCUP	0.0299
WBDN		WCDN	0.0035

Table 5. Hg Concentration vs. $\delta^{15}N$ within sites for all fish species.

Smallmouth bass shows a significant difference between Hg concentration and trophic position in sites OCDN ($P = .0012$), VRWB ($P = .0007$) and WBUP ($P = .0052$) while flathead catfish only shows this difference in site WBDN ($P = .0016$), indicating fish with a given trophic position has more Hg in one site than another, bioaccumulating Hg better in some sites than others (Table 6, 7). Furthermore, smallmouth bass showed a significant difference between Hg concentration and total length in sites OCDN ($P = .0214$), VRWC ($P = .0005$), WBDN ($P = .0099$), WBUP ($P = .0008$), and WCDN ($P = .0099$). While flathead catfish showed a significant difference in sites OCDN ($P = .0216$), WBDN ($P < .0001$), WCDN ($P = .0028$), indicating fish of a given size have higher mercury in some sites than others (FIG.7); Table 8, 9).

Site	Vs	Site	p-Value
WBDN		OCDN	<.0001
WBDN		VRWB	<.0001
WCDN		OCDN	<.0001
OCMD		VRWB	0.0066
WCDN		VRWB	0.0002
WCUP		OCDN	0.0054
WCUP		VRWB	0.0219
WBUP		OCDN	0.0017
WBDN		WBUP	0.0258
WBUP		VRWB	0.0162

Table 6. Difference between Hg Concentration and $\delta^{15}N$ within sites for MIDO.

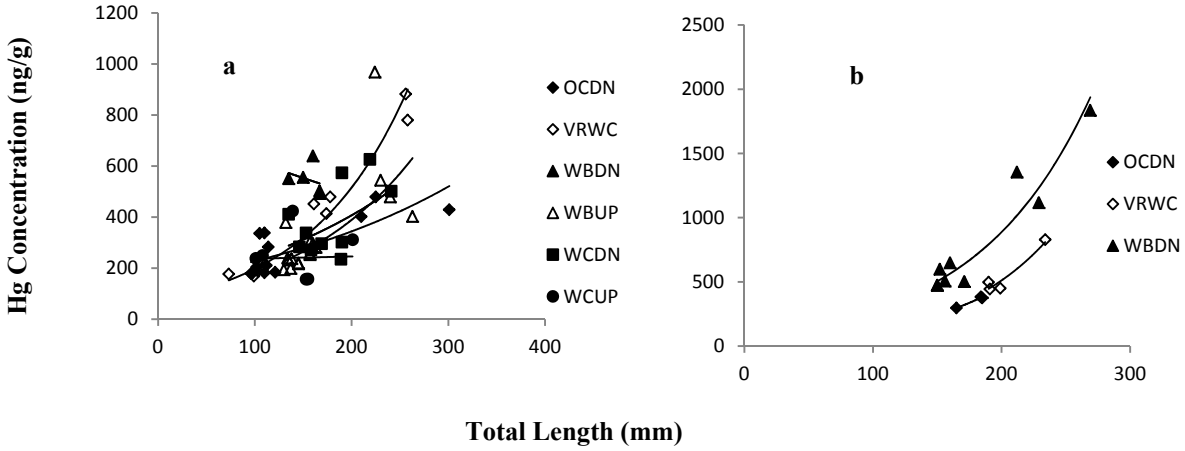


FIG. 7**.** Relationship between Hg concentration and fish length for different sites. (a) *Micropterus dolomieui*. (b) *Pylodictis olivaris.*

Table 7. Difference between Hg Concentration and $\delta^{15}N$ within sites for PYOL.

Site	Vs	Site	p-Value
WBDN		OCDN	0.0002
WBDN		VRWC	0.0009
WCUP		OCDN	0.0132
WCDN		OCDN	0.0183
WCUP		VRWC	0.0357
WCDN		VRWC	0.0481

Table 8. Difference between Hg Concentration and Total Length within sites for MIDO.

Site	Vs	Site	p-Value
WBDN		WCUP	0.0004
WBDN		WBUP	<.0001
WBDN		OCDN	0.0005
WBDN		WCDN	0.0025
WBDN		VRWB	0.0065

Table 9. Difference between Hg Concentration and Total Length

Site	Vs	Site	p-Value
WBDN		WCDN	0.0002
WBDN		OCDN	0.0001
WBDN		VRWC	0.0005

4.2 Differences in Hg Concentration and Stable Isotopes

Our results indicate that mercury concentrations are statistically different upstream from downstream at Wet Beaver Creek, downstream having higher mercury concentrations (P = .0003). Mercury concentrations are statistically different from upstream to downstream, downstream having higher mercury levels at Oak Creek (P < .0001). At West Clear Creek, mercury concentrations do not differ from upstream to downstream (P = .1123). In addition, there is no significant relationship among the Verde River sites in Hg concentration. However, this accepts our hypothesis of mercury concentrations in fish will increase along the RCC as streams shift from being a detrial to algal based and significant changes in mercury concentrations in fish will be seen based on leaf-litter or algal food-base for two of the aquatic ecosystems analyzed (FIG. 8).

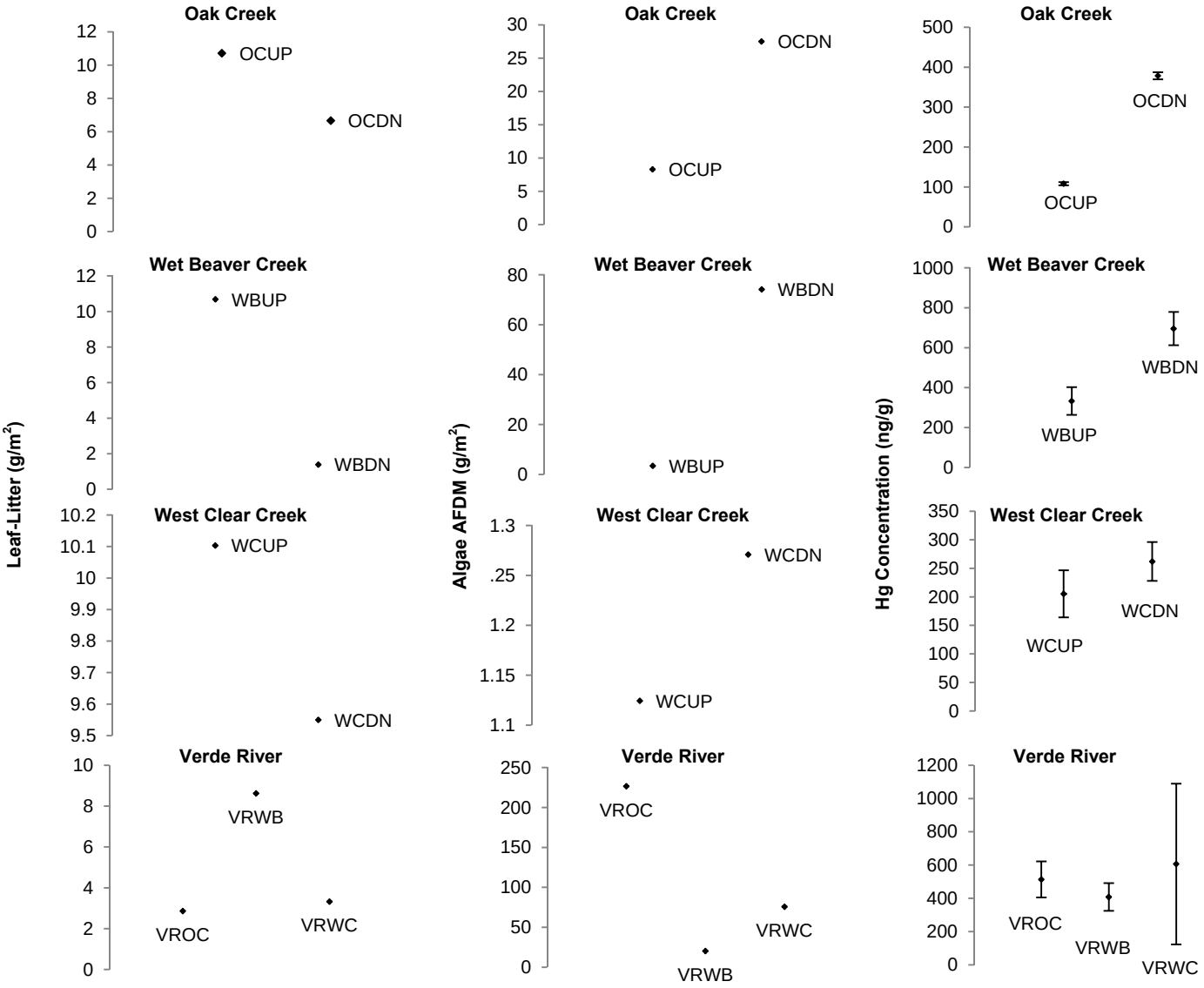


FIG. 8. Plots of mean values showing differences in position of leaf-litter, Hg concentration and algae AFDM in different locations of four aquatic ecosystems studied.

As the River Continuum Concept states, leaf-litter is expected to be higher upstream and decreasing downstream, while algae should be higher downstream and low upstream. This is shown in Oak Creek and Wet Beaver Creek, which is consistent with the two aquatic ecosystems with a significant Hg concentration difference upstream from downstream (FIG. 8).

Even though West Clear Creek and the Verde River showed no significant differences within sites for Hg concentration, leaf-litter mass and algae AFDM, they remained consistent for all these measurements. The data show that the food web in Wet Beaver Creek concentrates more Hg at high trophic positions than in West Clear Creek, the Verde River and Oak Creek. With significance to fish particularly, for a given trophic

position, there are lower concentrations of Hg in fish from Oak Creek, West Clear Creek and the Verde River than from Wet Beaver Creek. It is not yet clear whether this difference is due to differences in the supply of Hg to the streams, differences in the methylation of Hg between the streams, or differences in the trophic structures of the streams (FIG. 9).

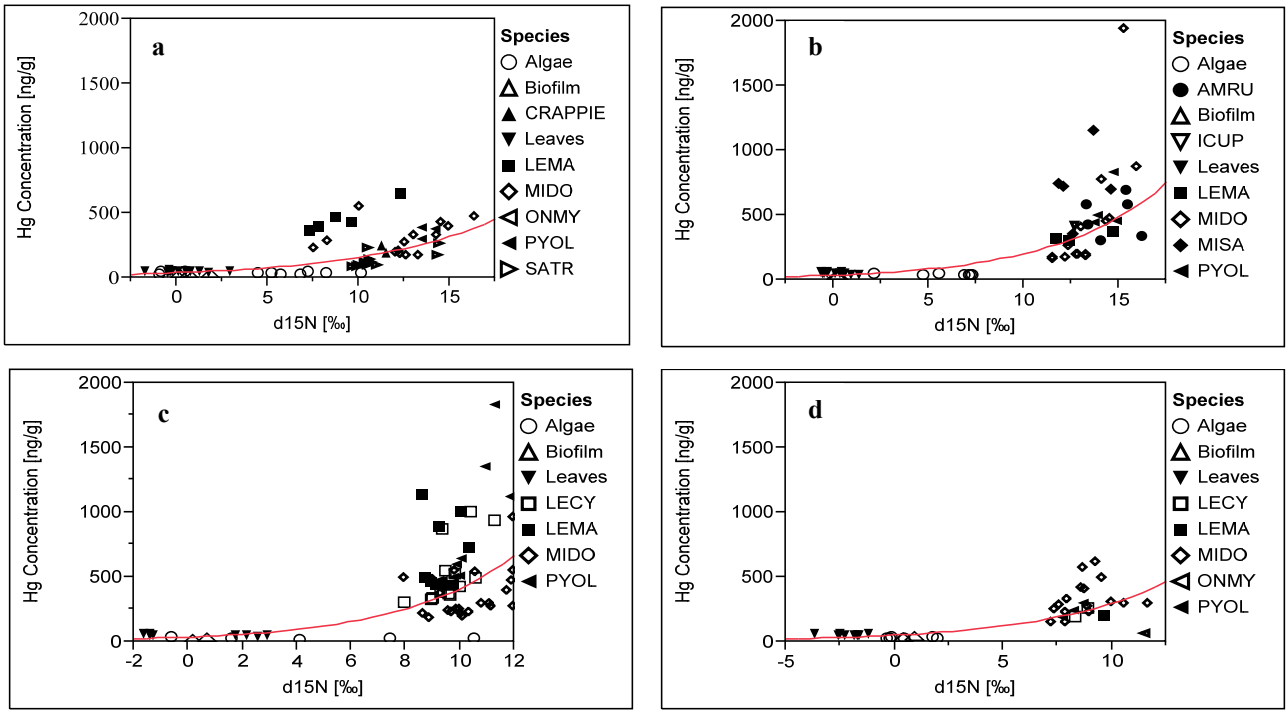


FIG. 9. Mercury Concentration versus $\delta^{15}N$ for food webs in (a) Oak Creek, (b) Verde River, (c) Wet Beaver Creek, (d) West Clear Creek.

The data for individual fish species were notable for the correlations between Hg concentration, fish length, and $\delta^{15}N$. Importantly, the results indicated that fractional changes in trophic position had a significant effect on the mercury concentrations of certain fish species. The use of stable isotope data describes the role of food web effects in mercury bioaccumulation. It is well established that mercury concentrations increase with fish age (therefore fish size) because of the very slow rate of elimination of MeHg from tissues compared with its rate of uptake, which is the basic process by which Hg is bioaccumulated (Swanson, et al, 2003). Most recent data indicate food uptake as the main pathway for mercury bioaccumulation in fish and the reason for this is because fish that occupy the same trophic position throughout their life are exposed to relatively constant mercury concentrations in their food, therefore Hg concentrations gradually increasing with age (Bowles, et al., 2001). Partial increases in trophic position potentially result in an increase in the Hg content of prey, corresponding to an increase in the change of Hg to the predator and, therefore, an increased Hg concentration in tissue. Therefore, increases in trophic position increases the bioaccumulation of Hg through time.

Data presented in this paper show that the length of the food chain and the associated biomagnifications factors between trophic levels were sufficient to biomagnify MeHg concentrations approximately 15-fold between the algae and fish. The relatively high Hg concentrations in the different streams fish are therefore explicable by the efficient bioaccumulation of Hg at the base of the food web, and the magnification of mercury concentrations given by the length of the food chain.

This study of mercury bioaccumulation focuses on analyzing contaminant concentrations and examining food-web interactions and changes in trophic position within individual fish species. The results show the utility of carbon and nitrogen stable isotope measurements in understanding food-web interactions, and their importance in separating the processes of mercury bioaccumulation. Used in conjunction with mercury concentration and fish-size data, these parameters allow conclusions to be made on the processes affecting bioaccumulation in individual fish species (Swanson, et al, 2003). Significant trophic position ($\delta^{15}N$) and body size relationships in some species were observed except for rainbow trout, brown trout, bluegill, channel catfish and crappie. These relationships differed among sites and among species; therefore comparisons of trophic position and total length were made at FIG. 3G to see a relationship between these two for all fish species collected. In order to see a more significant relationship between trophic position and total length, a larger size range of sampled fish would be needed to determine if this explains the differing Hg concentration and body size relationships for the studied species that did not have a significant relationship.

It was found that fin clips of δD for fish consumers, occupying the higher trophic levels in these aquatic ecosystems, were intermediate between δD of algae and leaves (FIG. 10). This suggests combined dependence on these energy sources for consumer biomass production (Doucett, 2007). It is clear that the results on δD stable isotopes demonstrate that they can be used to identify energy flow in aquatic food webs where both allochthonous and autochthonous sources of productivity are present.

4.3 Sampling and Monitoring Considerations

Mercury as a Pollutant

Mercury has been well known as an environmental pollutant for several decades (USGS, 1995). There is well documented information in the accumulation of mercury in fish to concentrations of concern for human consumption. Mercury concentrations in local aquatic organisms can provide an assessment of the availability of mercury in a particular area because of its potential human health concerns and because aquatic organisms can be the best indicators of availability of mercury under the specific conditions present at a site (Beckvar et al., 1996). The selection of target species, trophic level, size (age), weight, and energy sources of organisms are all important factors to consider when analyzing for Hg. It is valuable to use higher trophic-level fish species for determining whether a high mercury problem exists at a particular site since mercury bioaccumulates and thus may be found in the highest concentrations in predatory fish. Additionally, it is useful to perform long-term monitoring since mercury concentrations decrease at slower

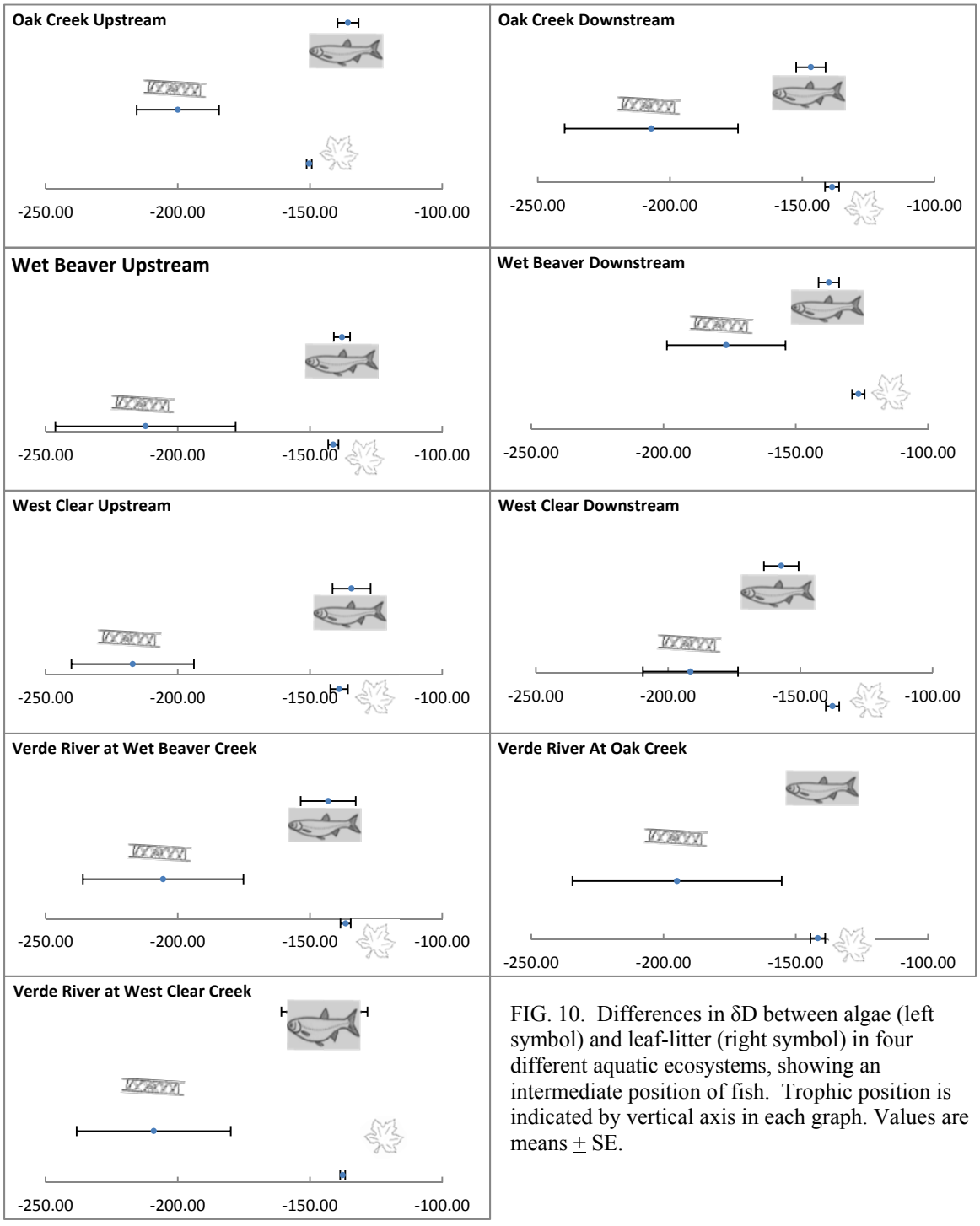


FIG. 10. Differences in δD between algae (left symbol) and leaf-litter (right symbol) in four different aquatic ecosystems, showing an intermediate position of fish. Trophic position is indicated by vertical axis in each graph. Values are means ± SE.

rates. Though, even fish occupying the same trophic level, with similar diets and feeding habits, can show different patterns of mercury accumulation due to differences in habitat preferences, behavior, and metabolic rate causing different exposures (Beckvar et al., 1996). It is also possible that mercury concentrations in biota may not show a

relationship with sediment mercury concentrations, therefore correlations between mercury concentrations in predator and prey species are useful to determine the food web pattern that connects the mercury in the sediment to the biota.

Environmental Sampling

Depending on the scope of the project, monitoring several different species from different trophic levels may be appropriate. Monitoring changes in abundance and diversity in macrobenthic community composition can provide useful information in estimating the toxicity of mercury in aquatic habitats. Macrobenthic communities may affect biogeochemical processes in sediments and provide a combination between contaminants and the food chain. Macroinvertebrates can provide the answer to understanding the level of impact that contaminants have on the food chain. When developing sampling objectives, the probability for direct toxicity to aquatic organisms should also be considered. Laboratory and *in situ* toxicity testing can be useful methods for estimating the direct biological effects of increasing mercury concentrations to aquatic organisms in sediment and water. Standard toxicity tests for testing toxicity at mercury sites should be included (Beckvar et al., 1996). Other important tests for mercury toxicity that could be done are: early life-stage tests which will test the species from post-fertilization through embryonic stage, larval, and early juvenile development; partial life-cycle tests from early juvenile through post-hatch of next generation.

Examinations of mercury concentrations should include both spatial and seasonal sampling. Seasonal and spatial variations in mercury concentrations, including its forms and distribution, within a single water body can be important to be able to observe significant changes. By determining environmental parameters that affect the activity of methylating bacteria such as nutrients, temperature, and dissolved oxygen as well as the factors that affect the availability of inorganic mercury for methylation of mercury such as the resuspension of sediment, total organic carbon (TOC), and sulfides, may be necessary when for the design of sampling and monitoring. To determine the relative magnitude of the individual inorganic and methylmercury species and their overall partition coefficients, data on chloride concentration and pH may be used (Mason et al., 1996). In determining the level of contamination at a site, it is important to consider that both resuspended contaminated sediment and dissolved mercury since they act as important sources of transportation (Beckvar et al., 1996).

Modeling of mercury in aquatic environments and the availability of mercury to aquatic organisms requires the collection of detailed information on the forms of mercury and their relative concentrations in different environmental compartments, for example the amounts of inorganic, methylmercuy, and elemental mercury in dissolved and particulate forms in water, sediment, and biota. The transfer of mercury through the food web has been modeled using a descriptive approach to explain the high levels of mercury of fish in several studies. This approach would be useful in identifying critical factors responsible for localized elevations in mercury concentrations, but also in demonstrating the limitations and large effort required for modeling.

4.4 Regulatory Context

The U.S. federal government has developed regulations enforced by law to protect public health. Some federal agencies that have developed these regulations for toxic substances are the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA). Regulations are usually expressed in "not-to-exceed" levels in air, water, soil, or food that are based on levels that affect animals which are then adjusted to help protect people (ATSDR, 1999). Sometimes these "not-to-exceed" levels differ among federal organizations because of different exposure times, the use of different animal studies, or other factors.

The most recent EPA and FDA regulations have set a limit of 2 parts per billion (ppb) of inorganic mercury in drinking water. The current U.S. Environmental Protection Agency criterion is based on mercury concentrations in fish because there is not enough information about mercury in water or about bioaccumulation of mercury in fish to establish a water standard. However, EPA is in the process of revising the Water Quality Criteria for mercury. It has determined that a daily exposure (for an adult of average weight) to inorganic mercury in drinking water at a level up to 2 ppb is not likely to cause any significant adverse health effects. EPA currently recommends that the level of inorganic mercury in rivers, lakes, and streams be no more than 144 parts per trillion (ppt) of mercury in water to protect human health (1 ppt is a thousand times less than 1 ppb). The FDA has set a maximum acceptable level of 1ppm of methylmercury of seafood products sold through interstate commerce (1 ppm is a thousand times more than 1 ppb). When shipments of fish and shellfish contain more than 1 ppm of methylmercury can be detained by the FDA. Different agencies use different criteria in mercury risk assessments. For example, the Department of Health and Environmental Control (DHEC) has set mercury detection limit in fish to be 0.25 ppm, the EPA at 0.30 ppm, the Health Canada at 0.50 ppm = 500 ppb and the FDA at 1.0 ppm as mentioned above.

OSHA regulates levels of mercury in the workplace. It has set limits of 0.1 milligrams of mercury per cubic meter of air (mg/m^3) for organic mercury and 0.05 mg/m^3 for elemental mercury vapor in workplace air to protect workers during an 8-hour shift and a 40-hour work week.

Approximately 75% of moisture content is removed from every sample when dried. For this reason we made a rough conversation from dry weight to wet weight to estimate mercury concentration detection limit (Table 10). Methylmercury in fish is often near to and sometimes exceeds the concentration deemed safe for human consumption (1 ppm). The average values are approximately 10 times below the U.S. Food and Drug Administration's (FDA) action level of 1000 ng/g (1 ppm) for fish tissue mercury content. Even the maximum mercury concentration measured in individual fish (486 ng/g) was well below the FDA action level. The data from this study are not readily comparable to the EPA guidance. However, based on the conservative assumption that 100% of total mercury was in the form of methylmercury, 3% fish analyzed, respectively, fell into the unrestricted consumption category established in the EPA guidance for methylmercury (Table 10).

Our findings will contribute to improved mercury criteria and standards and contribute with new information to federal and state agencies to continue with monitoring of these sites. More studies that relate concentrations of mercury in water to mercury concentrations in fish will eventually allow a more timely and cost-effective method for regulating mercury and setting water mercury standards. In addition, the data from this study and research may aid in the development of more thorough monitoring designs that relate water quality to mercury bioaccumulation, thereby enhancing capabilities for predicting mercury contamination in fish.

CHAPTER 5.0 CONCLUSION

The findings of this study indicate that Hg concentrations in fish muscle tissue vary according to the species, trophic level, and fish length. However, significant differences in the bioaccumulation of mercury are shown in two of the four freshwater ecosystems studied by comparing a leaf-litter food-base to an algal food-base ecosystem along the river continuum. Our results indicate that mercury concentrations are statistically different upstream from downstream at Wet Beaver Creek and Oak Creek, downstream having higher mercury concentrations. This accepts our hypothesis of mercury concentrations in fish will increase along the RCC as streams shift from being a detrial to algal based. No significant relationship in Hg concentration was found at West Clear Creek from upstream to downstream and among the Verde River sites. The River Continuum Concept states that leaf-litter is expected to be higher upstream and decreasing downstream, while algae should be higher downstream and low upstream and this is shown in the two aquatic ecosystems with a significant Hg concentration difference upstream from downstream. This study also showed mercury concentrations in fish increases with trophic level and correlate positively with $\delta^{15}N$ values as hypothesized. The data show that the food web in Wet Beaver Creek concentrates more Hg at high trophic positions than in West Clear Creek, the Verde River and Oak Creek. With significance to fish particularly, for a given trophic position, there are lower concentrations of Hg in fish from Oak Creek, West Clear Creek and the Verde River than from Wet Beaver Creek indicating differences in Hg bioaccumulation between streams. The results from this study establish baselines for future studies and monitoring of mercury.

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Table 10. Summary of total length, weight, Hg concentrations, and isotope data of fish.

Site	Fish Species	Total Length [mm]	Weight [g]	Hg Conc. [ng/g]	$d^{13}C$ [‰]	$d^{15}N$ [‰]	dD [‰]	Estimated Wet Weigt Hg Conc (ng/g)
VROC	AMRU	137.0	50.5	297.91	-23.88	14.05	-148.5	74.5
VROC	AMRU	150.0	64.1	330.29	-25.69	16.24	-147.8	82.6
VROC	AMRU	182.0	106.6	692.31	-24.17	15.43	-142.3	173.1
VROC	AMRU	200.0	155.8	574.51	-23.18	15.48	-149.8	143.6
VROC	AMRU	211.0	191.4	577.49	-22.75	13.34	-117.8	144.4
VRWC	AMRU	150.0	60.5	424.88	-24.56	13.43	-145.64	106.2
OCDN	CRAPPIE	61.0	4.1	184.05	-25.67	11.52	-174.0	46.0
OCDN	CRAPPIE	68.0	5.5	243.86	-24.68	11.21	-183.5	61.0
VROC	ICUP	334.0	291.8	420.49	-23.34	12.68	-135.4	105.1
WBDN	LECY	98.0	15.9	315.79	-22.34	7.91	-125.1	78.9
WBDN	LECY	106.0	20.5	551.99	-23.24	9.43	-138.21	138.0
WBDN	LECY	111.0	19.7	331.61	-22.96	8.93	-120.4	82.9
WBDN	LECY	112.0	23.8	381.72	-23.33	9.56	-120.6	95.4
WBDN	LECY	112.0	22.6	342.43	-23.32	8.99	-119.7	85.6
WBDN	LECY	112.0	23.8	530.16	-22.40	9.77	-107.78	132.5
WBDN	LECY	125.0	31.8	499.13	-21.79	10.55	-110.3	124.8
WBDN	LECY	133.0	46.7	368.27	-23.11	9.62	-116.7	92.1
WBDN	LECY	145.0	56.9	386.98	-22.50	9.23	-106.6	96.7
WBDN	LECY	154.0	70.9	431.07	-22.68	9.93	-132.5	107.8
WCDN	LECY	115	18.9	271.20	-22.54	8.89	-124.83	67.8
WCDN	LECY	130.0	34.3	201.64	-22.67	8.24	-133.6	50.4
WBDN	LEMA	126.0	31.9	873.11	-22.51	9.32	-117.47	218.3
WBDN	LEMA	126.0	31.8	947.93	-22.70	11.24	-100.2	237.0
WBDN	LEMA	126.0	31.1	1011.91	-21.65	10.36	-115.7	253.0
OCDN	LEMA	124.0	32.8	653.74	-22.08	12.28	-122.3	163.4
OCMD	LEMA	96.0	13.8	483.27	-22.10	8.65	-145.35	120.8
OCMD	LEMA	104.0	20.0	371.64	-22.72	7.22	-140.5	92.9
OCMD	LEMA	111.0	21.8	436.94	-21.01	9.61	-129.0	109.2
OCMD	LEMA	130.0	35.4	403.42	-21.57	7.73	-151.6	100.9
VRWB	LEMA	103.0	18.2	321.05	-23.33	11.70	-145.2	80.3
VRWB	LEMA	104.0	18.5	316.41	-24.16	12.33	-122.1	79.1
VRWC	LEMA	112.0	23.3	375.51	-24.52	14.65	-145.8	93.9
WBDN	LEMA	120.0	25.7	438.98	-24.57	9.73	-136.9	109.7
WBDN	LEMA	120.0	29.5	737.53	-23.61	10.30	-125.22	184.4
WBDN	LEMA	122.0	28.6	502.93	-21.62	8.67	-136.2	125.7
WBDN	LEMA	125.0	29.9	1008.90	-22.50	10.01	-137.9	252.2
WBDN	LEMA	131.0	34.3	466.94	-22.16	8.92	-148.2	116.7
WBDN	LEMA	134.0	41.7	1145.58	-22.71	8.57	-149.5	286.4
WBDN	LEMA	136.0	39.9	447.76	-23.08	9.11	-156.4	111.9
WBDN	LEMA	152.0	60.3	439.69	-24.61	9.40	-156.5	109.9
WBDN	LEMA	157.0	64.8	902.70	-23.83	9.18	-139.6	225.7
WCDN	LEMA	101.0	16.2	211.08	-24.70	9.60	-149.4	52.8
OCDN	MIDO	96.0	10.0	178.73	-26.15	12.61	-152.9	44.7
OCDN	MIDO	102.0	12.9	204.72	-26.54	11.96	-158.63	51.2
OCDN	MIDO	105.0	12.7	336.59	-23.68	12.99	-133.9	84.1
OCDN	MIDO	110.0	18.6	182.16	-25.91	13.21	-161.8	45.5
OCDN	MIDO	110.0	15.3	338.29	-24.59	14.23	-148.3	84.6
OCDN	MIDO	112.0	17.2	211.28	-25.52	12.16	-140.0	52.8
OCDN	MIDO	114.0	18.5	282.82	-26.17	12.48	-152.8	70.7

OCDN	MIDO	121.0	22.1	184.10	-26.48	12.27	-159.3	46.0
OCDN	MIDO	210.0	113.3	402.55	-23.82	14.89	-133.6	100.6
OCDN	MIDO	225.0	125.0	479.32	-23.39	16.31	-135.6	119.8
OCDN	MIDO	301.0	368.3	429.50	-22.99	14.53	-110.6	107.4
OCMD	MIDO	104.0	12.2	292.47	-24.87	8.26	-167.1	73.1
OCMD	MIDO	119.0	20.9	235.46	-24.83	7.53	-148.2	58.9
OCMD	MIDO	191.0	85.5	555.26	-22.50	9.97	-129.8	138.8
VROC	MIDO	91.0	8.3	265.91	-26.13	12.30	-164.6	66.5
VRWB	MIDO	108.0	14.3	195.25	-24.34	12.73	-147.2	48.8
VRWB	MIDO	161.0	46.7	451.85	-23.00	14.33	-126.6	113.0
VRWB	MIDO	174.0	64.7	414.33	-23.67	13.02	-156.3	103.6
VRWB	MIDO	256.0	207.6	882.87	-22.75	15.95	-144.6	220.7
VRWB	MIDO	258.0	205.7	780.28	-23.31	14.09	-158.8	195.1
WBDN	MIDO	135.0	29.6	550.91	-21.22	9.80	-129.7	137.7
WBDN	MIDO	150.0	41.9	555.60	-22.68	11.97	-136.4	138.9
WBDN	MIDO	160.0	51.9	639.83	N/A	N/A	N/A	160.0
WBDN	MIDO	167.0	60.6	502.94	-21.05	7.93	-128.1	125.7
WBDN	MIDO	167.0	64.3	492.80	-22.42	8.94	-124.3	123.2
WBUP	MIDO	130.0	21.6	194.10	-20.87	8.84	-134.2	48.5
WBUP	MIDO	132.0	24.8	379.05	-22.46	9.25	-125.0	94.8
WBUP	MIDO	134.0	25.3	239.72	-21.14	9.57	-133.2	59.9
WBUP	MIDO	135.0	25.7	232.32	-22.65	9.67	-163.9	58.1
WBUP	MIDO	137.0	30.0	198.88	-22.02	10.09	-126.7	49.7
WBUP	MIDO	138.0	26.6	237.57	-22.30	10.32	-140.3	59.4
WBUP	MIDO	145.0	31.5	220.30	-22.22	8.62	-147.8	55.1
WBUP	MIDO	145.0	32.7	217.33	-22.90	10.00	-134.5	54.3
WBUP	MIDO	157.0	48.3	252.76	-21.33	9.86	-135.0	63.2
WBUP	MIDO	157.0	38.7	302.52	-22.76	11.06	-140.2	75.6
WBUP	MIDO	157.0	46.3	257.55	-22.95	9.95	-156.63	64.4
WBUP	MIDO	160.0	43.8	281.71	-20.90	11.14	-130.69	70.4
WBUP	MIDO	160.0	45.7	296.99	-22.16	10.78	-141.7	74.2
WBUP	MIDO	163.0	47.7	281.65	-22.21	11.94	-157.2	70.4
WBUP	MIDO	224.0	135.7	968.44	-21.36	11.97	-117.1	242.1
WBUP	MIDO	230.0	144.6	544.50	-20.83	10.53	-128.1	136.1
WBUP	MIDO	240.0	169.5	479.39	-21.69	11.86	-121.1	119.8
WBUP	MIDO	263.0	229.7	402.99	-21.01	11.72	-149.2	100.7
WCDN	MIDO	135.0	25.6	411.57	-23.23	8.73	-135.3	102.9
WCDN	MIDO	146.0	32.2	283.66	-22.88	7.56	-152.6	70.9
WCDN	MIDO	153.0	36.8	338.15	-23.42	7.92	-131.7	84.5
WCDN	MIDO	158.0	42.5	272.32	-24.04	8.84	-151.2	68.1
WCDN	MIDO	169	56.1	296.11	-25.51	11.64	-154.1	74.0
WCDN	MIDO	189.0	83.3	235.35	-22.76	8.94	-138.83	58.8
WCDN	MIDO	190.0	80.4	302.75	-24.10	10.56	-141.9	75.7
WCDN	MIDO	190.0	81.8	574.00	-23.30	8.62	-145.9	143.5
WCDN	MIDO	219.0	107.1	626.77	-21.83	9.21	-119.9	156.7
WCDN	MIDO	241.0	146.5	502.20	-22.53	9.48	-156.3	125.5
WCUP	MIDO	101.0	12.2	238.41	-25.78	7.85	-165.3	59.6
WCUP	MIDO	108.0	15.7	250.12	-26.83	7.31	-177.5	62.5
WCUP	MIDO	139.0	29.3	423.94	-24.56	8.57	-173.9	106.0
WCUP	MIDO	153.0	44.8	156.81	-24.35	7.80	-145.3	39.2
WCUP	MIDO	155.0	44.3	157.61	-24.93	7.18	-163.2	39.4
WCUP	MIDO	201.0	98.7	311.76	-22.71	9.98	-131.6	77.9
VRWB	MIDO	73.0	5.2	176.95	-24.77	12.20	-167.60	44.2
VRWB	MIDO	98.0	9.7	182.06	-23.53	11.54	-158.0	45.5
VRWB	MIDO	99.0	11.4	168.99	-23.22	11.47	-156.2	42.2

VRWB	MIDO	103.0	14.4	189.02	-24.53	13.23	-161.7	47.3
VRWB	MIDO	108.0	14.2	202.08	-24.09	13.24	-137.6	50.5
VRWB	MIDO	178.0	62.4	479.11	-23.13	14.48	-129.97	119.8
VRWC	MIDO	93.0	8.7	195.86	-23.32	12.83	-157.2	49.0
VRWC	MIDO	379.0	920.3	1943.56	-22.79	15.29	-98.7	485.9
WBDN	MIDO	152	44.4	800.83	N/A	N/A	N/A	200.2
VROC	MISA	274.0	274.7	742.27	-23.86	11.84	-129.29	185.6
VROC	MISA	331.0	518.8	727.44	-24.70	12.06	-125.2	181.9
VROC	MISA	367.0	799.0	1154.90	-21.85	13.70	-92.4	288.7
VRWB	MISA	154.0	42.3	360.20	-23.30	12.57	-161.8	90.0
VRWB	MISA	210.0	121.8	701.86	-24.20	14.59	-130.6	175.5
OCUP	ONMY	172.0	49.0	113.71	-19.98	11.24	-153.5	28.4
OCUP	ONMY	232.0	128.7	68.13	-17.65	10.61	-109.5	17.0
OCUP	ONMY	232.0	165.0	66.80	N/A	N/A	N/A	16.7
OCUP	ONMY	233.0	111.0	81.86	-20.56	11.17	-141.1	20.5
OCUP	ONMY	234.0	142.0	71.24	N/A	N/A	N/A	17.8
OCUP	ONMY	236.0	149.1	79.02	-17.48	10.55	-109.57	19.8
OCUP	ONMY	244.0	135.2	76.38	-18.08	11.18	-117.4	19.1
OCUP	ONMY	245.0	145.8	67.74	-17.51	10.91	-124.1	16.9
OCUP	ONMY	249.0	147.0	83.42	-19.10	11.44	-117.6	20.9
OCUP	ONMY	256.0	188.7	65.30	-17.54	10.70	-114.7	16.3
OCUP	ONMY	257.0	177.7	81.20	-17.43	10.86	-114.0	20.3
OCUP	ONMY	260.0	157.1	80.93	-17.40	11.66	-113.2	20.2
WCUP	ONMY	217.0	100.1	64.75	-15.93	11.55	-105.1	16.2
OCDN	PYOL	165.0	60.8	297.13	-24.33	13.47	-146.6	74.3
OCDN	PYOL	184.0	71.0	383.77	-24.01	13.51	-127.0	95.9
OCDN	PYOL	185.0	75.8	376.02	-23.55	14.29	-150.71	94.0
VRWC	PYOL	190.0	82.4	498.39	-25.43	13.92	-168.1	124.6
VRWC	PYOL	191.0	84.6	443.76	-24.33	13.74	-157.6	110.9
VRWC	PYOL	199.0	99.3	450.44	-24.54	14.94	-156.6	112.6
VRWC	PYOL	234.0	172.8	829.37	-23.78	14.77	-152.7	207.3
WBDN	PYOL	150.0	42.6	473.85	-23.78	9.24	-178.6	118.5
WBDN	PYOL	150.0	38.6	473.19	-24.05	9.35	-164.2	118.3
WBDN	PYOL	152.0	37.1	598.89	-24.44	9.91	-181.7	149.7
WBDN	PYOL	156.0	45.2	506.41	-24.86	10.02	-161.8	126.6
WBDN	PYOL	160.0	43.2	649.05	-23.77	10.07	-140.13	162.3
WBDN	PYOL	171.0	57.6	503.28	-24.08	9.76	-175.4	125.8
WBDN	PYOL	212.0	127.8	1355.15	-25.16	10.95	-169.8	338.8
WBDN	PYOL	229.0	152.7	1117.07	-22.07	11.87	-124.1	279.3
WBDN	PYOL	269.0	267.2	1836.20	-23.31	11.30	-141.2	459.0
WCDN	PYOL	142.0	31.3	240.62	-25.14	8.25	-219.1	60.2
WCDN	PYOL	154.0	42.1	192.48	-25.98	7.75	-195.8	48.1
WCUP	PYOL	127.0	22.5	294.93	-24.52	8.69	-138.34	73.7
OCUP	SATR	170.0	53.9	101.46	-27.28	10.42	-163.4	25.4
OCUP	SATR	172.0	53.6	105.34	-27.24	9.95	-150.91	26.3
OCUP	SATR	195.0	67.5	92.77	-29.74	9.60	-158.0	23.2
OCUP	SATR	196.0	90.6	86.16	-26.37	9.80	-158.1	21.5
OCUP	SATR	203.0	85.9	116.86	-26.16	10.55	-167.4	29.2
OCUP	SATR	233.0	92.2	123.93	-26.94	10.21	-159.8	31.0
OCUP	SATR	235.0	132.1	232.79	-24.91	10.41	-130.87	58.2
OCUP	SATR	237.0	145.5	98.69	-27.68	10.89	-149.4	24.7
OCUP	SATR	248.0	136.1	146.37	-25.29	10.47	-143.9	36.6
OCUP	SATR	285.0	208.6	265.76	-20.43	14.36	-136.7	66.4
OCUP	SATR	295.0	232.0	110.55	-26.17	10.37	-140.3	27.6
OCUP	SATR	355.0	473.0	174.82	-16.56	14.24	-138.7	43.7