FACTORS INFLUENCING BIOACCUMULATION OF POLYCHLORINATED

BIPHENYLS IN SIX FISH SPECIES IN LOGAN MARTIN

RESERVOIR, ALABAMA

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FACTORS INFLUENCING BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS IN SIX FISH SPECIES IN LOGAN MARTIN RESERVOIR, ALABAMA

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VITA

Justin Bradford Mitchell, son of William and Linda Mitchell, was born on July 31, 1977, in Dothan, Alabama. He graduated from Cottonwood High School in 1995. He attended Auburn University in Auburn, Alabama, and graduated with a Bachelor of Science degree in Fisheries Management in 1999. After graduation, he worked as a hydrologic technician with the Alabama district of the U.S. Geological Survey – Water Resources Division in Montgomery, Alabama for two years. In 2001, he entered the Auburn University Graduate School. He married Rebecca Joy Dean, daughter of Rodger Dean and Faye Bozone, on August 17, 2002. They have one child, Kaitlyn LeAnne. In January 2004, he began working as a Fisheries Biologist for the Environmental Affairs Department of Alabama Power Company in Birmingham, Alabama.

THESIS ABSTRACT

FACTORS INFLUENCING BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS IN SIX FISH SPECIES IN LOGAN MARTIN RESERVOIR, ALABAMA

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The objective of this project was to evaluate the biological and environmental factors that influenced the bioaccumulation of polychlorinated biphenyls (PCB) in the consumptive portions of fish. I investigated the relationships of seven different factors that might potentially impact the uptake of PCBs in fillets in largemouth bass *Micropterus salmoides*, spotted bass *Micropterus punctulatus*, striped bass *Morone saxatilus*, black crappie *Pomoxis nigromaculatus*, and freshwater drum *Aplodinotus grunniens* from Logan Martin Reservoir, Alabama (N=1022). These factors include temporal trends, spatial trends (distance at capture), lipid content, total length, relative weight, age, and gender.

Regression techniques were employed to identify those variables having the most influence on PCB concentrations in fish fillets of each species. Regression modeling was evaluated at two contrasting conditions: relatively high (1996) and low concentrations (2001-2002). In these models, lipid content and distance at capture appeared to be the dominant predictors of PCBs in all six species. Total length, relative weight, and gender (2001-2002 only) also explained some additional variability in a few species. In addition, PCB concentrations were not related to fish age for any species in the relatively low concentration period (2001-2002).

Multiple regression modeling for PCB trends concluded that the natural log transformation of PCB concentrations (lnPCB) declined significantly ($p<0.05$) from 1996 to 2002 in all six species when evaluated for differences between the three sampling years (1996, 2001, and 2002). In these final models, all six species were positively related to lipid content and every species except striped bass were negatively related to distance at capture. However, the influence of each of these variables on PCB concentrations was species specific and highly variable. ANOVA testing along with Dunnett's C post hoc tests showed PCB concentrations in striped bass and spotted bass were significantly greater than the other species evaluated. Species specific regression models that include these bioaccumulative factors should be integrated into PCB monitoring plans associated with fish consumption advisories.

ACKNOWLEDGEMENTS

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I. Introduction

Polychlorinated biphenyls (PCBs) are complex molecules of chlorinated substituted biphenyls that act similar to pesticides (Laws 1993). PCBs are extremely stable, thus resistant to degradation (Laws 1993). Different mixtures of congeners called Aroclors have different toxic potencies (Cogliano 2000). Little is known about the specific toxicity of each Aroclor. Although, studies conducted by the Environmental Protection Agency (EPA) hint that every Aroclor possesses a threat of cancer to humans (Cogliano 2000). In regard to human health toxicity, PCBs may rank third behind dioxins and furans (Sun 1983; Laws 1993). Other detrimental health risks to humans include neurotoxicity, developmental toxicity, reproductive toxicity, immune system suppression, liver damage, skin irritation, and endocrine disruption (Cogliano 2000). PCBs also have been proven to adversely affect the health of other animals including laboratory rats, fish (marine and freshwater), and waterfowl (Maugh 1975; Laws 1993). Peakall (1973) reported that PCBs were even more toxic at similar levels to birds than DDE, the degradation product of the banned pesticide DDT. Because PCBs pose such health risks, the U.S. Food and Drug Administration (USFDA) mandated that PCB concentrations for consumptive fish flesh cannot exceed 2.0 parts per million (ppm) (Dycus and Lowery 1985; Laws 1993, Zlokovitz and Secor 1999).

PCBs were primarily used in semi-closed or closed electrical transformers, hydraulic fluids, and heat transfer systems (EPA 1979; Laws 1993). In 1971, Monsanto

Corporation initiated a voluntarily manufacturing restriction to limit the use of PCBs to electrical capacitors and transformers only (Laws 1993). Six years later, in 1977, all manufacturing of PCBs in the United States were terminated (Laws 1993). In July 1979, all manufacturing, distribution, and use of PCBs was banned by the EPA under the authorization of the Toxic Substances Control Act (Laws 1993).

PCBs are widespread because of poor technological measures taken in the past and ignorance about the environmental persistency during early years of manufacture (Kocan et al. 2001). As with many contaminants, the persistence of PCBs is governed by its environmental chemistry. PCBs are lipophillic, thus highly soluble in fats and virtually insoluble in water (Spigareill et al. 1983; Olsson et al. 2000). In addition to its solubility, PCBs also naturally bind to sediment, making removal from water bodies extremely difficult. Fluctuations in PCB concentrations have occurred because of changes in sedimentation rates, atmospheric transport, biodegradation, and continued loading from industrial or municipal facilities contaminated with PCBs (Stow 1995).

A chemical company (Monsanto Corp.) produced PCBs at a site adjacent to Snow Creek in Anniston, Alabama from 1935 to 1971 (ADEM 2001, Bayne et al. 2002). PCB waste was also disposed of in unlined landfills at this facility. As a result PCBs have been detected in portions of Snow Creek and Choccolocco Creeks flowing from Anniston to Logan Martin Reservoir on the Coosa River. The Anniston plant was one of two PCB manufacturing plants in the United States.

Statewide PCB monitoring (including Logan Martin) has been conducted by the Alabama Department of Environmental Management (ADEM) since 1991 (personal communication w/ Fred Leslie, ADEM). ADEM has collected two species (a predator species and a benthic species) and six fish per species from four locations in Choccolocco Creek and eight sites on Logan Martin Reservoir annually since 1991 (personal communication w/ Michael Len, ADEM). Monitoring by ADEM in the early 1990s revealed a resurgence of PCBs in fish tissue from Logan Martin Reservoir that was attributed to dredging and snagging operations by the Natural Resources Conservation Service (NRCS) in the upper reaches of Choccolocco Creek (ADEM 2001; Bayne et al 2002). To reduce excessive human consumption of PCBs, advisories limiting or banning fish consumption on Logan Martin Reservoir have been imposed since the early 1990's by the Alabama Department of Public Health (ADPH).

Identifying and understanding factors that affect PCB accumulation in fish could be a useful management tool to reduce the risk of human exposure to PCBs. The objective of this study was to: 1) examine variables that might be related to the uptake and storage of PCBs by the warmwater fish community of Lake Logan Martin, Alabama. Variables examined included fish species, age, total length, fillet lipid content, relative weight and distance at capture, of the fish to the PCBs point source. 2) Explore the relationships of these variables on PCB concentrations of fish under conditions of relatively high PCB environmental conditions (1996 data set and Choccolocco Creek data set) and under relatively low environmental PCB concentrations (2001-2002 data set and Lake station data set). This spatial and temporal comparison of PCBs concentrations under varying environmental PCB concentrations is unique in that it was all accomplished in the same reservoir. 3) Discuss any management implications that may be relevant to present and future PCB monitoring in fish.

II. Literature Review

Aquatic biota is often used as a PCB indicator because they tend to have higher concentrations than the surrounding water they live in (Nebeker et al. 1974). Aquatic organisms obtain these higher concentration values because PCBs are bioaccumulated through the food web (Olsson et al. 2000). Bioaccumulation rates in fish vary according to diet, growth rates, habitat, trophic status, motility, sex, concentrations in prey, and in body lipid content (Madenjian et al. 1998; Khan 1999; Bayne et al. 2002). PCBs are similar to DDT and other organochlorine substances also have been shown to biomagnify in aquatic food webs (Olsson et al. 2000). Biomagnification is defined as an increase in concentrations of contaminants due to diet, above the concentrations expected from equilibrium partitioning of the water in the environment (Olsson et al. 2000). Fish are dominant aquatic organisms that can bioconcentrate PCBS directly from the water and also bioaccumulate PCBs through the food web. Therefore, fish must be monitored for PCB content because of human health concerns. The biomagnification and bioaccumulation of PCBs in fish is dependent on various biological factors such as dietary lipid, body lipid content, species, age, and gender (Olsson et al. 2000). Environmental factors including exposure rate, distance from PCB source (point or non-point source), time since environmental exposure or reexposure also affects the bioaccumulation rate of PCBs in fish.

Diets seem to be the primary pathway for uptake of PCBs in many freshwater fishes (Wszolek et al. 1979; Jackson and Schindler 1996; Maruya and Lee 1998; Bayne et al. 2002). Spigareill et al. (1983) reported from laboratory simulations that natural foraging was the major source of PCBs in adult brown trout (Salmo trutta) from Lake Michigan. Bioaccumulation of PCBs can rapidly occur in piscivores if the PCB levels found in forage species are elevated (Madenjian et al. 1998).

Like other organochlorine substances, PCBs are lipophillic, so biological factors which affect the lipid content of the fish are known to affect the overall PCB burden in fish (Spigareill et al. 1983; Olsson et al. 2000). PCB concentrations have a direct relation with body lipid content when located near a point source of PCBs (Harding et al. 1997; Khan 1999). Lipid content was also highly correlated to PCB concentrations in fishes from the Hudson River (Brown et al. 1985; Larsson et al. 1991). In contrast, Stow (1995) did not find a significant relationship between lipid content and PCB concentrations in fillets among five Lake Michigan salmonid species. Lipid content is highly variable among species (Henderson and Tocher 1987). Larsson et al. (1991) reported that lipids comprise up to 40% body weight for the eel (*Anguilla anguilla*). In contrast, northern pike (*Esox lucius*) and many other North European fish species contain as little as 2 percent lipid per body weight (Larsson 1993). Seasonal changes in diet, due to changes in water temperature, daylight length, prey type, prey abundance, and prey availability can heavily impact lipid content in fish therefore altering PCB concentrations (Ludsin and Devries 1997). Lipids are used for energy through winter and ultimately reach a minimum just after spawning, while maximum lipids accumulation is probably reached in late autumn (Olsson et al. 1977). Seasonal changes in lipids can also be linked to spawning effects. Niimi (1983) reported that lipid deposition in eggs can significantly reduce parental lipid content causing PCB concentration changes in adult females. Fish tend to store lipids in adipose tissue and in internal organs such as the liver (Wszolek 1979), so a whole body sample could show a different PCBs/percent lipid relationship compared to a skinless fillet (Stow 1995).

Age, gender, total length, and trophic status are other factors that influence lipid content in fish (Olsson et al. 2000). Perch (*Perca fluviatilis*) greater than 20 cm expressed a higher trophic status and led to an increase in PCB levels due to larger, older fish having more lipid reserves (Olsson et al. 2000). Lipid content can be highly variable depending upon the type of sample (Stow 1995).

PCB bioaccumulation rates can vary between genders. Male walleye (*Stizostedion vitreum*) had higher PCB concentrations than females because males spent more time in the contaminated Saginaw River (Madenjian et al. 1998). Zlokovitz and Secor (1999) also reported higher PCB concentrations for male striped bass (*Morone saxatilis*) because the males had more time of exposure to PCBs in the heavily contaminated Hudson River. Using muscle segments located below the dorsal fin, Larsson et al. (1993) found a reduction in PCB concentrations in female northern pike (*Esox lucius*) after females became sexually mature because of gonadal release after spawning. Aguilar and Borrell (1988) reported a decline in PCB concentrations for females after sexual maturity but an increase in PCBs for males with age and body size in fin whales (*Balaenoptera physalus*). Alternatively, no gender difference was found in perch from Lake Burtnieku, Latvia (Olsson et al. 2000).

Because exposure time to PCBs in the environment is directly related to age, PCB concentrations in fish generally increase with age for most fish species (Wszolek 1979). Striped bass (Morone saxatilis) from the Hudson River (Fabrizio et al. 1991); the anadromous eel (*Anguilla anguilla*) (Larsson et al. 1991); channel catfish from Logan Martin Reservoir; and five salmonid species from Lake Michigan (Stow 1995) showed this pattern. However, Larsson et al. (1993) found age negatively related to PCB concentrations in northern pike.

Environmental factors associated with the discontinued use and the reexposure of PCBs has a major effect on fish bioaccumulation. In many areas contaminated with PCBs, the location of the source is known. Since PCBs have a propensity to bind to sediment and settle out, a spatial concentration gradient often develops causing PCB concentrations to decrease with increased distance from the source (Dycus and Lowery 1986; Stow 1995; Bremle and Larsson 1998; Bayne et al. 2002). Bayne et al. (2002) showed that PCBs could be used to imply relative fish motility in systems having a distinct concentration gradient. Zlokovitz and Secor (1999) reported that PCB concentrations for the highly motile striped bass (*Morone saxatilis*) were inversely related to salinity, showing a concentration gradient existed from the Hudson River downstream into New York Harbor.

Since the ban in 1977, PCBs in water and in fish tissues have declined over time (Swackhamer and Hites 1988). A sharp decline in the late 1970's and early 1980's was largely due to decreases in loading from point sources and non-point sources (Swackhamer and Hites 1988). However, PCB concentration trends in water and in aquatic life at many contaminated sites have shown little decline since the mid 1980's (Stow 1995).

The typical management strategy thus far has been to impose fish consumption limits while simultaneously allowing the contaminated sediment to become buried naturally until concentrations in fishes are reduced to levels below the USFDA guideline of 2.0 ppm. Nevertheless, in some river systems such as the Hudson River, NY and the Shiawasse River, Sweden, the contaminated sediments have been dredged and landfilled (Bremle and Larsson 1998; Zlokovitz and Secor 1999). However, dredging typically removes the top layer of contaminated sediment, exposing, and redistributing PCBs in buried sediment that was previously unavailable to the environment (Bremle and Larsson 1998; Zlokovitz and Secor 1999). Dredging has had some success in reducing PCB concentrations in aquatic organisms over time (Bremle and Larsson 1998). Surprisingly, the Hudson River showed no change in PCB concentrations in the water after dredging (Zlokovitz and Secor 1999). However, PCB levels in fish increased significantly downstream and in the region of the dredging site (Bremle and Larsson 1998; Zlokovitz and Secor 1999).

Ironically, previously published literature pertaining to aquatic organisms and their relationship with PCBs is limited to northern latitudes and/or cooler climates. No studies have examined temporal differences in PCB concentrations of fish in a southeastern U.S. reservoir. Most studies only investigated the PCB bioaccumulative relationship of one fish species in the same ecosystem. Previous studies did not investigate multiple environmental variables that might affect bioaccumulation rates of PCBs using a modeling approach. Only a limited few studies have examined fish PCB bioaccumulation rates spatially from a known point source. There has been no examination of the rate of uptake of PCBs in just the consumable portion (fillet) of fish. Logan Martin Reservoir provided an excellent location and opportunity to investigate these questions.

III. Materials and Methods

Study Site

Logan Martin Reservoir is a eutrophic, 6,179 ha impoundment on the Coosa River near Anniston, Alabama (Bayne et al. 2002). The reservoir was created in 1964 by Alabama Power Company for hydroelectric power generation and flood control (ADEM 1984; Bayne et al. 2002). Reservoir characteristics include occasional thermal and chemical stratification during summer months, a short mean hydraulic retention time of 11 days, a shallow mean depth of 5.5 meters, and 442 kilometers of shoreline (Bayne et al. 2002).

Fish Collection

Fish were collected at a total of seven sites on or near Logan Martin Reservoir (Stations 33, 35-36, 38-39 and 43-44) using both boat electrofishing and gillnetting (Figure 1). Two lotic sites were located in Choccolocco Creek, the closest sampling sites to Anniston, Alabama. One station was at the confluence of Choccolocco Creek and Cheaha Creek (Stations 43) and the other was at the confluence of Choccolocco Creek and Coldwater Creek (station 44). Station 35 and 36 were lentic sites located further downstream toward the mouth of Choccolocco Creek and the reservoir. Station 36, was just a few kilometers downstream of station 35. Station 33 (Blue-eye Creek) was located upstream of the mouth of Choccolocco Creek. Station 38, Cropwell Creek embayment,

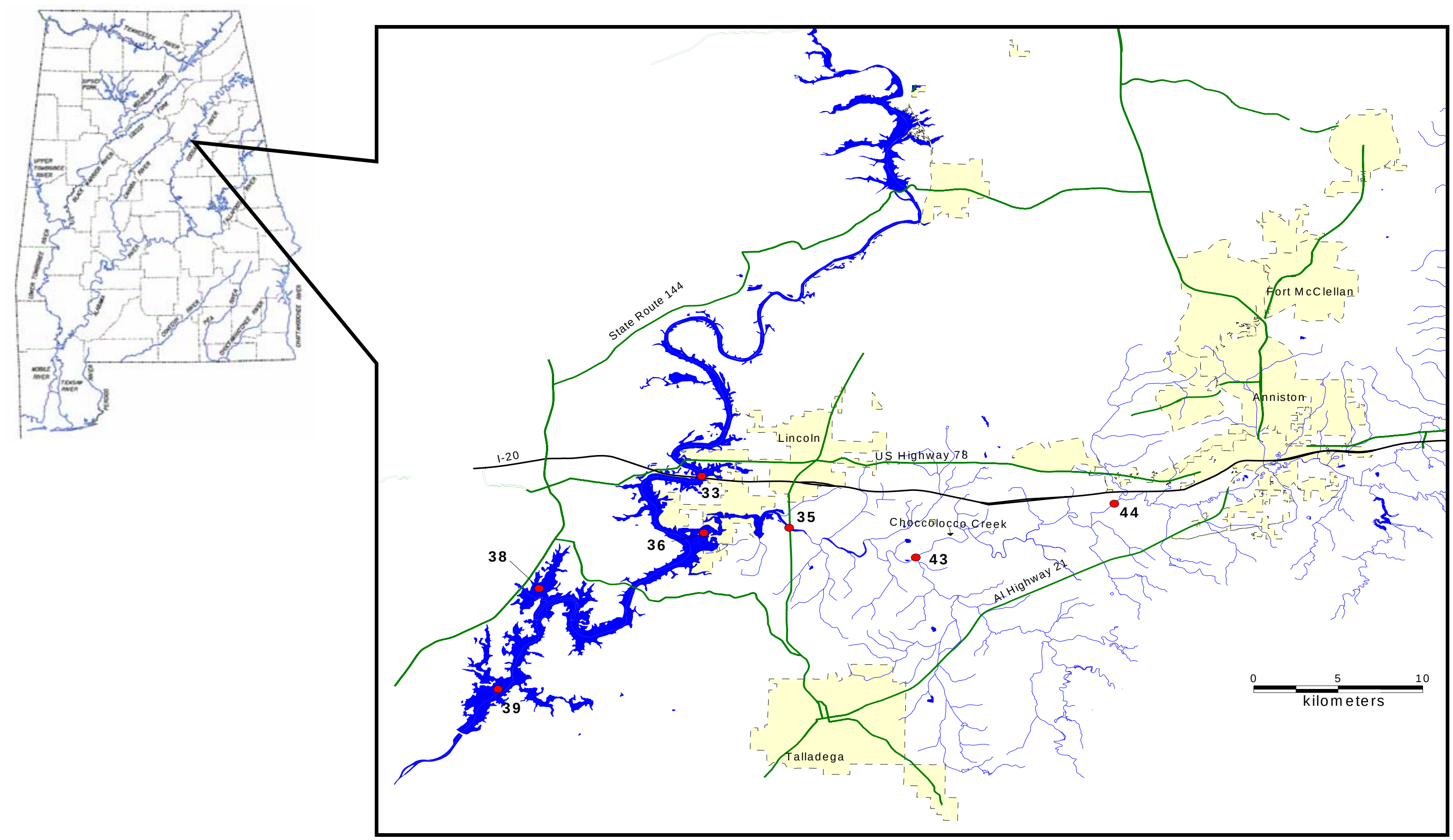

Figure 1. Map of Logan Martin Reservoir, Alabama, a main stream impoundment of the Coosa River.

was located downstream of Choccolocco Creek. Station 39 was located in the vicinity of Rabbit Branch and the Logan Martin dam forebay.

Sampling was conducted October – December in 1996, 2001 and 2002. Adult fish species targeted annually at each site included 6-12 individuals from each of the following species: largemouth bass *Micropterus salmoides*, spotted bass *Micropterus punctulatus*, striped bass *Morone saxatilis*, black crappie *Pomoxis nigromaculatus*, freshwater drum *Aplodinotus grunniens*, and channel catfish *Ictalurus punctatus*. After capture, all fish were wrapped individually in aluminum foil and sealed in a plastic bag. All fish were placed on ice, in coolers for transportation back to an Auburn University laboratory for processing. Species identity, total weight (g) and total length (mm) were determined for all fish. In 2001 and 2002, gender and age were also determined for all fish.

All fish were skinned, filleted, packaged, and frozen according to a U. S. Environmental Protection Agency (EPA) protocol (ADEM 1991). All frozen fillets were shipped to Savannah Laboratories in Savannah, Georgia for analysis of fillet PCBs (ppm) and fillet percent lipid composition (%). Each fillet was analyzed individually for total PCBs using gas chromatography. (Bayne et al. 2002)

Sagittal otoliths from all fish and pectoral spines from catfish were removed to estimate age. Otoliths were preserved in a 40:60 glycerin/alcohol solution and placed in 4 mm vials. Both catfish spines were placed in an individually labeled coin envelope. Otoliths from all black bass, striped bass, and black crappie were prepared following Maceina (1988). Otoliths from all freshwater drum were prepared according to Mitchell et al. (in progress). Channel catfish pectoral spines were prepared using the cut spine method according to Buckmeier et al. (2002). Two independent readers with similar experience aged otoliths and spines. A third independent reader aged each fish the first two readers disagreed upon. Ages were assigned after the third independent read based upon agreement between readers.

Relative weight (Wr) was calculated for each fish as an index of individual body condition following the procedures of Anderson and Neumann (1996). Fish that have a relative weight less than 80 of the standard are considered severely thin, relative weight between 80 – 100 are considered normal, and relative weight greater than 100 are considered plump (Anderson and Neumann, 1996). To explain a possible PCB concentration gradient, a variable called distance was computed by measuring the distance in river kilometers to each station from the most upstream station (Station 44) in Choccolocco Creek closest to Anniston, Alabama (Table 1). Fish were sampled over a 60 km spatial gradient.

Table 1. A detailed location of the seven sampling sites on or near Logan Martin Reservoir.

Station	Station Description	Distance from St. 44 (km)
44	Choccolocco Creek at the confluence of Coldwater Creek	0.00
43	Choccolocco Creek at the confluence of Cheaha Creek	21.85
35	Logan Martin Reservoir near the mouth of Choccolocco Creek	33.99
36	Logan Martin Reservoir near the embayment of Choccolocco Creek	39.36
33	Logan Martin Reservoir near Blue-eye Creek	49.66
38	Logan Martin Reservoir near Cropwell Creek	54.07
39	Logan Martin Reservoir near Rabbit Branch	61.31

The SAS (2004) system and SPSS version 14.0 (2005) was used to conduct statistical analyses. In an attempt to select for fish with a relatively high probability of exposure to PCBs, separate regression analyses were performed on fish collected from stations Choccolocco Creek (Stations. 35, 36, 43, and 44) where PCB concentrations in fish have been high. Analysis of Variance (ANOVA) tests and regression analyses were used to determine if the biological and environmental variables were significantly ($p<0.05$) related to fillet PCB concentrations within each of the fish species for the different collection years (1996, 2001, and 2002) and locations of capture (lake and creek). Fillet PCB concentrations for all fish within each species were regressed against six variables including percent lipid content, total length, relative weight, distance from the uppermost sampling station in the Choccolocco Creek watershed, age, and gender. Age and gender data were only collected during 2001-2002. Analysis of Covariance (ANCOVA) was used to determine if significant ($P<0.05$) PCB concentration differences exist between sample periods and capture locations for each species. Sexual differences in the accumulation of PCBs of the same six fish species from Logan Martin Reservoir was conducted by Rypel (2004).

A multiple regression analysis approach was used to identify variables related to differences in PCB concentrations for each of the six fish species (SAS 2004). Variables were excluded in the regression model if they were not significantly ($P > 0.05$) related to PCB concentrations in fish fillets. Multicollinearity among all independent variables was checked using the variance inflation factor. In an attempt to select the best overall model to predict PCB concentrations, separate multiple regression analyses for relatively high

(1996) and low concentrations (2001-2002) were performed. The 2001-2002 model included the addition of the variables “fish age” and “gender”.

For each species, multiple regression analyses were also used to develop a final model to determine if significant changes in PCB concentrations had occurred over time and what factors are related to PCBs in Logan Martin Reservoir. Scatter plots of the residual values versus the observed values were created to determine if any transformations would be appropriate. A two-way ANOVA was employed to test for differences in PCB concentrations between Choccolocco Creek and the lake stations. The interaction term was also included in the ANOVA. The ANOVA testing was followed by a set of Dunnett’s C multiple comparison tests to determine significant differences in PCB concentrations by species and sampling location.

IV. Results

Biological and environmental data were highly variable among the six fish species (Table 2). During the collection years of 1996, 2001, and 2002, fillets from 1,022 fish among the six species were analyzed for PCBs (Figure 2). Fillet PCB concentrations were highly variable among species (Figure 3). These concentrations ranged from a low of 0.02 ppm which was found in freshwater drum to a high of 33.9 ppm which was also found in freshwater drum (Table 2). Striped bass ($\bar{x}$=2.59 ppm) and channel catfish ($\bar{x}$=2.21 ppm) had the highest mean fillet PCB concentrations, while black crappie ($\bar{x}$=0.84 ppm) and largemouth bass ($\bar{x}$=1.01 ppm) had the lowest (Table 2). Likewise, striped bass had the highest mean percent fillet lipid content ($\bar{x}$=2.68 %) and largemouth bass ($\bar{x}$=0.57%) had the lowest among all six species (Table 2). Comparison of mean percent lipid found in the fillets indicated lipid content was highly variable among species and collection periods (Figure 4). Comparison of PCB concentration bioaccumulated in the fillet divided by the percent lipid available in the fillet within each species continues to indicate that differences in the PCB levels existed between the collection years (Figure 5).

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Table 2. Range, mean, standard deviation, and number of samples for six fish species from Logan Martin Reservoir, AL, 1996, 2001, and 2002.

Species	Statistic	Length (mm)	Age (yrs)	Relative Weight	Lipid (%)	PCB conc. (ppm)
Largemouth Bass	Range	258-530	1-7	32.10-123.39	0.03-4.80	0.03-16.8
	Mean	375	3	95.31	0.57	1.01
	STD	50	1	12.26	0.68	2.08
	N	185	123	185	185	185
Spotted Bass	Range	272-512	1-14	63.57-111.42	0.04-12.0	0.05-15.7
	Mean	390	3	89.03	1.22	1.66
	STD	52	2	8.29	1.61	2.42
	N	201	147	201	201	201
Striped Bass	Range	286-702	1-6	54.29-114.45	0.10-10.0	0.21-34.8
	Mean	520	2	87.29	2.68	2.59
	STD	98	1	12.90	1.94	4.31
	N	112	91	112	112	112
Black Crappie	Range	206-323	1-8	72.76-115.73	0.03-4.0	0.04-9.9
	Mean	273	3	92.87	1.11	0.84
	STD	29	2	7.75	0.81	1.42
	N	155	111	155	155	155
Channel Catfish	Range	241-510	3-12	59.61-115.42	0.0-7.8	0.03-20.6
	Mean	343	5	85.06	1.10	2.21
	STD	67	2	9.90	1.17	3.58
	N	193	148	193	193	193
Freshwater Drum	Range	234-489	2-21	39.86-125.37	0.03-9.0	0.02-33.9
	Mean	351	8	90.51	0.89	1.81
	STD	58	4	13.03	1.44	4.24
	N	176	135	176	176	176

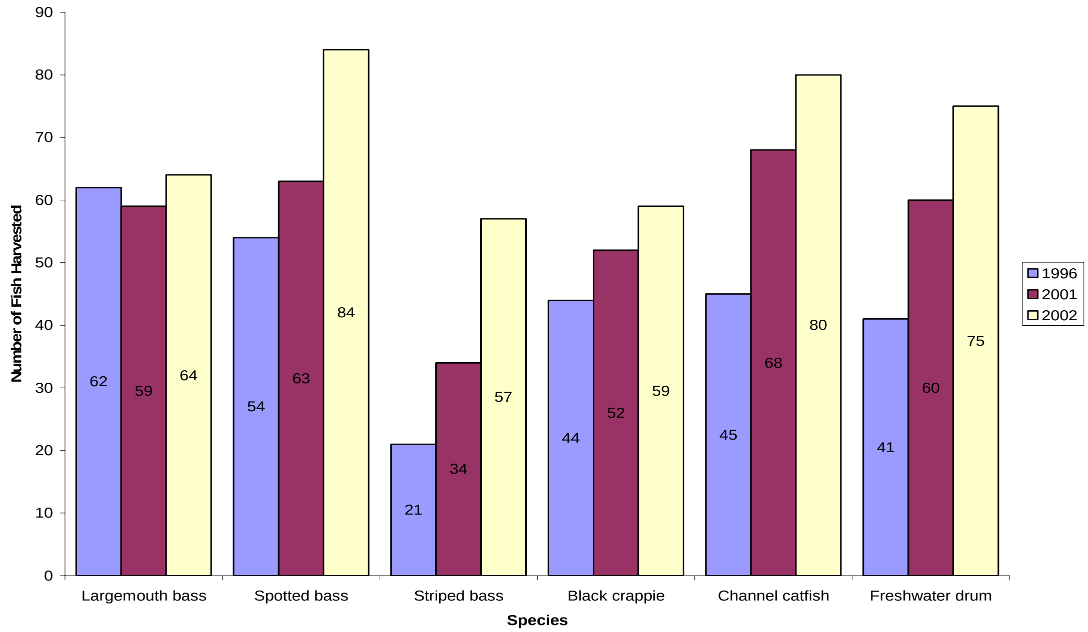

Figure 2. Total number of fish harvested for PCB analysis during 1996, 2001, and 2002 from Logan Martin Reservoir, AL.

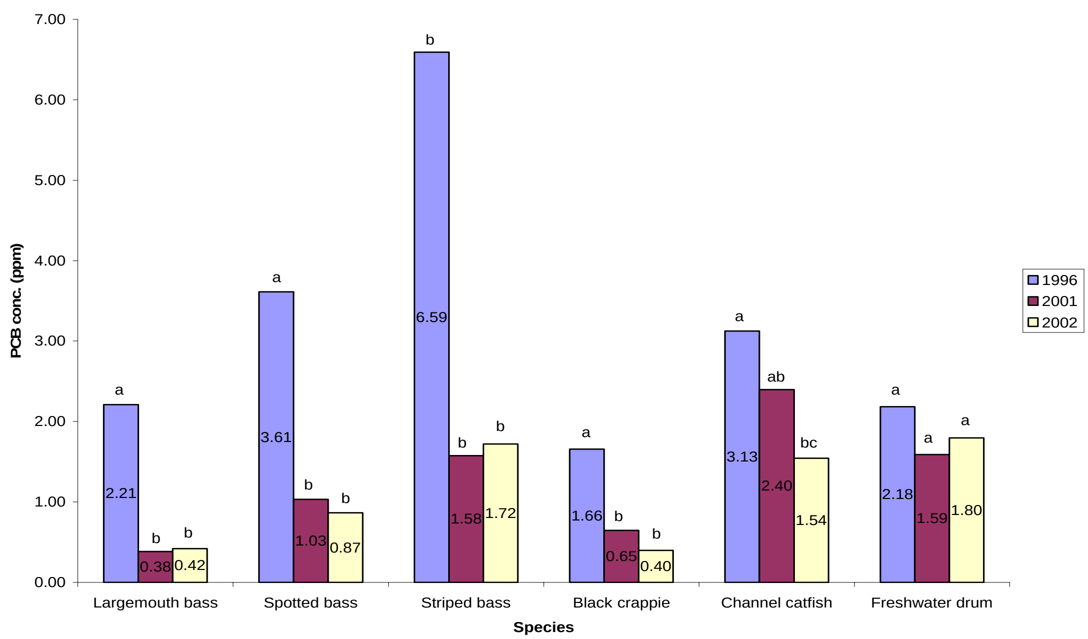

Figure 3. Mean PCB concentrations in fish fillets for each species collected in 1996, 2001, and 2002 from Logan Martin Reservoir, AL. Bars with different letters are statistically ($p<0.05$) different.

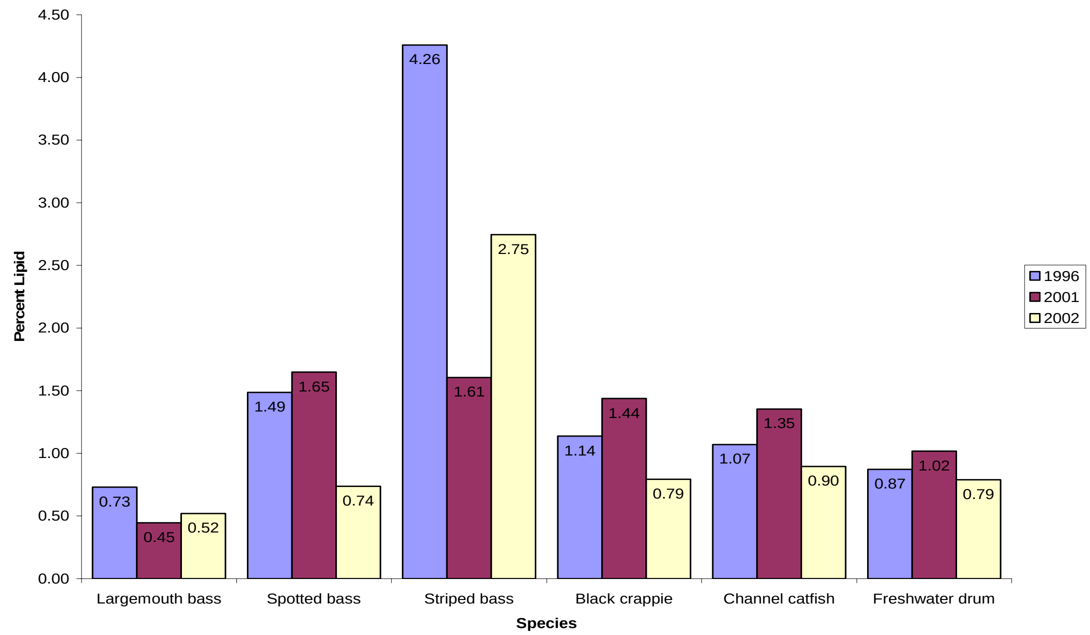

Figure 4. Mean percent fillet lipid content in fish fillets for each species collected in 1996, 2001, and 2002 from Logan Martin Reservoir, AL.

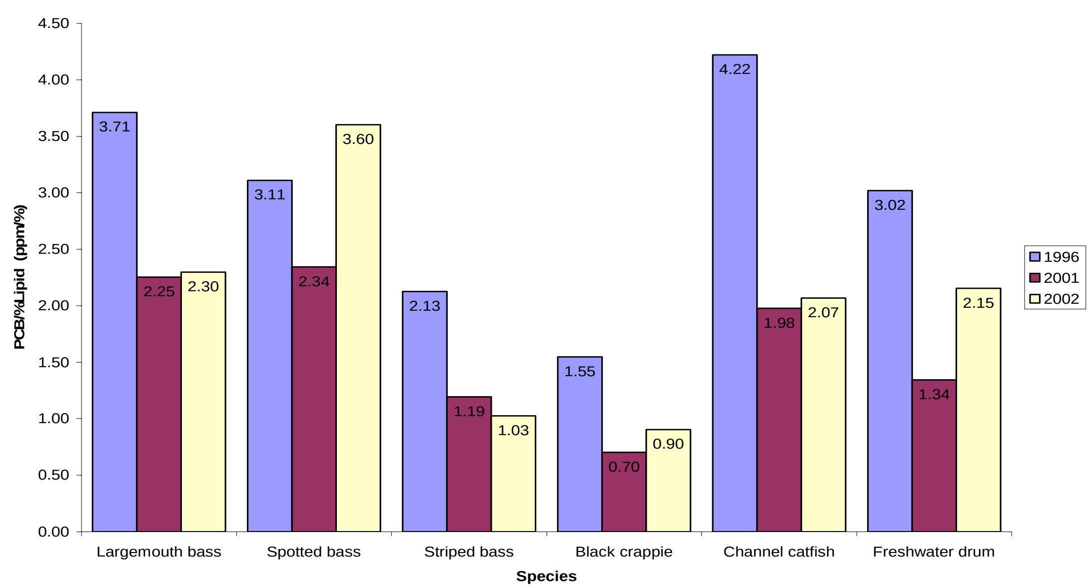

Figure 5. Mean PCB concentrations per percent unit extracted in fish fillets for each species collected in 1996, 2001, and 2002 from Logan Martin Reservoir, AL.

Fillet concentrations of PCBs were significantly ($p<0.05$) higher in fish collected during 1996 compared to either 2001 or 2002 with the exception of freshwater drum (Figure 3). Therefore, fish data collected in 2001 were combined with data collected in 2002, creating two distinct sampling periods (i.e. 1996 and 2001-2002) for some of the regression analyses. Multiple regression techniques were used to determine how PCB concentrations are affected by lipid content, distance (relative to the upper most station on Choccolocco Creek), total length, relative weight, age (2001-2002 only) and gender (2001-2002 only). Each model was conducted for each species during the 1996 and 2001-2002 collection periods. The evaluation of how these variables affect PCB level in the individual species was followed by multiple regression analyses which determined if significant reduction in PCB contaminant levels has occurred since 1996 and if significant differences in PCB concentrations existed between species. The variable weight (g) was excluded from all analyses because it was strongly correlated with total length (mm) ($r=0.94$; $p<0.0001$).

Largemouth Bass

During the entire study period, 185 largemouth bass were collected that ranged from 258 to 530 mm total length (Table 2). During the 2001 and 2002 sampling years, age ranged from 1 to 7 years with a mean of 3 years (Table 2). Fish were not aged prior to 2001. Data collected during 1996 to 2002 indicated that the mean PCB concentrations in largemouth bass declined from 1996 to 2001, but did not significantly ($p<0.05$) change from 2001 to 2002 (Figure 3). Fish age, a strong predictor of PCBs in many studies, was not significantly (p=0.41) related to fillet PCB concentrations in largemouth bass collected in 2001 and 2002.

Analyses of both collection periods indicated that percent fillet lipids was the strongest, positive predictor and distance at capture from the point source (distance) was a secondary negative predictor of PCBs in largemouth bass (Table 3). These variables explained 59 and 31% of the variability in PCBs during the 1996 and 2001-2002 collection periods, respectively. The inclusion of the variable fish gender improved the model by only 3% and the inclusion of fish age had no affect on the prediction of PCB concentrations.

Table 3. Regression models for largemouth bass from Logan Martin Reservoir, AL, 1996 and 2001-2002.

1996 R^2=0.587			2001-2002 R^2=0.312		
Variable	**Coefficients**	**Semi-partial r coefficient**	**Variable**	**Coefficients**	**Semi-partial r coefficient**
Constant	7.819		Constant	0.593	
Lipid	2.565	0.60	Lipid	0.305	0.44
Distance	-0.158	-0.47	Distance	-0.010	-0.39
			Gender	0.138	0.16

The relationship between PCB concentrations and the various independent variables are presented in Figures 6-11. PCB residues were not related to total fish length during 1996 (R^2=0.049; p=0.0851) or in the 2001-2002 study period (R^2=0.021; p=0.189). Fish collected in 1996 had significantly (R^2=0.369; p<0.0001) higher proportion of PCBs to lipid relation compared to 2001-2002 fish (R^2=0.136; p<0.0001) (ANCOVA p<0.0001) (Figure 6). A stronger spatial concentration gradient was expressed in 1996 (R^2=0.224;

p<0.0001), compared to 2001-2002 (R^2=0.346; p<0.0001) (ANCOVA p<0.0001) (Figure 7). PCBs positively increased with relative weight (R^2=0.079; p=0.0266) during 1996 (R^2=0.079; p=0.0266) and 2001-2002 (R^2=0.035; p=0.0375) (Figure 8). Covariate analysis also revealed PCB concentrations in fish with the same relative weight were significantly (ANCOVA p=0.002) higher during the 1996 sample period compared to the 2001-2002 sample period.

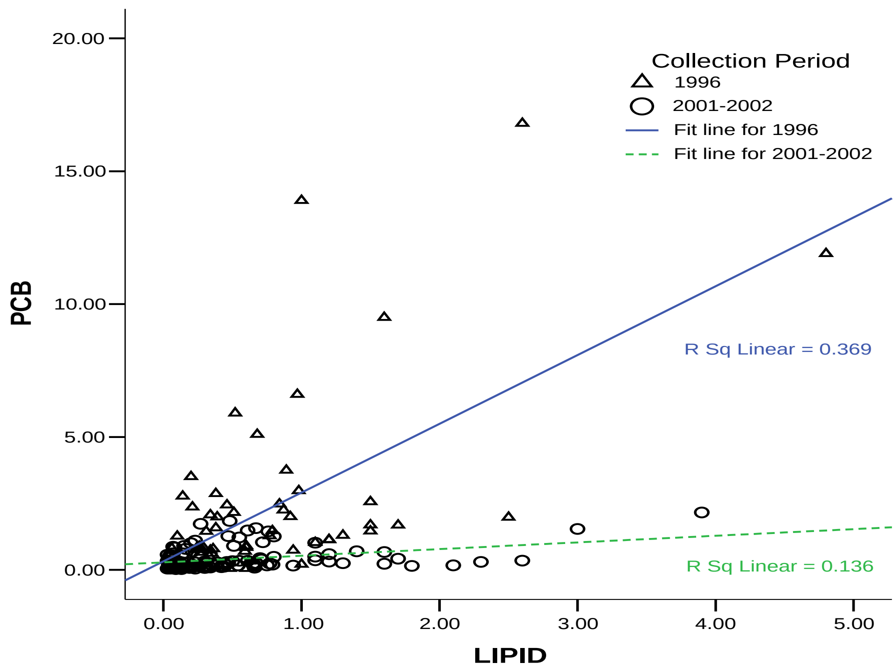

Figure 6. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of largemouth bass from 1996 (p<0.0001) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

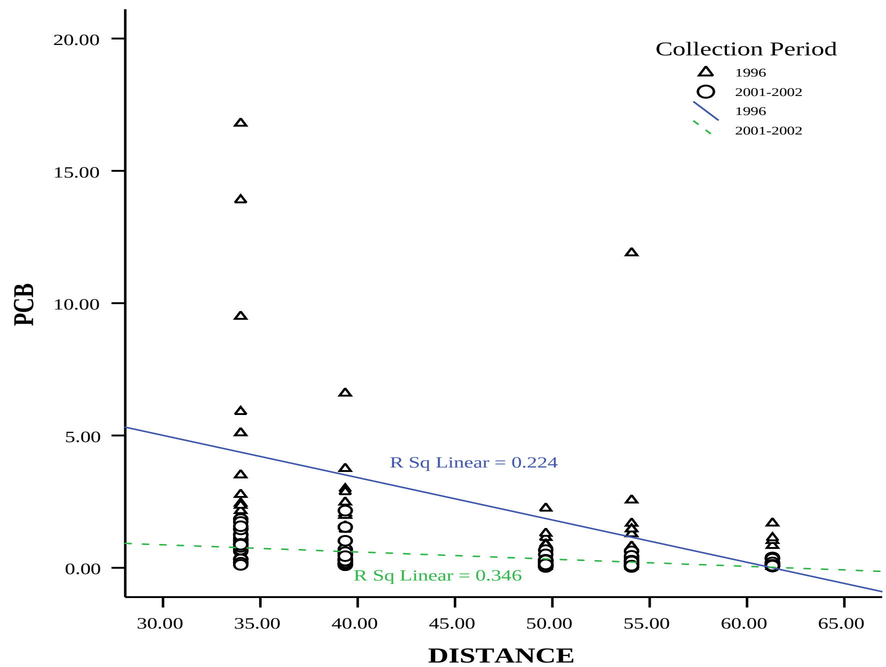

Figure 7. Relation between PCB concentrations (ppm) and distance at capture (km) of largemouth bass from 1996 (p=0.0001) and 2001-2002 (p=0.0005) collection periods in Logan Martin Reservoir, AL, 1996-2002.

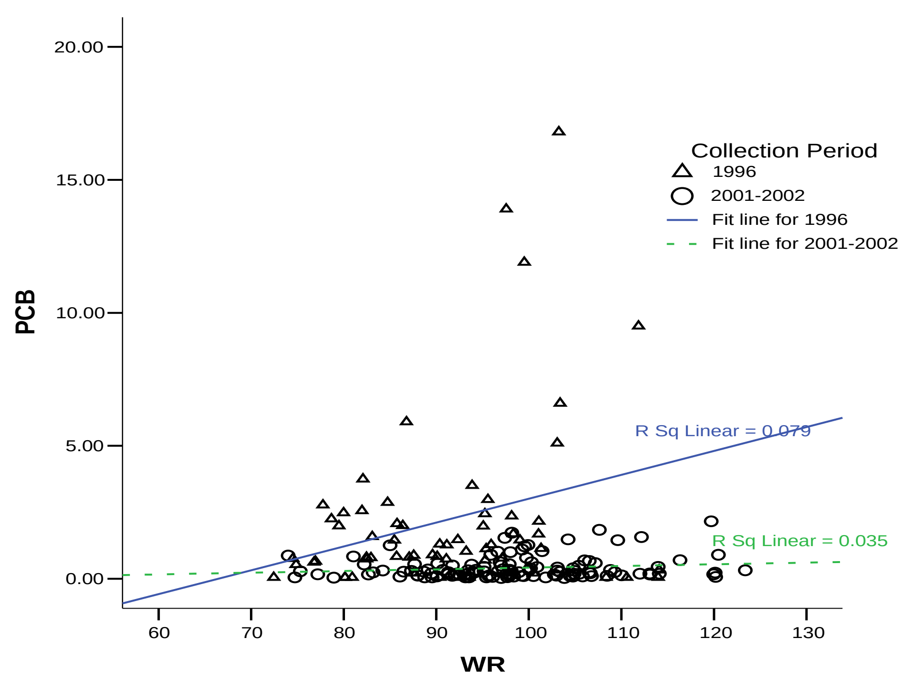

Figure 8. Relation between PCB concentrations (ppm) and relative weights (Wr) of largemouth bass from 1996 (p=0.0266) and 2001-2002 (p=0.0375) collection periods in Logan Martin Reservoir, AL, 1996-2002.

Largemouth bass collected from Choccolocco Creek (Creek stations 35, 36, 43, and 44) had significantly ($p<0.05$) higher mean PCB concentrations than largemouth bass from the Lake stations (Lake stations 33, 38, and 39) during both collection periods (1996 and 2001-2002) (Figure 9). Mean PCB concentrations in fish collected in 1996 were also found to be significantly ($p<0.05$) higher than fish collected in 2001-2002 for both Lake and Creek stations (Figure 9). Largemouth bass from Lake stations were significantly (R^2=0.0619; p=0.0126) lower in PCBs for fish of the same total length than Creek stations fish (R^2=0.166; $p<0.0001$) (ANCOVA $p<0.0039$) (Figure 10). However, there were no differences between Creek (R^2=0.190; $p<0.0147$) and Lake stations (R^2=0.483; $p<0.0001$) in the PCB to lipid content (ANCOVA p=0.2161) (Figure 11). Relative weight (Wr) of fish collected from Creek (p=0.7220) or Lake stations (p=0.9364) did not influence PCB concentrations in fish fillets.

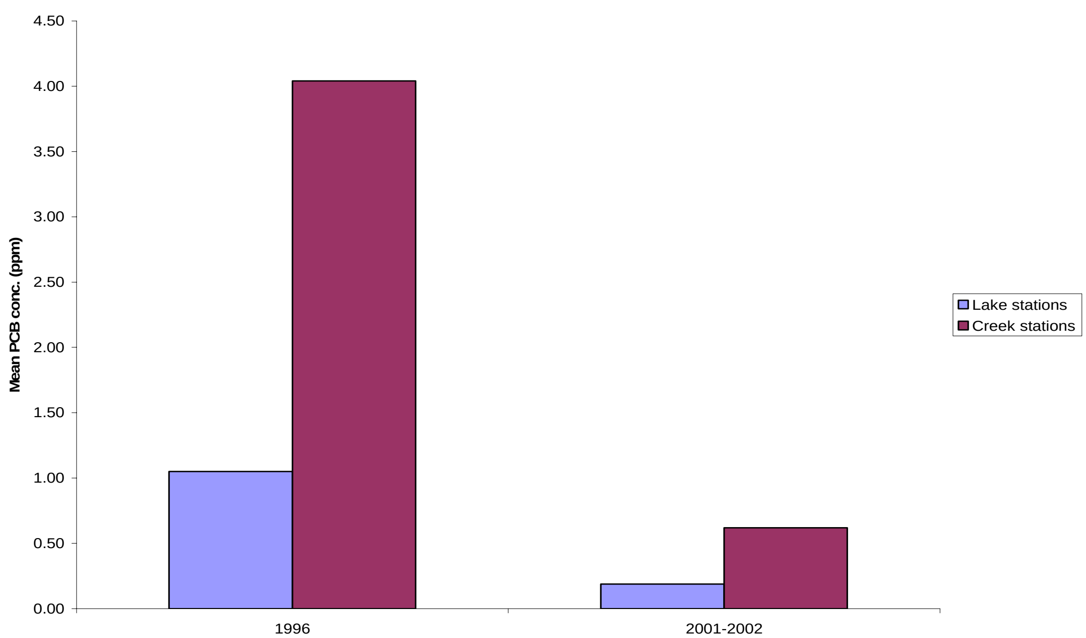

Figure 9. Mean PCB concentrations (ppm) for largemouth bass collected at Lake and Creek stations during the 1996 and 2001-2002 collection periods from Logan Martin Reservoir, AL, 1996-2002.

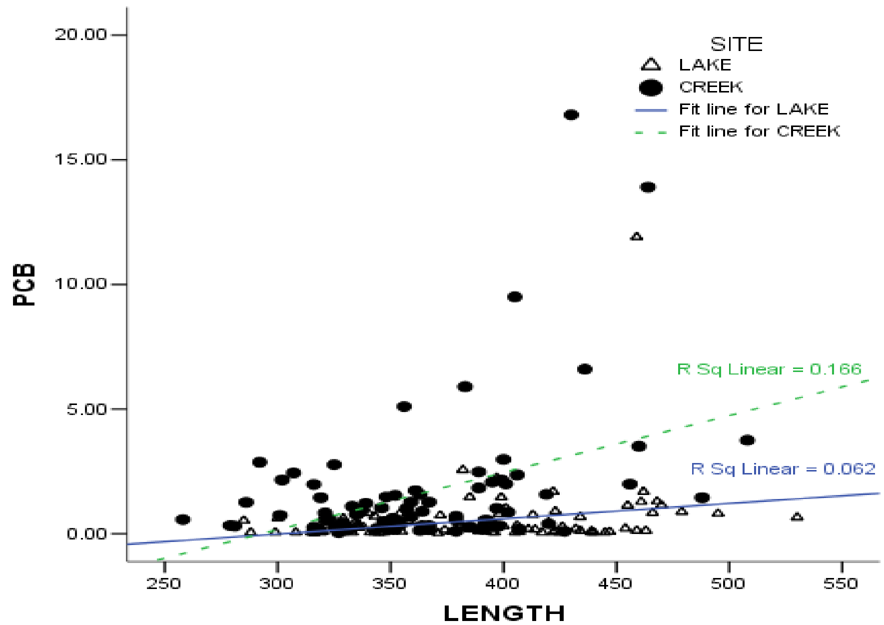

Figure 10. Relation between PCB concentrations (ppm) and total length (mm) of largemouth bass from Lake (p=0.0126) and Creek (p=0.0001) stations in Logan Martin Reservoir, AL, 1996-2002.

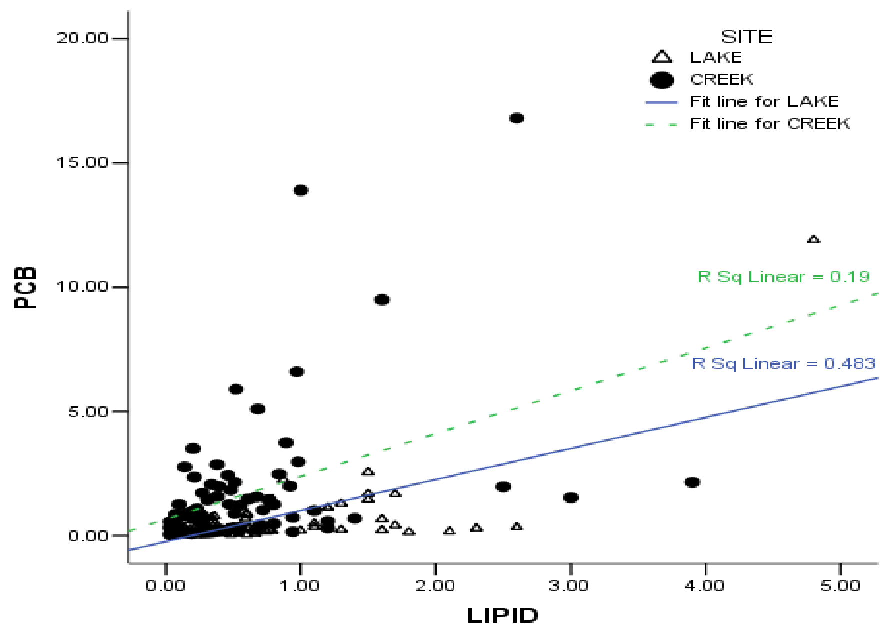

Figure 11. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of largemouth bass from Lake (p<0.0001) and Creek (p<0.0001) stations in Logan Martin Reservoir, AL, 1996-2002.

Spotted Bass

A total of 201 spotted bass were collected that ranged from 272 to 512 mm total length (Table 2). During the 2001 and 2002 sampling years, age ranged from 1 to 14 years with a mean of 3 years (Table 2). Data collected during 1996 to 2002 indicated that the mean PCB concentrations in spotted bass declined from 1996 to 2001, but did not significantly ($p<0.05$) change from 2001 to 2002 (Figure 3). In contrast to other studies, fish age was not significantly ($p=0.2396$) related to fillet PCB concentrations in fish collected in 2001 and 2002.

An analysis of both models indicated the majority of the variability explained resulted from a combination of distance at capture and percent lipid in fillets (Table 4.). Total fish length explained a small portion of the variability in 2001-2002 (Table 4). These variables explained 57 and 54 % of the variability in PCB concentrations during the 1996 and 2001-2002 collection periods, respectively. The inclusion of fish age and gender into the model did not improve the 2001-2002 model.

Table 4. Multiple regression models for spotted bass from Logan Martin Reservoir, AL, 1996 and 2001-2002.

1996 R^2=0.569			2001-2002 R^2=0.537		
Variable	**Coefficients**	**Semi-partial r coefficient**	**Variable**	**Coefficients**	**Semi-partial r coefficient**
Constant	15.756		Constant	-0.152	
Distance	-0.299	0.42	Lipid	0.315	0.62
Lipid	1.552	-0.73	Distance	-0.027	-0.52
			Length	0.005	0.21

The relationship between PCB concentrations and the various independent variables are presented in Figures 12-15. PCB concentrations increased with increasing total length in 1996 (R^2=0.077; p=0.0427) but not for the 2001-2002 sampling period (p=0.7324). The PCB concentration to percent lipid relationship was significant (R^2=0.271; p<0.0001) in the 2001-2002 sample period but not during the 1996 sample period (p=0.1552). Percent lipids increased significantly (R^2=0.2991; p<0.0001) as total length increased in 1996 but not in 2001-2002 (p=0.8630). A weaker spatial concentration gradient was expressed in the 2001-2002 (R^2=0.245; p<0.0001) sampling period compared to 1996 (R^2=0.394; p<0.0001) (ANCOVA p<0.0001) (Figure 12). There were no significant (p>0.05) relationships between relative weight (Wr) and PCB concentrations in 1996 or in 2001-2002.

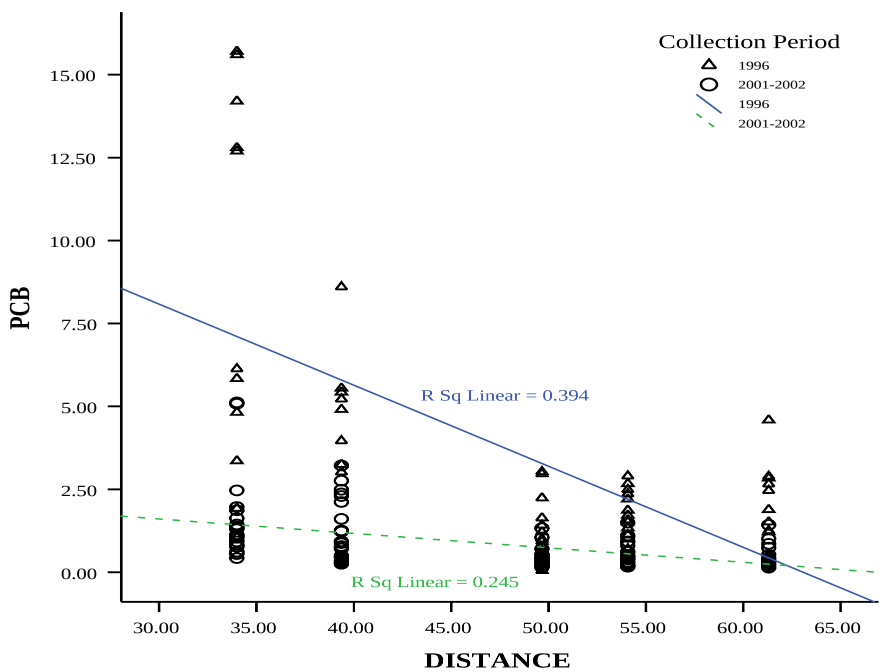

Figure 12. Relation between PCB concentrations (ppm) and distance at capture (km) of spotted bass from 1996 (p<0.0001) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

Spotted bass collected from Choccolocco Creek had significantly ($p<0.05$) higher mean PCB concentrations than spotted bass from the Lake stations (Figure 13). Mean PCB concentrations in fish collected in 1996 were also found to be significantly ($p<0.05$) higher than fish collected in 2001-2002 for both Lake and Creek stations (Figure 13).Creek stations were statistically ($R^2=0.064$; $p=0.0115$) similar in PCBs for fish of the same total length compared to Lake stations ($R^2=0.142$; $p<0.0001$) (ANCOVA $p=0.1050$) (Figure 14). No differences were observed in the PCB to lipid content relationship (ANCOVA $p=0.4123$) between Creek stations ($R^2=0.059$; $p=0.0147$) and Lake stations (($R^2=0.440$; $p<0.0001$) (Figure 15). PCB concentrations in fish increased ($R^2=0.049$; $p<0.0270$) as distance decreased for Creek stations. PCBs to distance relationship did not exist for Lake stations ($p=0.4597$). As relative weight (Wr) increased, fillet PCB concentrations also increased ($R^2=0.042$; $p=0.0412$) at Creek stations but not at Lake stations ($p=0.6335$).

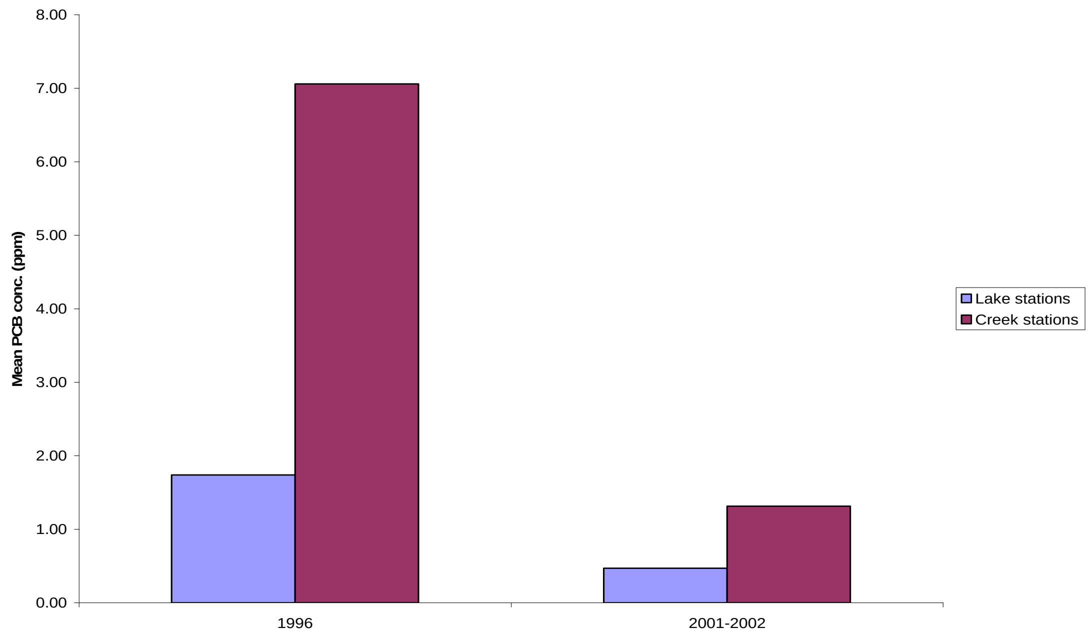

Figure 13. Mean PCB concentrations (ppm) for spotted bass collected at Lake and Creek stations during the 1996 and 2001-2002 collection periods from Logan Martin Reservoir, AL, 1996-2002.

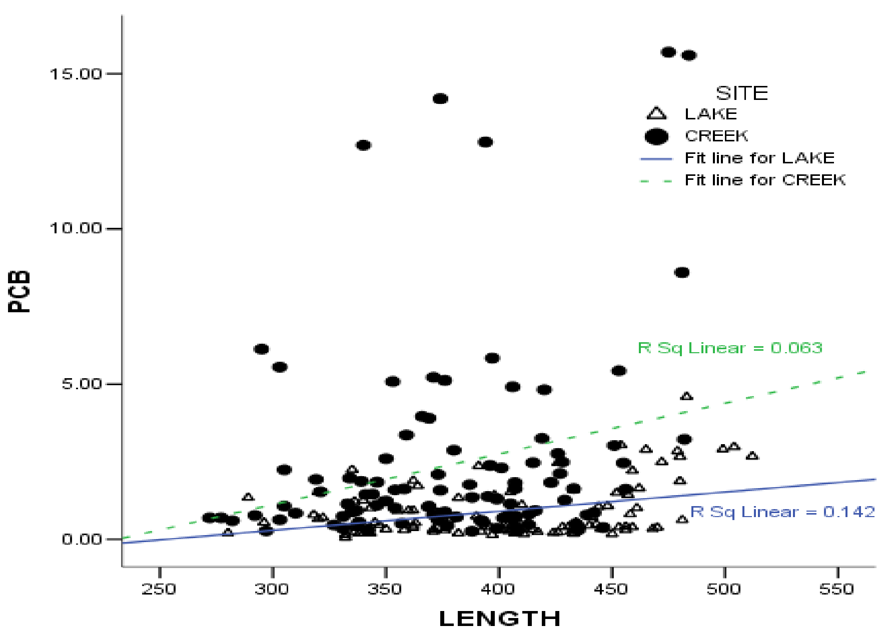

Figure 14. Relation between PCB concentrations (ppm) and total length (mm) of spotted bass from Lake (p=0.0001) and Creek (p=0.0115) stations in Logan Martin Reservoir, AL, 1996-2002.

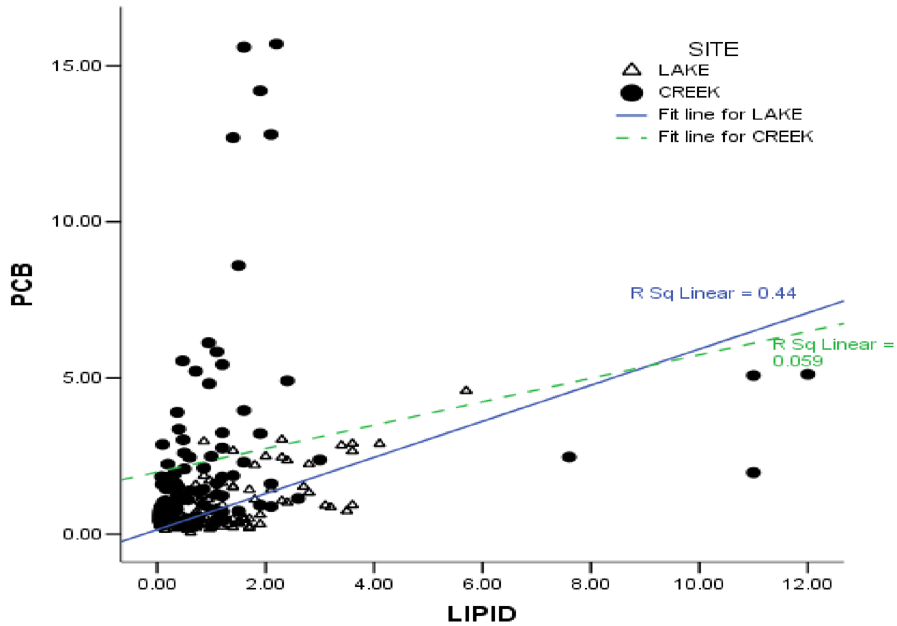

Figure 15. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of spotted bass from Lake ($p<0.0001$) and Creek ($p=0.0147$) stations in Logan Martin Reservoir, AL, 1996-2002.

Striped Bass

A total of 112 striped bass were collected that ranged from 286 to 702 mm total length (Table 2). During the 2001 and 2002 sampling years, age ranged from 1 to 6 years with a mean of 2 years (Table 2). Data collected during 1996 to 2002 indicated that the mean PCB concentrations in striped bass declined from 1996 to 2001, but did not significantly ($p<0.05$) change from 2001 to 2002 (Figure 3). PCBs increased (R^2=0.1071; p=0.0015) with age for all striped bass collected during 2001 and 2002 (Figure 16).

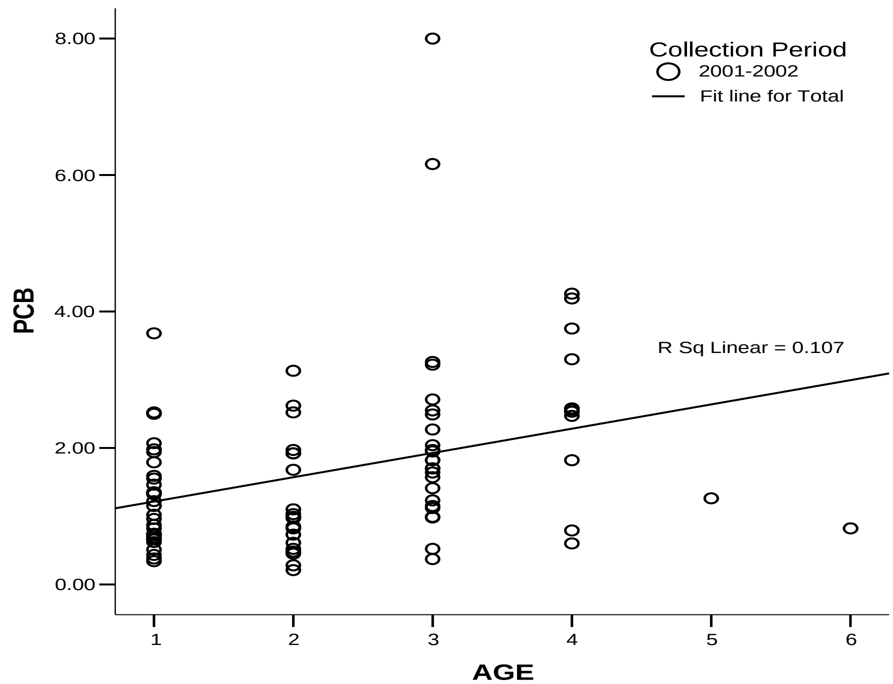

Figure 16. Relation between PCB concentrations (ppm) and age (yrs) of striped bass from all stations (p=0.0015) in Logan Martin Reservoir, AL, 2001-2002.

Analyses of both time periods revealed that the majority of the variability explained resulted from a combination of percent lipid in fillet, distance at capture, and total length (Table 5). Total fish length and lipids explained a majority of the variability in 2001-2002 (Table 5). These variables explained 74 and 42% of the variability in PCB concentrations during the 1996 and 2001-2002 collection periods, respectively. The inclusion of fish age and gender into the model did not improve the2001-2002 model.

Table 5. Regression models for striped bass from Logan Martin Reservoir, AL, 1996 and 2001-2002.

1996 R^2=0.740			2001-2002 R^2=0.417		
Variable	Coefficients	Semi-partial r coefficient	Variable	Coefficients	Semi-partial r coefficient
Constant	4.51		Constant	-0.853	
Lipid	2.301	0.67	Length	0.007	0.43
Distance	-0.386	-0.47	Lipid	0.385	-0.30
Length	0.022	0.27	Distance	-0.04	0.44

PCBs and their relationship with the various independent variables are displayed in Figures 17-20. PCB concentrations were significantly (R^2=0.216; p<0.0001) higher for fish of the same total length in 1996 compared to 2001-2002 (R^2=0.189; p=0.0337) (ANCOVA p<0.0001) (Figure 17). Similarly, PCB concentrations were significantly (R^2=0.410; p=0.0018) higher for the same percent fillet lipid in 1996 compared to 2001-2002 (R^2=0.056; p=0.0245) (ANCOVA p<0.0001) (Figure 18). Percent fillet lipids declined significantly (R^2=0.0752; p=0.0085) as total length increased in 2001-2002 but not (R^2=0.0475; p=0.3427) in 1996. A spatial concentration gradient exists in 2001-2002 (R^2=0.098; p=0.0026) but does not exist in 1996 (p=0.1846). No PCB relationships existed with relative weight in 1996(p=0.8667). However, PCBs slightly increased with decreasing relative weight (R^2=0.054; p=0.0265) in 2001-2002.

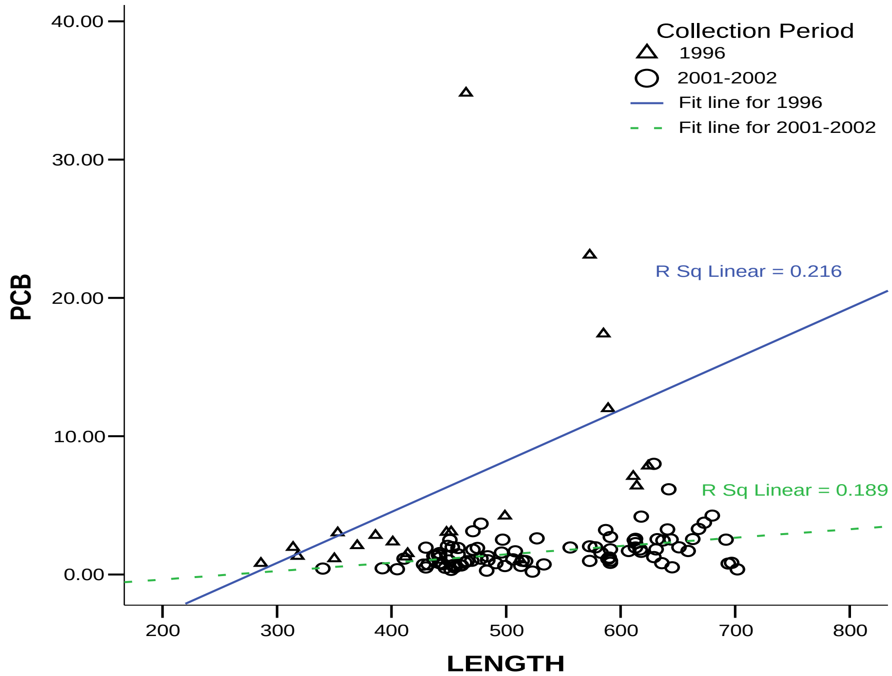

Figure 17. Relation between PCB concentrations (ppm) and total length (mm) of striped bass from 1996 (p<0.0001) and 2001-2002 (p=0.0337) collection periods in Logan Martin Reservoir, AL, 1996-2002.

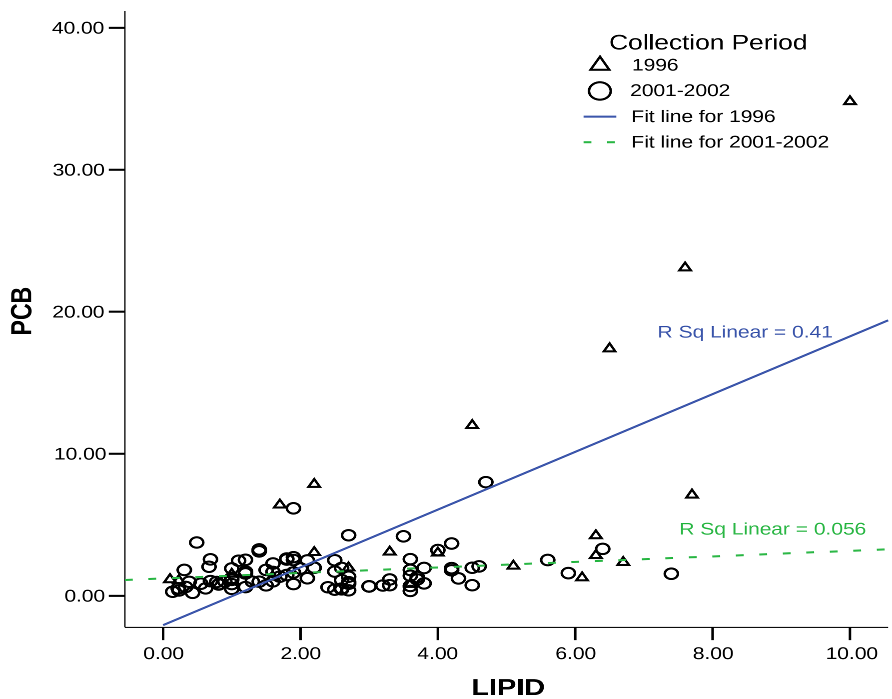

Figure 18. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of striped bass from 1996 (p=0.0018) and 2001-2002 (p=0.0245) collection periods in Logan Martin Reservoir, AL, 1996-2002.

Striped bass collected from Choccolocco Creek had significantly ($p<0.05$) higher mean PCB concentrations than striped bass from the Lake stations (Figure 19). Mean PCB concentrations in fish collected in 1996 were also found to be significantly ($p<0.05$) higher than in fish collected in 2001-2002 for both Lake and Creek stations (Figure 19). PCB concentration increased some with total fish length for Lake stations (R^2=0.0698; p=0.0396) but not for Creek stations (p=0.6469). Creek stations were significantly (R^2=0.683; p=0.0065) higher in PCBs for the same percent lipid content than Lake stations (R^2=0.119; p<0.0001) (ANCOVA p<0.0001) (Figure 20). PCBs in striped bass were not related to distance for Creek (p=0.6588) or Lake stations (p=0.8369). Relative weight (Wr) did not influence PCB concentrations at either Creek stations (p=0.0810) or at Lake stations (p=0.1044).

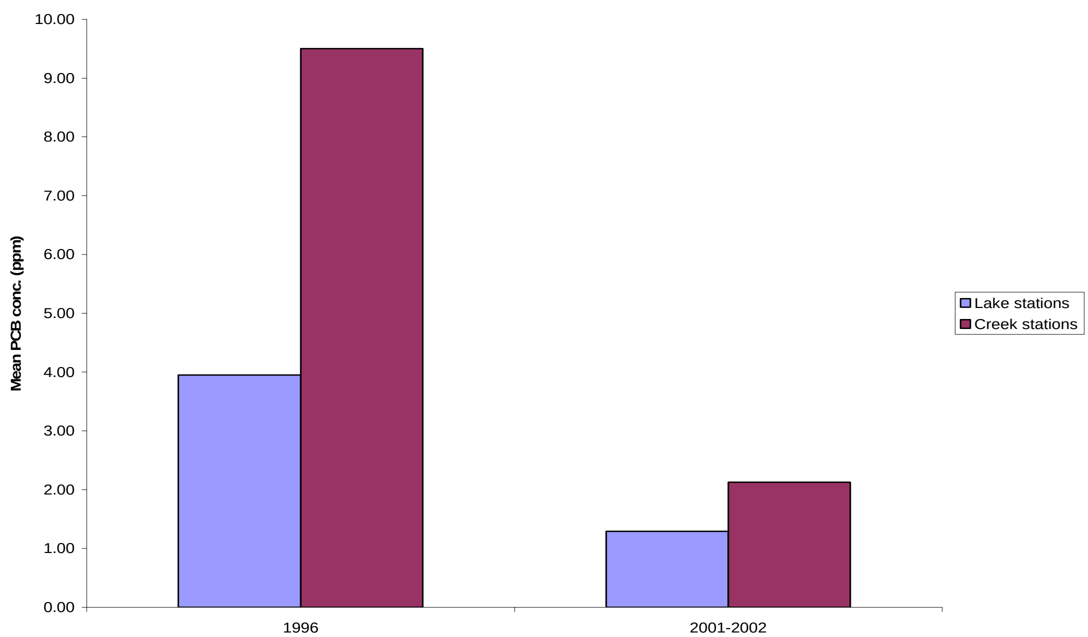

Figure 19. Mean PCB concentrations (ppm) for striped bass collected at Lake and Creek stations during the 1996 and 2001-2002 collection periods from Logan Martin Reservoir, AL, 1996-2002.

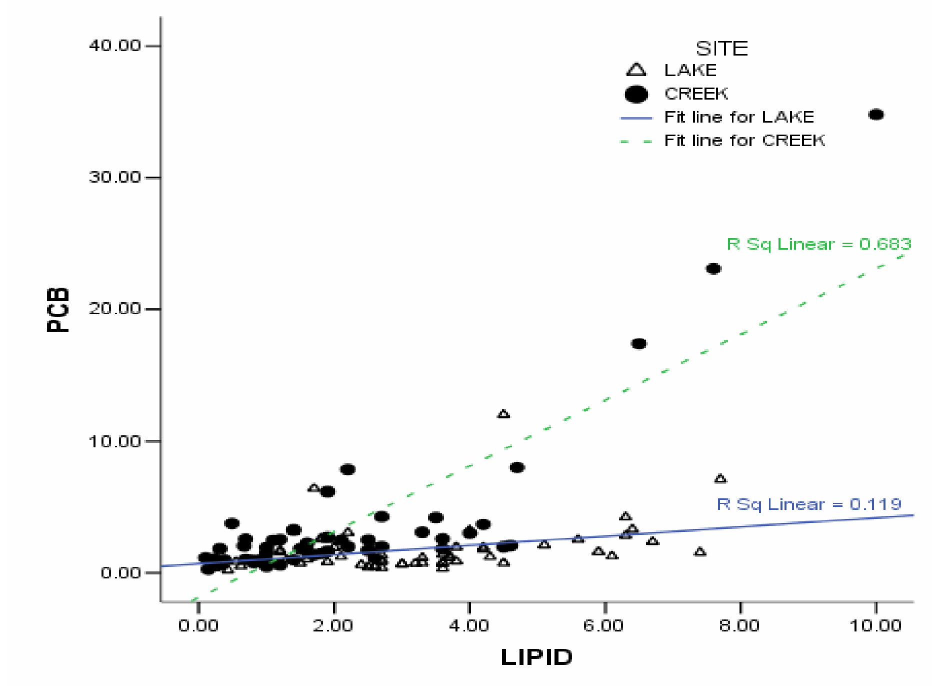

Figure 20. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of striped bass from Lake (p=0.0065) and Creek (p<0.0001) stations in Logan Martin Reservoir, AL, 1996-2002.

Black Crappie

A total of 155 black crappie were collected that ranged from 206 to 323 mm total length (Table 2). During the 2001 and 2002 sampling years, age ranged from 1 to 8 years with a mean of 3 years (Table 2). Data collected during 1996 to 2002 indicated that the mean PCB concentrations in black crappie declined from 1996 to 2001, but did not significantly (p<0.05) change from 2001 to 2002 (Figure 3). PCBs were not significantly (p=0.1171) related to fish age.

Analyses of both collection periods suggested that distance at capture was the dominant, negative predictor of PCBs (Table 6). Although less influential, percent fillet lipids was an important positive regressor in the prediction of PCB concentrations (Table 6). Relative weight (in 1996) or total length (in 2001-2002) increased the amount of variability explained by the models (Table 6). These variables explained 61% and 50% of the variability in PCB concentrations during the 1996 and 2001-2002 collection periods, respectively. Gender and age were not related to PCB levels in black crappie because neither improved the model.

Table 6. Regression models for black crappie from Logan Martin Reservoir, AL, 1996 and 2001-2002.

1996 R^2=0.612			2001-2002 R^2=0.495		
Variable	**Coefficients**	**Semi-partial r coefficient**	**Variable**	**Coefficients**	**Semi-partial r coefficient**
Constant	15.461		Constant	0.147	
Distance	-0.171	0.41	Distance	-0.029	0.36
Lipid	1.241	-0.68	Lipid	0.318	-0.55
Wr	-0.081	-0.25	Length	0.005	0.19

The relationship between PCB concentrations and each independent variable are shown in Figures 21-26. No differences (ANCOVA p=0.0543) in PCB concentrations for fish of the same total length occurred during 1996 (R^2=0.098; p=0.0389) or the 2001-2002 (R^2=0.132; p<0.0001) sample period (Figure 21). PCB residues increased (p=0.0082) with increasing percent lipids in 1996 and 2001-2002 (p<0.0001); with the 2001-2002 sample period being significantly (ANCOVA p=0.0023) lower than the 1996 sampling period (Figure 22). Percent fillet lipids increased positively with total length in 1996 ($R^2$0.26; p=0.0004) and in 2001-2002 (R^2=0.14; p=0.1623) (Figure 23). However, significant (ANCOVA p=0.7152) differences in fillet lipids were not observed between collection periods. A spatial concentration gradient was displayed in both 1996 (R^2=0.374; p<0.0001) and in 2001-2002 (R^2=0.302; p<0.0001) sampling periods, however a stronger (ANCOVA p<0.0001), more pronounced gradient occurred in 1996

(Figure 24). There was no significant relation between relative weight (Wr) and PCB concentrations for fish collected in 1996 (p=0.9204) or 2001-2002 (p=0.2354).

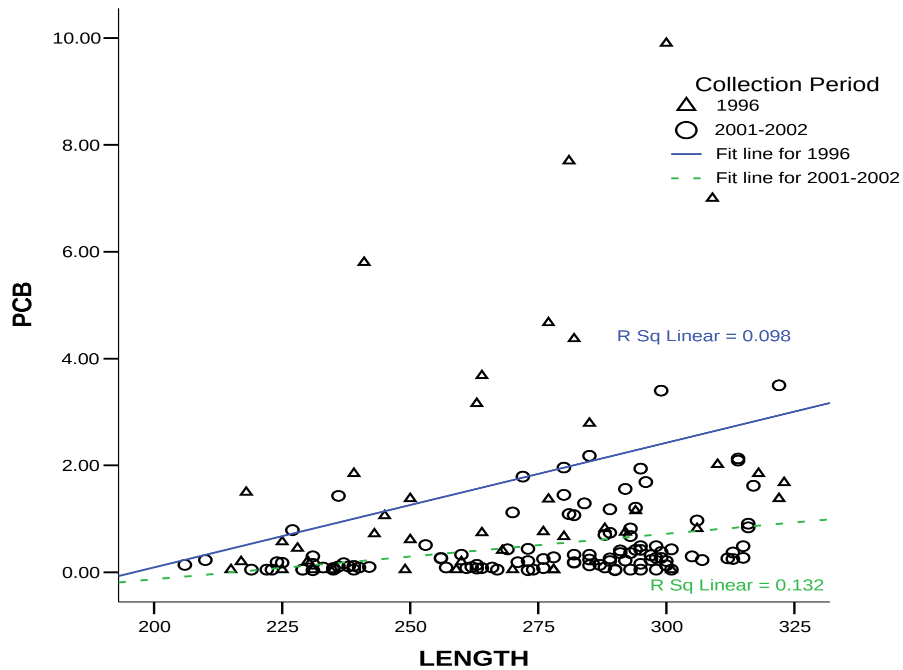

Figure 21. Relation between PCB concentrations (ppm) and total length (mm) of black crappie from 1996 (p=0.0389) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

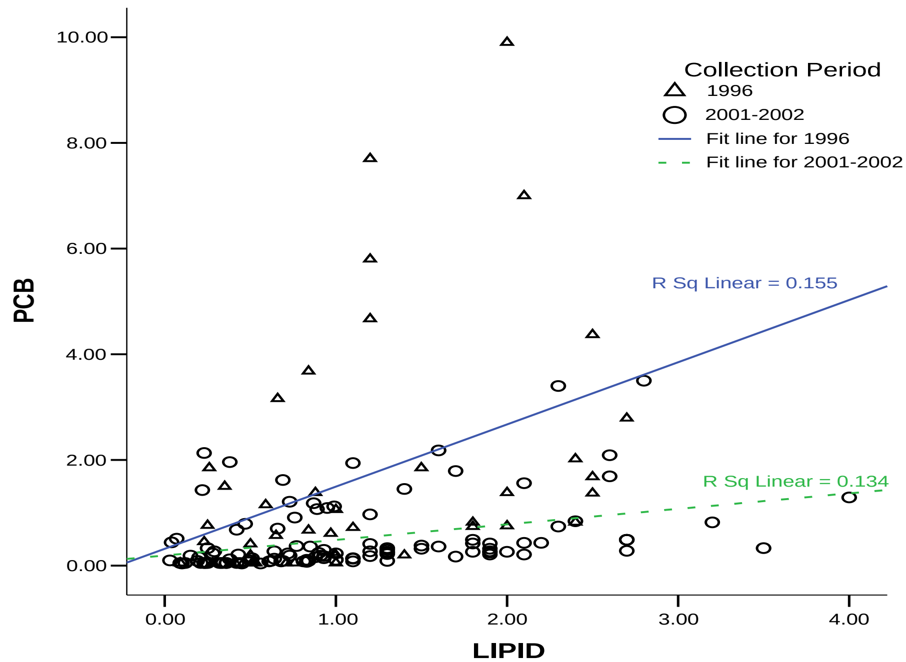

Figure 22. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of black crappie from 1996 (p=0.0082) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

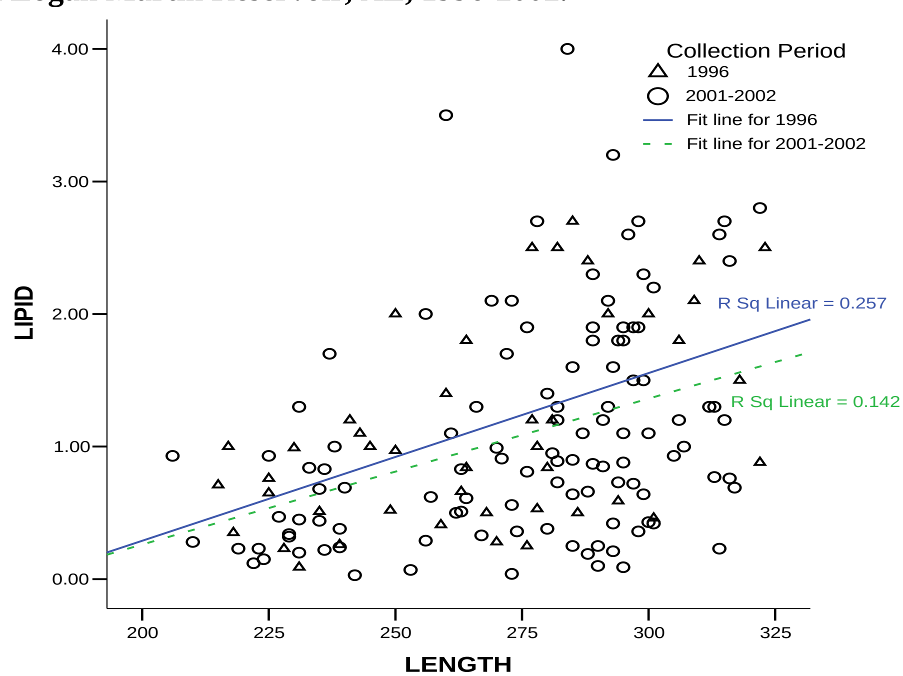

Figure 23. Relation between percent lipid content (%) and total length (mm) of black crappie from 1996 (p=0.0004) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

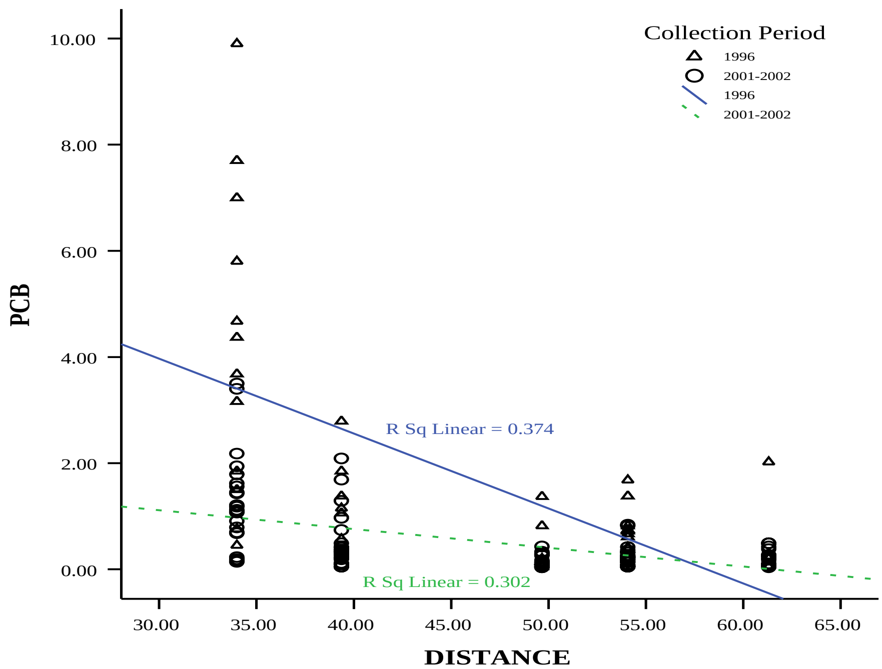

Figure 24. Relation between PCB concentrations (ppm) and distance (km) at capture of black crappie from 1996 (p<0.0001) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002

Black crappie collected from Choccolocco Creek had significantly ($p<0.05$) higher mean PCB concentrations compared to black crappie from the Lake stations (Figure 25). Mean PCB concentrations in fish collected in 1996 were also found to be significantly ($p<0.05$) higher than in fish collected in 2001-2002 for both Lake and Creek stations (Figure 25). PCB concentrations were positively related to length at Lake stations (R^2=0.129; $p<0.0006$). No PCBs to length relationship was present for Creek stations (R^2=0.041; p=0.0994). Nevertheless, the PCBs to lipid relationship was significantly (ANCOVA p=0.0042) higher at Creek stations (R^2=0.1824; p=0.0003) compared to Lake stations (R^2=0.4113; $p<0.0001$) (Figure 26). Fillet PCB residues were not related to distance for either Creek stations (p=0.6752) or Lake stations (p=0.9833). No PCBs to relative weight relationship was present from fish collected at Creek stations (p=0.0531). Relative weight (Wr) declined (p=0.0279) with increasing PCB concentrations in fish collected from Lake stations.

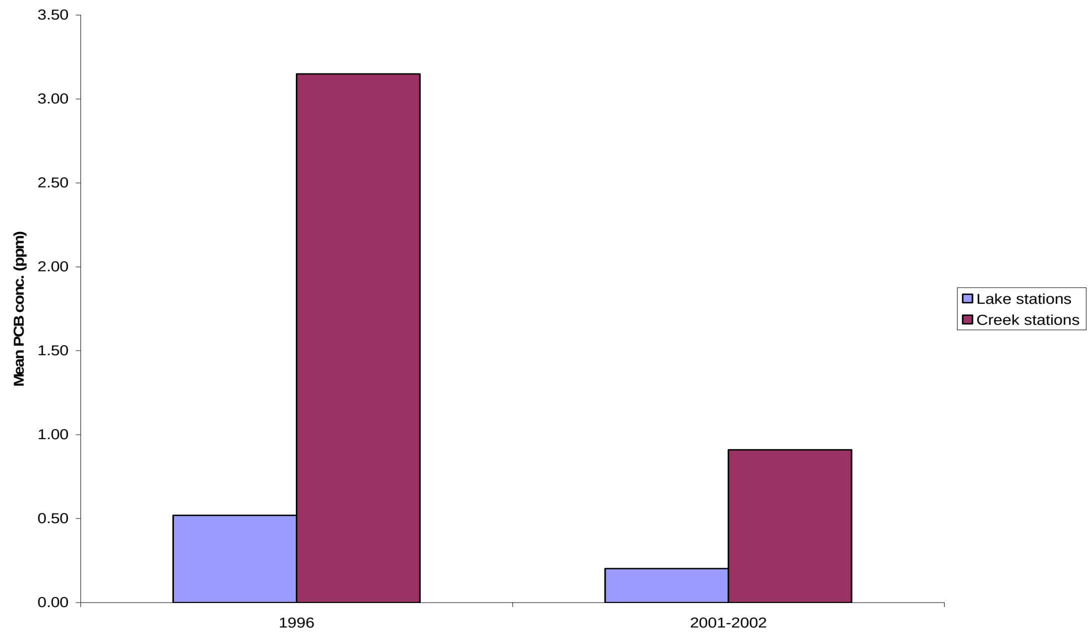

Figure 25. Mean PCB concentrations (ppm) for black crappie collected at Lake and Creek stations during the 1996 and 2001-2002 collection periods from Logan Martin Reservoir, AL, 1996-2002.

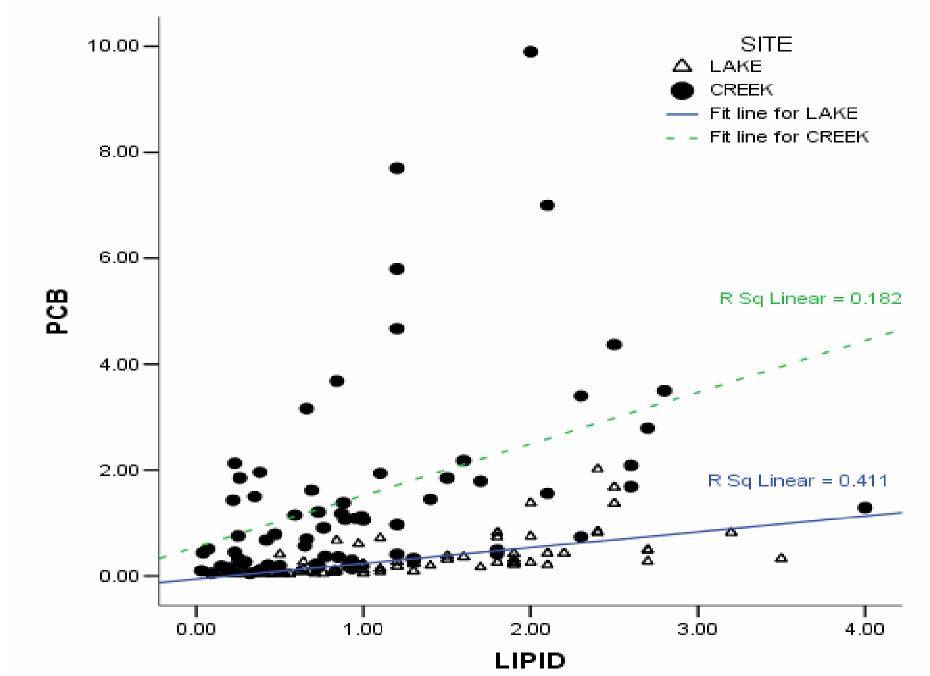

Figure 26. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of black crappie from Lake (p<0.0001) and Creek (p=0.0003) stations in Logan Martin Reservoir, AL, 1996-2002.

Channel Catfish

Three hundred and twenty channel catfish were collected that ranged from 241 to 510 mm total length (Table 2). During the 2001 and 2002 sampling years, age ranged from 1 to 12 years with a mean of 5 years (Table 2). Data collected during 1996 to 2002 indicated that the mean PCB concentrations in channel catfish were significantly (p<0.05) greater in 1996 compared to 2002 (Figure 3). However, mean PCB concentrations during 2001 were not statistically (p<0.05) different than mean PCB concentrations in 1996 or 2002 (Figure 3). Fish age, a strong predictor of PCBs in many other species, does not demonstrate a significant (p=0.3636) relationship with fillet PCB concentrations collected in 2001 and 2002.

Similar to other species evaluated in this study, channel catfish regression analyses revealed distance at capture and percent lipids controlled the uptake of PCBs (Table 7). Both models indicate distance at capture was negatively related to PCBs while lipid content was positively related to PCB concentrations. These variables explained 66 and 49 % of the variability in PCBs during 1996 and 2001-2002, respectively. Gender or age did not improve the model for the prediction of PCBs in 2001-2002.

Table 7. Regression models for channel catfish from Logan Martin Reservoir, AL, 1996 and 2001-2002.

1996 R^2=0.660			2001-2002 R^2=0.489		
Variable	**Coefficients**	**Semi-partial r coefficient**	**Variable**	**Coefficients**	**Semi-partial r coefficient**
Constant	11.788		Constant	3.358	
Distance	-0.210	0.46	Lipid	1.179	0.34
Lipid	1.548	-0.56	Distance	-0.073	-0.36

The relationship between PCB concentrations and the independent variables are presented in Figures 27-33. PCB concentrations increased with length only in 2001-2002 (R^2=0.364; p<0.0001). No significant differences (ANCOVA p=0.7705) were present in PCBs to lipid relationships between sampling periods (1996 R^2=0.343; p<0.0001) (2001-2002 R^2=0.369; p<0.0001) (Figure 27). Percent fillet lipids in channel catfish increased as length increased during 1996 (R^2<0.1651; p=0.0056) and in 2001-2002 (R^2=0.2939; p<0.0001) (Figure 28). However, significant (ANCOVA p=0.6535) differences in fillet lipid content were not observed between collection periods. A weaker spatial concentration gradient was present in 2001-2002 (R^2=0.361; p<0.0001) compared to 1996 (R^2=0.448; p<0.0001) (ANCOVA p=0.0032) (Figure 29). Fish relative weight (Wr) decreased significantly (R^2=0.075; p=0.0007) with PCB concentrations in 2001-2002, but not in 1996 (p=0.1427).

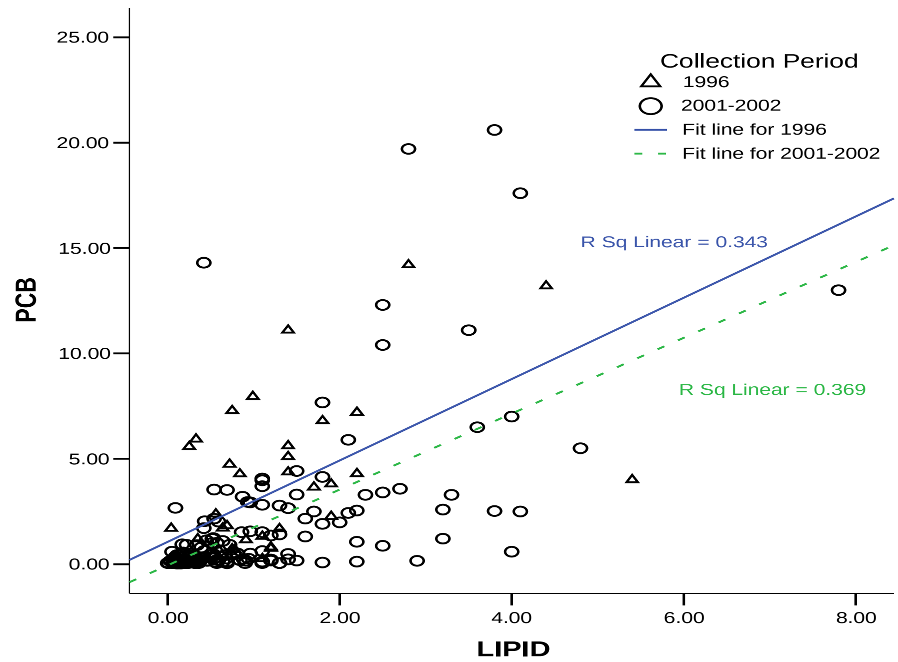

Figure 27. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of channel catfish from 1996 (p<0.0001) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

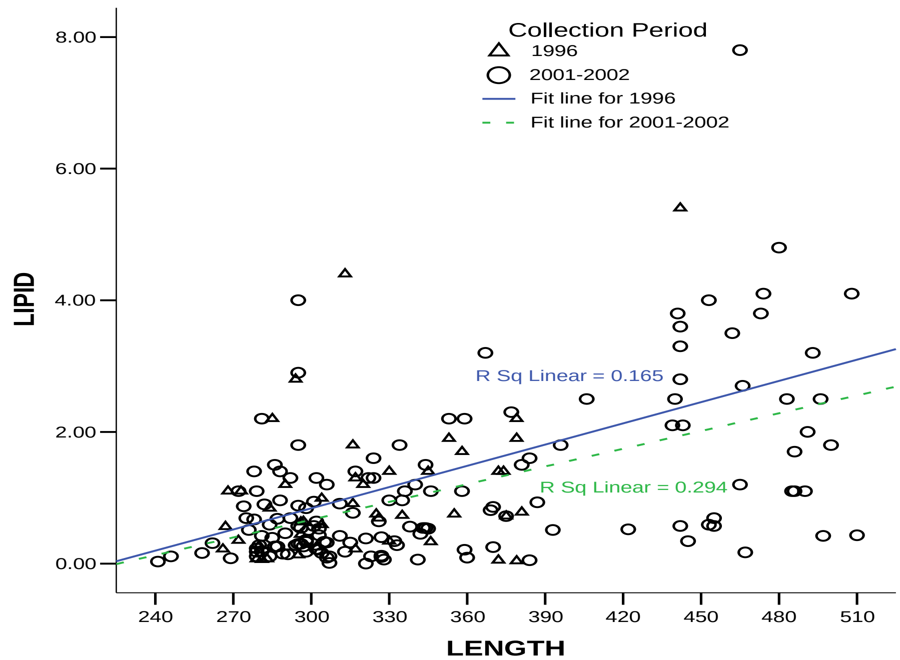

Figure 28. Relation between percent lipid content (%) and total length (mm) of channel catfish from 1996 (p=0.0056) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

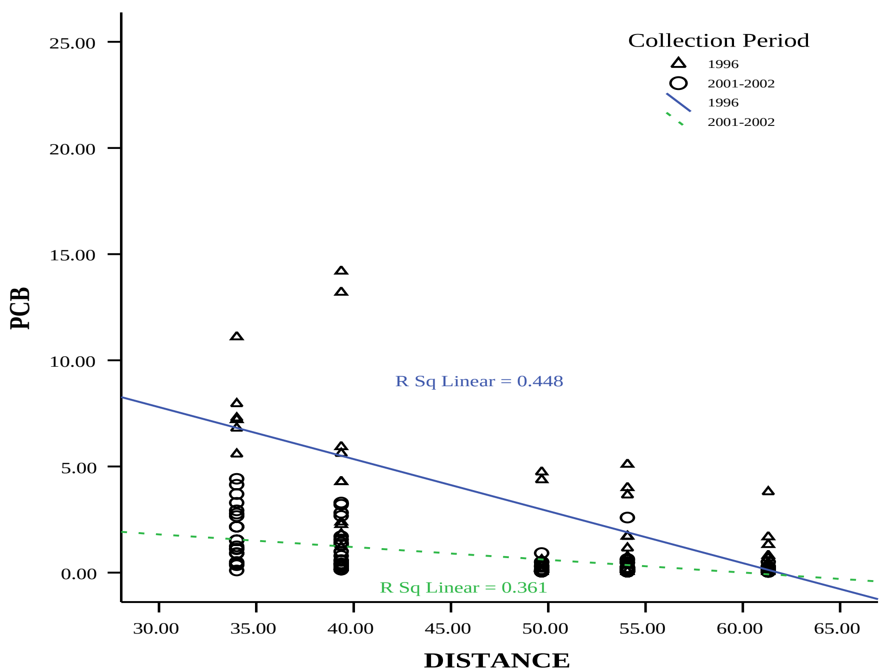

Figure 29. Relation between PCB concentrations (ppm) and distance at capture (km) of channel catfish from 1996 (p<0.0001) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

Channel catfish collected from Choccolocco Creek had significantly ($p<0.05$) higher mean PCB concentrations than channel catfish from the Lake stations (Figure 30). Mean PCB concentrations in fish collected in 1996 were also found to be significantly ($p<0.05$) higher than in fish collected in 2001-2002 for both Lake and Creek stations (Figure 30). Fish collected from Creek stations ($R^2=0.132$; $p<0.0002$) were not significantly (ANCOVA $p=0.9103$) different in PCB concentrations for fish of the same total length compared to fish from Lake stations ($R^2=0.345$; $p<0.0001$) (Figure 31). However, fish from Lake stations ($R^2=0.218$; $p<0.0001$) were significantly (ANCOVA $p=0.0005$) lower in PCBs for the same percent lipid content than Creek stations ($R^2=0.354$; $p<0.0001$) (Figure 32). PCBs weakly declined as distance increased in the Creek stations ($R^2=0.1217$; $p=0.0004$). PCB concentrations at Lake stations did not display a relationship with distance ($p=0.1949$). Fish from Creek and Lake stations were statistically similar (ANCOVA $p=0.3256$) in PCB concentrations in respect to relative weight (Wr) ($R^2=0.051$; $p=0.0244$; $R^2=0.158$; $p<0.0001$, respectively) (Figure 33).

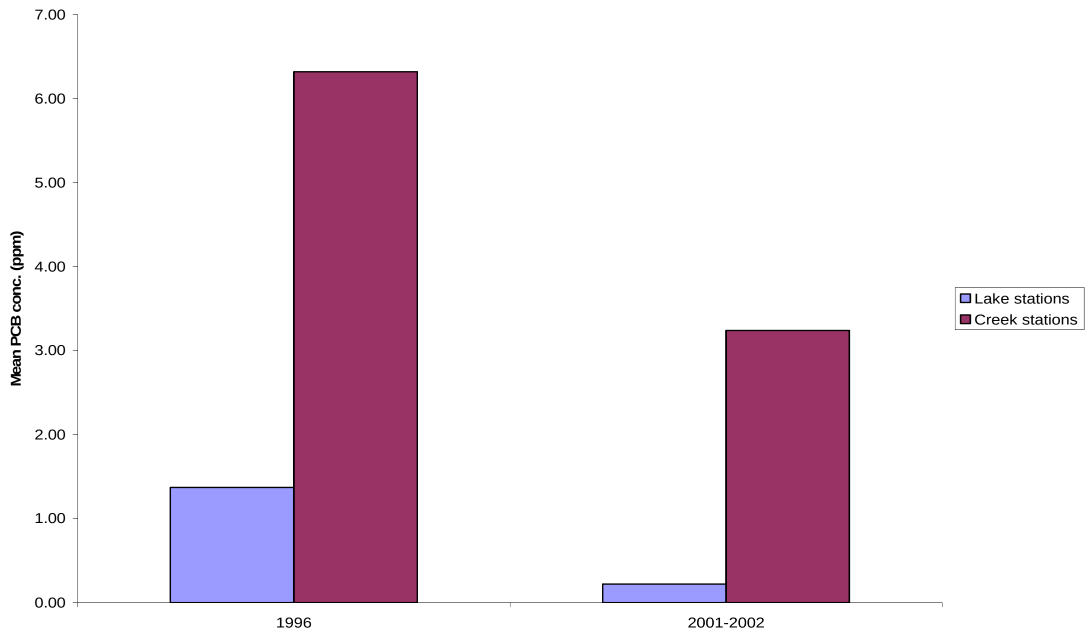

Figure 30. Mean PCB concentrations (ppm) for channel catfish collected at Lake and Creek stations during the 1996 and 2001-2002 collection periods from Logan Martin Reservoir, AL, 1996-2002.

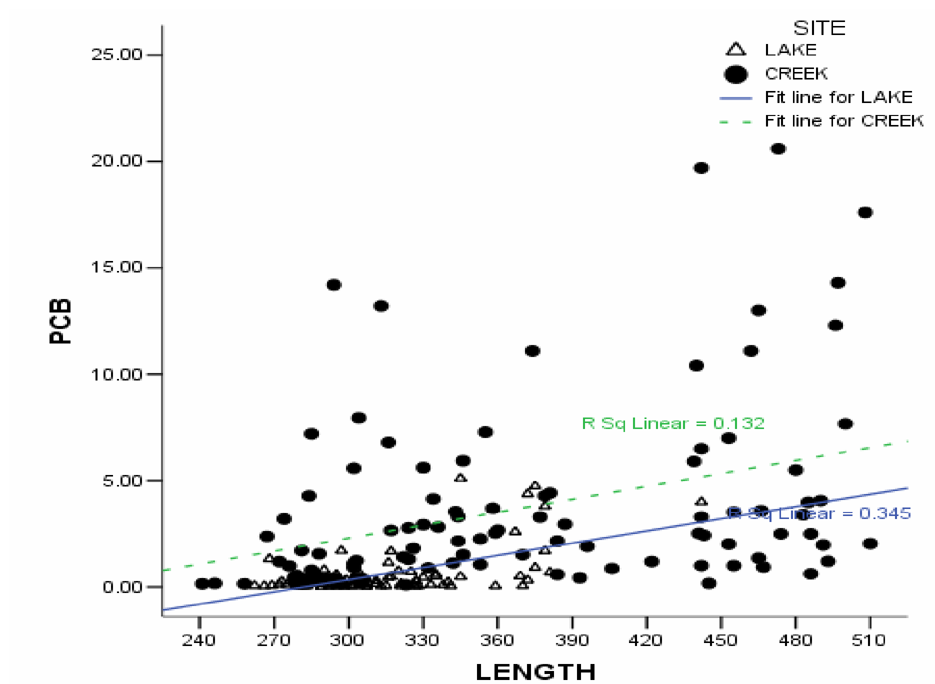

Figure 31. Relation between PCB concentrations (ppm) and total length (mm) of channel catfish from Lake (p<0.0001) and Creek (p<0.0002) stations in Logan Martin Reservoir, AL, 1996-2002.

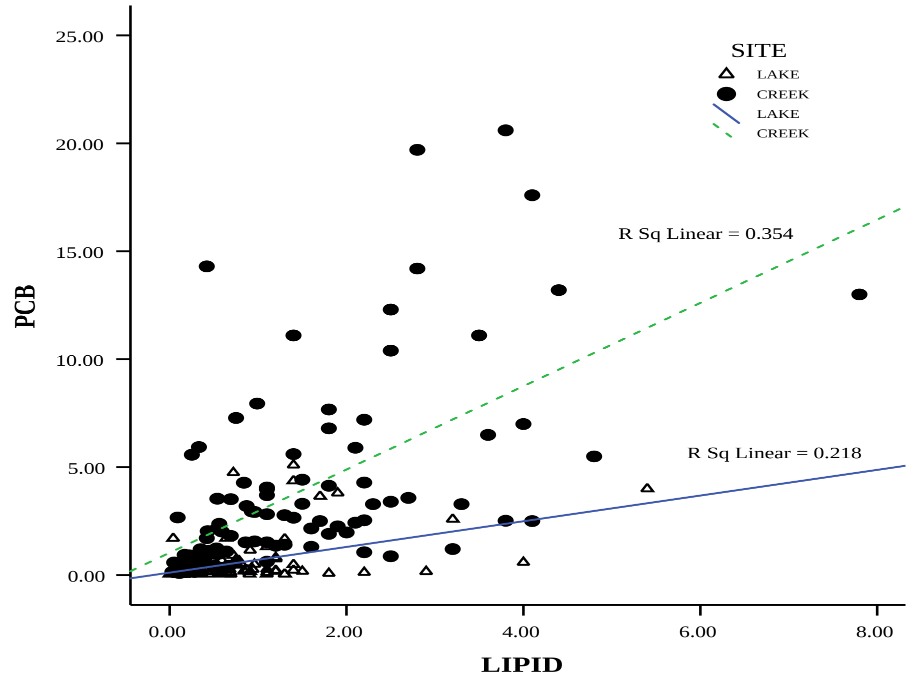

Figure 32. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of channel catfish from Lake (p<0.0001) and Creek (p<0.0001) stations in Logan Martin Reservoir, AL, 1996-2002.

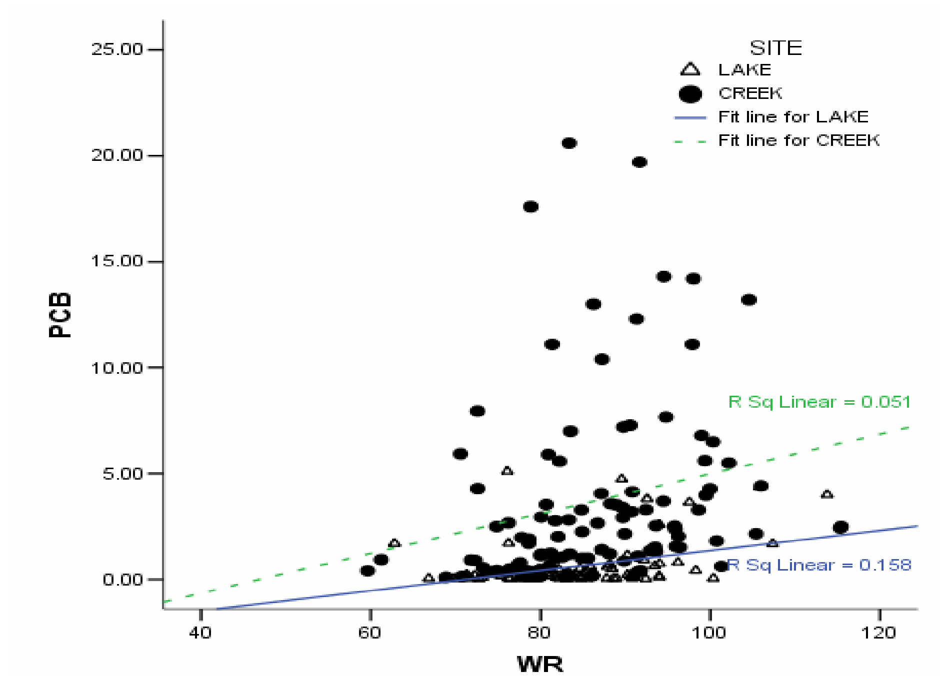

Figure 33. Relation between PCB concentrations (ppm) and relative weights (Wr) of channel catfish from Lake (p<0.0001) and Creek (p=0.0244) stations in Logan Martin Reservoir, AL, 1996-2002.

Freshwater Drum

A total of 176 freshwater drum were collected that ranged from 234 to 489 mm total length (Table 2). During the 2001 and 2002 sampling years, age ranged from 2 to 21 years with a mean of 8 years (Table 2). Mean age of freshwater drum was nearly doubled that of the other five species evaluated during this study. Data collected in 1996, 2001, and 2002 suggests that the mean fillet PCB concentrations have not significantly (p=0.788) changed during the seven year period (Figure 3). Regression analysis from fish collected in 2001-2002 showed PCB bioaccumulation was not related (p=0.5419) to fish age.

Unlike the other species evaluated in this study, strong regressors were highly variable among both models (Table 8). In the 1996 model, the dominant predictor of PCBs was relative weight (Wr) (Table 8). The dominant predictor in the 2001-2002 model was percent fillet lipids (Table 8). Distance at capture, however, was the second strongest predictor in both the 1996 and the 2001-2002 models (Table 8). Length explained a small portion of the variability in both the 1996 and the 2001-2002 models (6.0% and 2.0%, respectively) (Table 8). These variables explained 59 and 69 % of the variability in PCBs during 1996 and 2001-2002, respectively. The inclusion of age and gender did not explain additional variability in the model for the prediction of PCB concentrations.

Table 8. Regression models for freshwater drum from Logan Martin Reservoir, AL, 1996 and 2001-2002.

1996 R^2=0.587			2001-2002 R^2=0.681		
Variable	Coefficients	Semi-partial r coefficient	Variable	Coefficients	Semi-partial r coefficient
Constant	-12.616		Constant	-1.016	
Wr	0.178	0.23	Lipid	1.944	0.65
Distance	-0.192	-0.35	Distance	-0.049	-0.24
Length	0.019	0.28	Length	0.008	0.10
Lipid	0.891	0.28			

The relationship between PCB concentrations and the independent variables are presented in Figures 34-38. Length dependent differences (ANCOVA p=0.9420) in PCB concentrations were not observed between sampling periods (1996 R^2=0.1297; p=0.0207) (2001-2002 R^2=0.0920; p=0.0003) (Figure 34). Contrary to the other fish species examined, the PCBs to lipid relationship in 2001-2002(R^2=0.617; p<0.0001) was higher (ANCOVA p<0.01) than in 1996 (R^2=0.130; p=0.0208) (Figure 35). Lipids were positively related to length in the 2001-2002 sampling period (R^2=0.0756; p=0.0012) but were not in the 1996 sampling period (R^2=0.0001; p=0.950). A spatial PCB concentration gradient was displayed in both 1996 (R^2=0.1887; p=0.0045) and in 2001-2002 (R^2=0.0354; p=0.05) sampling periods, however a stronger, more pronounced (ANCOVA p=0.0417) gradient existed in 1996 (Figure 36). The PCB to relative weight (Wr) relationship (ANCOVA p=0.0417) was significantly higher in 1996 (R^2=0.400; p<0.0045) compared to the 2001-2002 sampling period, (R^2=0.245; p<0.0001) (Figure 37).

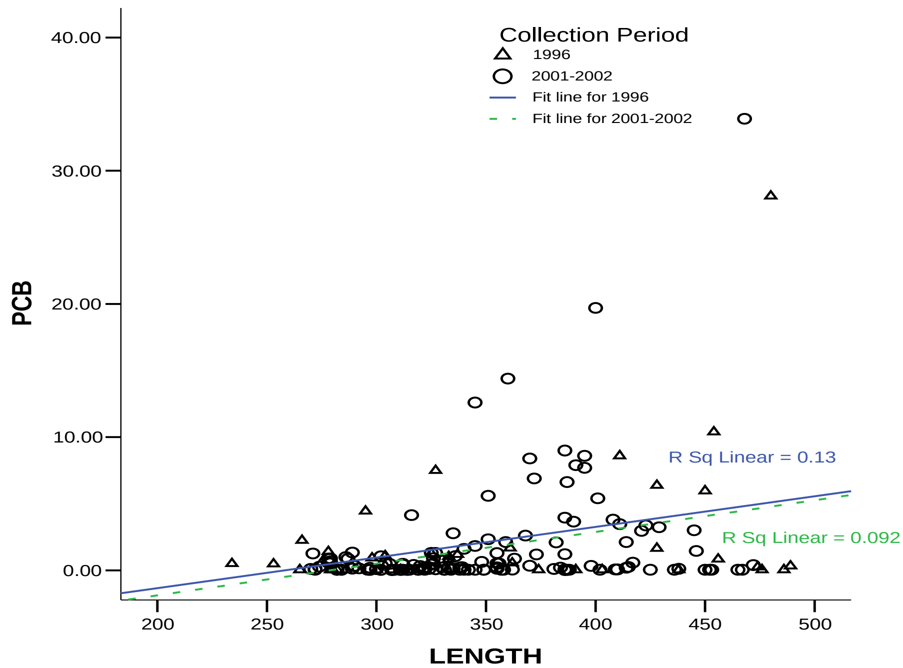

Figure 34. Relation between PCB concentrations (ppm) and total length (mm) of freshwater drum from 1996 ($p=0.0207$) and 2001-2002 ($p=0.003$) collection periods in Logan Martin Reservoir, AL, 1996-2002.

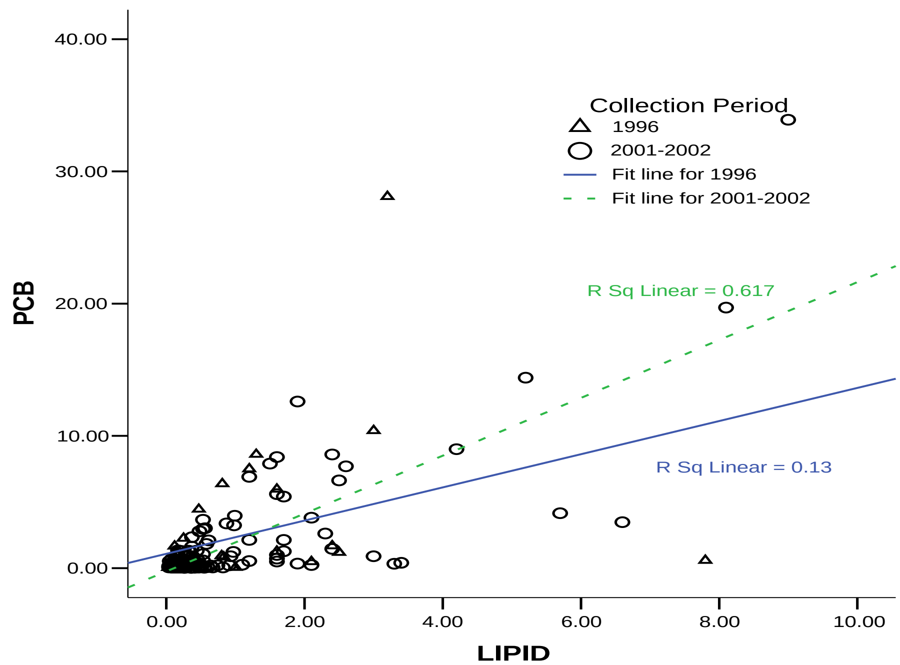

Figure 35. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of freshwater drum from 1996 ($p=0.0208$) and 2001-2002 ($p<0.0001$) collection periods in Logan Martin Reservoir, AL, 1996-2002.

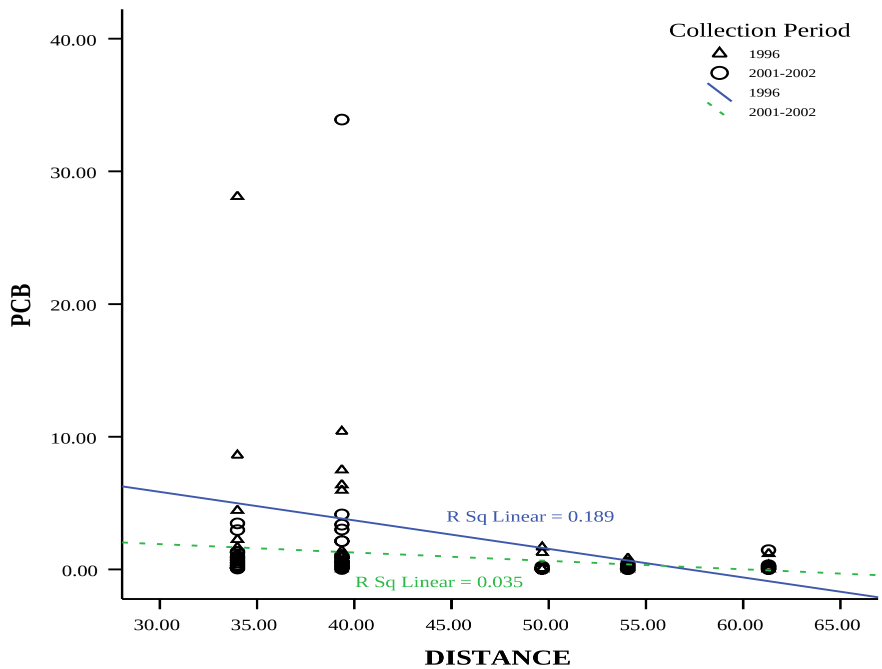

Figure 36. Relation between PCB concentrations (ppm) and distance at capture (km) of freshwater drum from 1996 (p=0.0045) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

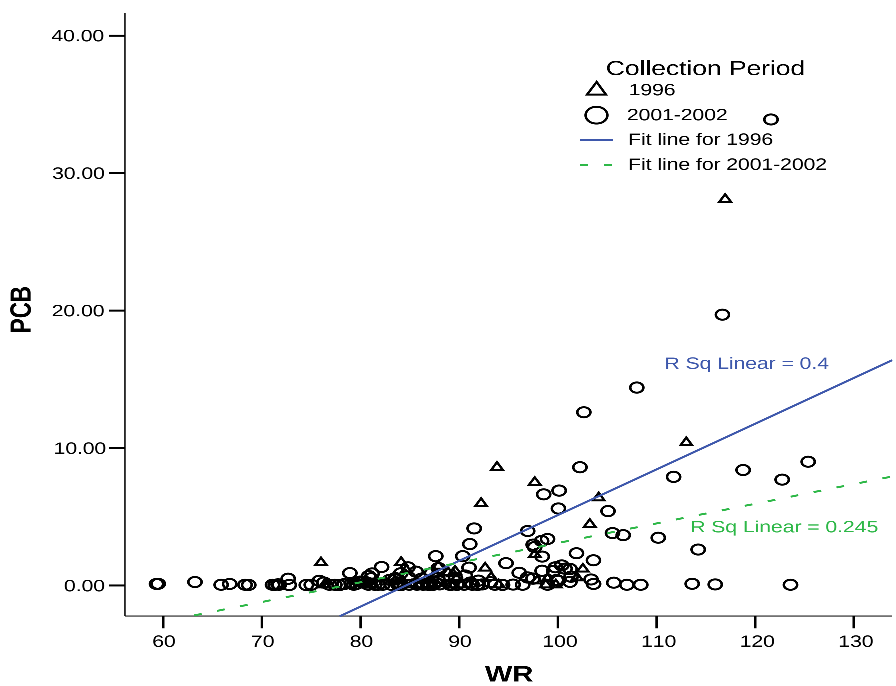

Figure 37. Relation between PCB concentrations (ppm) and relative weights (Wr) of freshwater drum from 1996 (p<0.0001) and 2001-2002 (p<0.0001) collection periods in Logan Martin Reservoir, AL, 1996-2002.

Mean concentrations of PCBs were significantly ($p<0.05$) higher in freshwater drum from Choccolocco Creek (Creek stations) compared to freshwater drum from Lake stations. Mean PCB concentrations between collection periods did not change ($p=0.237$) in the Creek stations and displayed only a slight reduction over time in the Lake stations ($p=0.004$). PCB concentration from Creek stations were higher as length increased ($R^2=0.2727$; $p<0.0001$). However, fish from Lake stations did not follow this trend ($p=0.2803$). Creek station fish ($R^2=0.0890$; $p<0.0001$) were significantly (ANCOVA $p<0.0001$) higher in PCBs for the same percent lipid content than Lake station fish ($R^2=0.3032$; $p<0.0001$) (Figure 38). PCBs were not related to distance for either Creek ($p=0.1653$) or Lake stations ($p=.6675$). PCB concentrations in fish fillets increased as fish relative weight (Wr) increased at Creek stations ($R^2=0.381$; $p<0.0001$). No PCBs to relative weight relationship was expressed from fish collected at Lake stations ($p=0.536$).

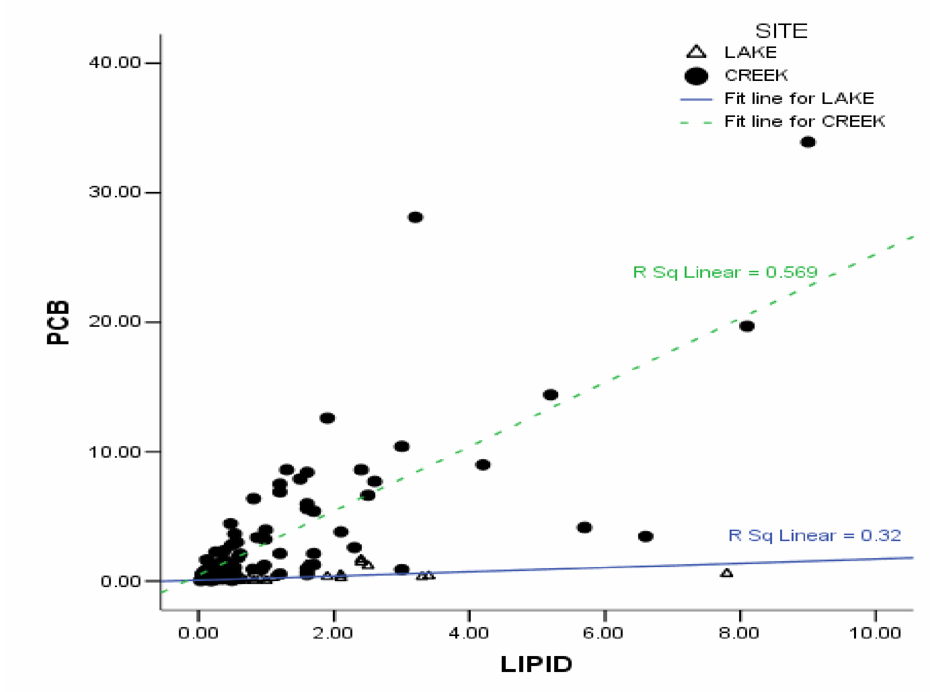

Figure 38. Relation between PCB concentrations (ppm) and percent lipid in fillet (%) of freshwater drum from Lake ($p<0.0001$) and Creek ($p<0.0001$) stations in Logan Martin Reservoir, AL, 1996-2002.

<u>Temporal Effects on PCB Concentrations</u>

Regression models for predicting PCB levels over time were developed using the natural log transformation of the PCBs (lnPCB). The natural log transformation was used to improve the normality of the model residuals. The samples collected at flowing water reaches of Choccolocco Creek (stations 43 and 44) were not included in the analyses because samples were not collected at these sites during 1996. Plots of the residuals confirmed that the natural log transformed model was improved over the untransformed model. Models developed for all six species were able to explain 58 to 73 percent of the variability associated with lnPCB (Table 9). Three or four variable models were found to best explain the differences in PCB concentrations throughout Logan Martin Reservoir. The number of years after the 1996 collection period was found to significantly ($p<0.05$) reduce PCB levels and the lipid content was found to significantly ($p<0.05$) increase PCB levels in all six fish species. Interestingly, the collection location did not seem to influence PCB concentrations in striped bass. Fish length does not appear to affect PCB levels in black crappie or channel catfish.

Table 9. Regression coefficients for predicting the natural log transformation of PCB concentrations in fish fillets for each species collected in 1996, 2001, and 2002 from Logan Martin Reservoir, AL. The semi-partial correlation coefficients are in parentheses.

	Constant	Distance (km)	Years (after 1996)	Lipid content (%)	Fish Length (mm)	R^2
Largemouth bass	1.508	-0.081 (-0.55)	-0.200 (-0.38)	0.727 (0.36)	0.004 (0.15)	0.67
Spotted bass	0.668	-0.056 (-0.48)	-0.252 (-0.60)	0.197 (0.29)	0.006 (0.29)	0.73
Striped bass	-1.762		-0.165 (-0.39)	0.214 (0.44)	0.005 (0.49)	0.58
Black crappie	2.171	-0.081 (-0.59)	-0.131 (-0.24)	0.917 (0.54)		0.69
Channel catfish	4.118	-0.094 (-0.58)	-0.235 (-0.36)	0.676 (0.34)		0.67
Freshwater drum	1.386	-0.096 (-0.56)	-0.121 (-0.19)	0.520 (0.41)	0.005 (0.19)	0.64

<u>Comparison of PCB Levels Between Species</u>

Two-way Analysis of Variance (ANOVA) and Dunnett's C multiple comparison analyses were used to determine if significant differences existed between species within each of the collection periods (1996 and 2001-2002) and across collection locations (Lake and Creek stations). Analyses were preformed on the natural log transformed PCB concentrations in order to normalize the concentration values. The geometric mean concentration of PCBs for each species collected during 1996 and 2001-2002 in the vicinity of Choccolocco Creek and Logan Martin Reservoir are presented in Figures 39 and 40, respectively.

Significant ($p<0.05$) differences in PCB concentrations were found between species during each collection period. Significant ($p<0.05$) differences in PCB concentrations were also found between the Lake and Creek stations. Significant interaction effects between species and sampling stations existed. Therefore, Dunnett's C multiple comparison tests were preformed to determine significant differences between species within each collection period and sampling stations.

The transformed PCB levels in decreasing order for the six fish species collected during 1996 in Choccolocco Creek were: spotted bass, channel catfish, striped bass, largemouth bass, freshwater drum, and black crappie. Dunnett's C multiple comparison tests indicates that significant ($p<0.05$) differences only existed between spotted bass and black crappie (Figure 41). After five years (2001-2002), the relative order of decreasing PCB concentrations were similar with largemouth bass and black crappie having significantly ($p<0.05$) lower PCB concentrations than the other four species (Figure 41). Again, the order of decreasing concentrations between species was similar for fish collected from the Lake stations during 1996. Striped bass and spotted bass had significantly ($p<0.05$) higher concentrations of PCBs when compared to freshwater drum and black crappie. Similarly, the order in decreasing PCB concentrations from the Lake stations during 2001-2002 followed those found in 1996 with striped bass and spotted bass having significantly ($p<0.05$) higher concentrations than the other species.

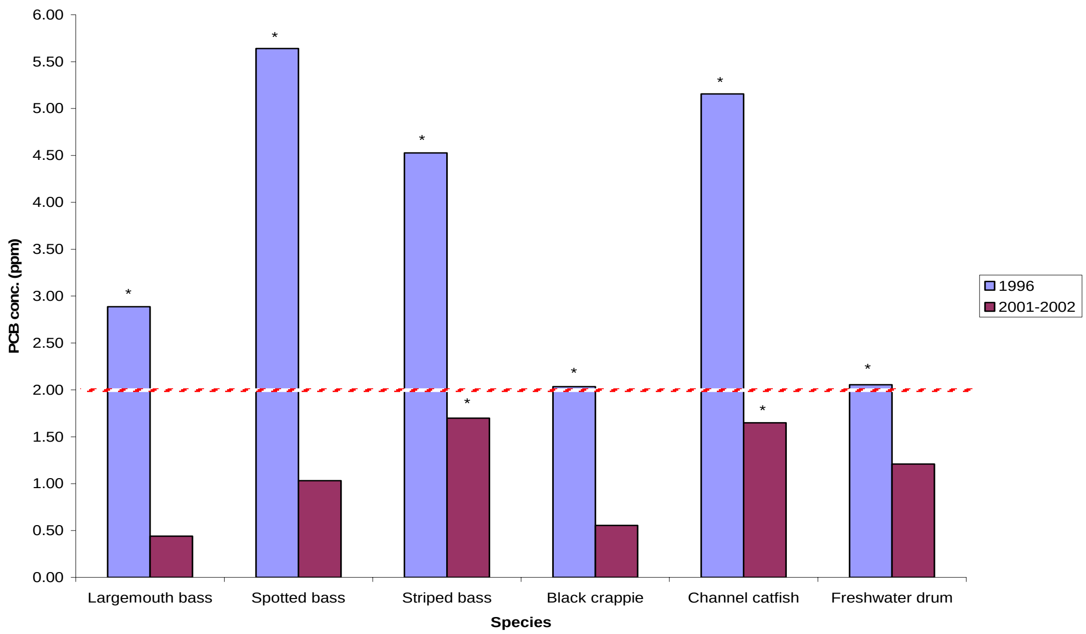

Figure 39. Geometric mean of PCB concentrations (ppm) in six species of fish collected from Choccolocco Creek during the 1996 and 2001-2002 collecting periods from Logan Martin, AL. Reference line is equivalent to the USFDA fish consumption advisory. Asterisk(s) (*) indicates that PCB conc. are not significantly (p<0.05) less than the USFDA advisory.

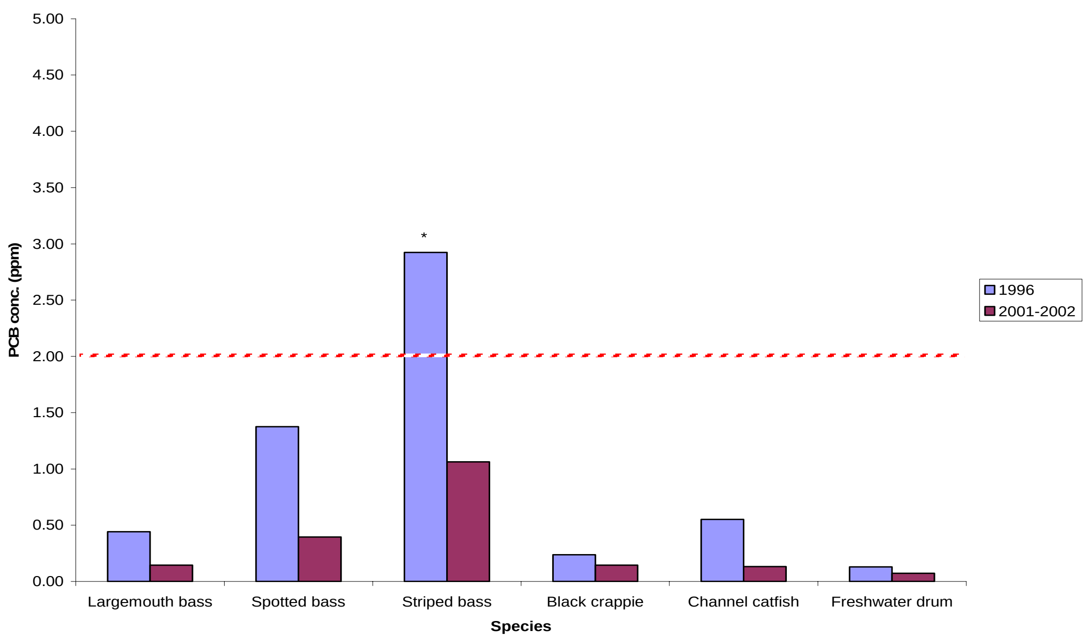

Figure 40. Geometric mean of PCB concentrations (ppm) in six species of fish collected away from the Choccolocco Creek during the 1996 and 2001-2002 collecting periods from Logan Martin, AL. Reference line is equivalent to the USFDA fish consumption advisory. Asterisk(s) (*) indicates that PCB conc. are not significantly (p<0.05) less than the USFDA advisory.

V. Discussion

Fillet lipid content, distance at capture and fish length were found to influence tissue PCB levels. Other variables such as age, gender and relative weight had less affect on PCB levels in Logan Martin Reservoir. An evaluation or screening of these variables allowed for the development of more rigorous regression analyses which concluded that PCB levels decreased in Logan Martin Reservoir and that different species are bioaccumulating PCB at varying rates.

Percent Lipids

Largemouth bass, striped bass, black crappie, and freshwater drum appeared to bioaccumulate PCBs via lipid deposition. This was consistent with previous studies (Brown et al. 1985; Larsson et al. 1991; Harding et al. 1997; Khan 1999). Fish collected in 2001 and 2002 had lower concentrations of both PCBs and lipids compared to those collected in 1996, as well as lower PCBs per unit of lipid. A decline in the concentrations of PCB accumulated in fillet lipids were likely caused by a reduction in prey PCB concentrations or a decrease in prey abundance. Conversely, concentrations of PCBs in channel catfish were higher as percent lipids increased during both collection periods with no significant ($p>0.05$) differences in PCBs between collection periods.

Since PCBs bind to suspended sediment and settle out over time, bottom feeding species such as channel catfish may experience a greater exposure to PCBs than other fish species.

Striped bass, black crappie, channel catfish and freshwater drum collected from Choccolocco Creek had higher concentrations of PCBs for the same percent lipid content compared to fish collected at stations in the reservoir. Environmental exposure to higher PCB concentrations is greater in Choccolocco Creek due to close proximity of the point source and greater concentrations of PCBs in the forage community (ADEM 2001; Bayne et al. 2002; Rypel 2004). In contrast, both black bass species (largemouth and spotted) examined in this study did not follow this trend.

Distance at Capture

PCBs were negatively related to distance at capture from the primary source of PCBs in largemouth bass, spotted bass, black crappie, channel catfish, and freshwater drum. This suggested that these five fish species are relatively immotile as more motile fish would tend to encounter both high and low concentrations of PCBs (Bayne et al. 2002). Striped bass, however, had an insignificant relationship with distance at capture in 1996 and a weak, negative relationship in 2001-2002 with PCB concentrations. This regression supports a similar conclusion arrived at by Bayne et al. (2002), who documented that striped bass from this same reservoir were relatively motile. Large numbers of striped bass are known to move out of the reservoir into Choccolocco Creek during warmer months to seek cooler water refuges. This usually occurs before the lake begins to thermally and chemically stratify. This stratification forced the cooler and welloxygenated water that striped bass prefer to deeper areas of the reservoir, confining them in the hypolimnion (Wilkerson and Fisher 1997; Bayne et al 2002). As hot weather prevails, the hypolimnion shrinks and dissolved oxygen declines. This shrinkage of the hypolimnion leads to stressful conditions and frequently causes fish mortality (Coutant and Carroll 1980).

Length

PCB concentrations were positively associated with length in all six species. Olsson et al. (2000) concluded that other species such as European perch accumulate PCBs according to length. The variable, length, generally was a poor predictor of PCB concentrations because too many other factors such as growth rate, population dynamics, food availability, bioenergetics, habitat, exploitation rates can influence fish length. Fish consumption advisories are frequently based solely on length. These data suggest that limiting consumption of fish in excess of a specified length may not reduce exposure to PCBs by humans.

Fish Age

Five of the six fish species examined did not accumulate PCBs with age. These species include largemouth bass, spotted bass, black crappie, channel catfish and freshwater drum. These findings contradict those of Wszolek (1979), Fabrizio et al. (1991) and Stow (1995). My samples were collected within a restricted length size range for ease of comparison. Therefore, the PCB/age relationship was limited to the size range collected for each species. Smaller, younger and larger, older fish may have altered this relationship. In addition, none of the previously mentioned studies were conducted in southern reservoirs. Another explanation may be related to species longevity. Freshwater drum from Logan Martin Reservoir were found to have greater longevity compared to many freshwater fish species. The oldest drum collected in my study was 21 years. However, drum from other locations in Alabama are known to reach ages of 30+ years (A. L. Rypel, unpublished data).

Unlike other fish species evaluated in this study, PCBs were positively correlated to age in 2001 and 2002 for striped bass. Fabrizio et al. (1991) also reported that PCBs in striped bass (from the Hudson River, NY) increased with fish age.

Relative Weight (Wr)

PCB concentrations were positively related ($p<0.05$) to relative weight in largemouth bass and freshwater drum but no relationships were noted for spotted bass, striped bass, black crappie, or channel catfish. Ironically, this would suggest that PCB concentrations increased as the physiological condition of the fish improved. Correlation analysis concluded relative weight was positively related to lipid content for all species but black crappie. An indication that PCBs stored in body lipids might be the mechanism for significant ($p<0.05$) PCB to relative weight relationships in largemouth bass and freshwater drum.

In addition, largemouth bass and freshwater drum exhibited higher concentrations of PCBs in 1996 compared to 2001-2002 for similar relative weight. Higher concentrations of PCBs in 1996 are probably a direct result of more bioaccumulative PCBs in the environment during 1996. No differences in PCB concentrations for fish with similar relative weight were observed between 1996 and 2001-2002 collection periods in spotted bass, striped bass, black crappie, and channel catfish.

PCB Levels Over Time

Modeling using multiple regression analyses demonstrated that a combination of factors played an important role in the accumulation of PCBs in fish from Logan Martin Reservoir. Two separate models were developed to evaluate PCB (untransformed) accumulation during relatively high (1996) and low (2001-2002) exposure. The amount of variability explained by the models was higher in 1996 for largemouth bass, spotted bass, striped bass, black crappie, and channel catfish compared to 2001-2002. The best PCB (untransformed) model for freshwater drum was computed under low PCB concentrations.

With few exceptions, lipids and distance at capture were generally the dominant predictors of PCBs (untransformed) in both the high concentration (1996) and low concentration (2001-2002) models. Fish length and relative weight (Wr) also influenced the prediction of PCBs in several species. Factors such as age and gender were never strong predictors of PCBs in either model. PCBs are highly variable in the environment; therefore, constructing accurate models to predict the uptake of PCBs by fish is extremely difficult.

Information from the screening of variables which were found to improve the predictability of the untransformed PCB levels were used to develop final models using the log transformed levels to determine if significant reductions in PCB concentrations had occurred in Logan Martin Reservoir over time. Regression analyses predicting the natural log transformation of PCB concentrations in fish fillets for all species found that PCB levels had significantly decreased since 1996 with lipid content acting as an important variable positively affecting PCBs. However, distance at capture was also an important factor negatively dictating the uptake of PCBs in all species except striped bass. This was likely a result of the motility of this species. Length also seemed to play a less dominant role controlling the uptake of PCBs.

PCB Bioaccumulation Between Species

When the data were separated by the two sampling areas and two collecting periods the one-way ANOVA found that significant differences in natural log transformation of PCB levels existed between species. Based on Dunnett's C multiple range analyses found that PCB levels in striped bass and spotted bass were significantly higher than levels in most of the other species indicating that certain species in Logan Martin Reservoir do bioaccumulate PCBs at different rates.

Management Implications

Future monitoring for trends in PCBs over time should control for distance from the former point source (Snow Creek), lipid content of the fish, and fish length. Monitoring for PCB trends should be conducted on Choccolocco Creek and the rest of the reservoir independently. PCB monitoring on Choccolocco Creek and Logan Martin Reservoir should target striped bass and spotted bass because these species seemed to have elevated PCB concentrations.

Based on 2001-2002 data, fillet PCB concentrations in all species from the lake stations were well below the United States Food and Drug Administration 2.0 ppm guideline for consumption. PCBs in black crappie and largemouth bass were also below the 2.0 ppm guideline in Choccolocco Creek. However, striped bass, spotted bass, channel catfish, and freshwater drum from Choccolocco Creek were not below the guideline.

Summary

Multiple regression analyses were employed to identify those variables having the most influence on untransformed PCB concentrations in fillets of six species. Modeling was evaluated under two contrasting conditions: relatively high PCB concentrations (1996) and low PCB concentrations (2001-2002). In these models, percent fillet lipids and distance at capture appeared to be the dominant predictors of PCBs in all six species. Total length, gender, and relative weight also explained some additional variability in a few species. Fish age was not related to PCB concentrations from Logan Martin Reservoir fish in these multiple regression models.

Multiple regression modeling for PCB trends using the natural log transformation of PCB concentrations, revealed a decline over time for all six species. All species were positively related to lipid content and all species except striped bass were negatively related to distance at capture. However, the effect of each bioaccumulative factor examined was species specific and highly variable. ANOVA tests along with Dunnett's C tests confirmed that PCB concentrations in striped bass and spotted bass were significantly greater than the other species evaluated. Such species specific regression models should be considered in monitoring plans for PCBs that are associated with fish consumption advisories. Future monitoring should not only evaluate the trends of environmental PCBs alone, but include these species specific bioaccumulative factors that appear to be controlling the uptake of PCBs in each species.

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