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Bioaccumulation of Polychlorinated Biphenyls (PCBs) in Atlantic Sea Bream (*Archosargus rhomboidalis*) from Kingston Harbour, Jamaica.

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Abstract.

Multiple sizes of Sea bream were collected from Kingston Harbour, Jamaica, to assess steady state bioaccumulation of polychlorinated biphenyls (PCBs) in a tropical fish. Sea beam fork lengths ranged from 7.3-21.5 cm (n=36 fish) and tissue lipids decreased with body length. Larger fish had lower $\delta^{13}C$ isotopes compared to smaller fish, suggesting a change in diet. Linear regressions showed no differences in lipid equivalent sum PCB concentrations with size. However, differences in individual congener bioaccumulation trajectories occurred. Less hydrophobic PCBs decreased with increasing body length, intermediate PCBs showed no trend, whereas highly hydrophobic (above log K_{OW} of 6.5) PCBs increased. The different congener patterns were interpreted to be a result of decreases in overall diet PCB concentrations with increased fish length coupled with differences in PCB toxicokinetics as a function of hydrophobicity yielding dilution, pseudo-steady state and non-steady state bioaccumulation patterns.

Keywords. Kingston Harbour, stable isotopes, biomagnification, toxicokinetics, POPs

INTRODUCTION

Bioaccumulation and food web biomagnification of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) is well established (Oliver and Niimi, 1988; Connolly and Pedersen, 1988; Rassmussen et al., 1990). Hydrophobic POPs compounds distribute primarily to lipids, and to a lesser extent hydrophobic non-lipid organic matter (Debruyn and Gobas, 2007), while their elimination is inversely related to chemical hydrophobicity and animal lipid pool size (Bruggeman et al., 1984; Hawker and Connell, 1985). Despite strong mechanistic understanding of toxicokinetics processes (Gobas et al., 1988) and prevalence of POPs bioaccumulation models (Arnot and Gobas, 2004), most aquatic food web models operate under the assumption that steady state is achieved between fish and their environment and diet. Yet, the majority of field studies examining POPs bioaccumulation in the field have emphasized sampling entire food webs (Borgå et al., 2012) and there are few studies that sampled different size and age classes of the same population of fish necessary to test the steady state assumption (Olsson et al., 2000; Paterson et al., 2006 a,b; Burtnyk et al., 2009; Paterson et al., 2016).

Steady-state is defined as the condition of constant chemical concentrations with time which is achieved when uptake and elimination kinetics become balanced under constant water and dietary POPs concentrations, constant growth and lack of change in tissue composition such as % lipids. Paterson et al. (2007) demonstrated that time to steady state in temperate fish is also dependent on seasonal temperature cycles; such that for yellow perch *(Perca flavascens*), steady state

requirements exceed the life of the species. Tropical fish, however, do not experience large changes in water temperature and their metabolic rates remain closer to their thermal optima. A lack of winter metabolic minimum means that chemical toxicokinetics remain more consistent, and annually elevated, compared to what is experienced by temperate fish. Thus, tropical fish are more likely to achieve steady state. Most studies testing steady state POPs bioaccumulation focused on temperate fish. This study examines steady state in the tropical Atlantic Sea Bream (*Archosargus rhomboidalis*) to determine if steady state dynamics predominant in a tropical fish.

Materials and Methods.

Atlantic sea bream were collected from the Kingston Harbour, Jamaica in 2010-2012 between June to September (Figure 1). The harbour has a total surface area of 51km^2 and extends 16.5 km east-west and 6.5km north-south. It receives industrial and residential waste via a number of gullies, rivers and outlets. These gullies and other outlets are a major source of solid waste, heavy metals, chemical contaminants and sewage (Goodbody, 2003). Temperature and salinity ranges from 26.66-27.11^{0}C and 33.51-34.96 ppt, respectively (Buddo, 2012).

Fig. 1 Study area in the Kingston Harbour, Jamaica. Rectangle indicates boundary of the sampling area.

Samples were collected during the day using trawl nets. The nets were dragged for 30 minutes. All "Brim" species were retained while bycatch were released. Sea bream were stored in a cooler over ice for transport to the laboratory. Species identification was later verified using the Fish Base online database and the Jamaica National Marine Fisheries Atlas. At the laboratory, each fish was given a unique identification number and fork length (cm) and weight (g) was recorded. Ten grams of dorsal muscle was removed stored frozen for stable isotope analyses. The remaining carcass was homogenized in stainless steel blenders and about 20 g was stored frozen until PCB analysis.

Chemical analyses was carried out according to modifications of Burtnyk et al. (2009). Approximately 1g of homogenate was ground with 10g of sodium sulfate and spiked with PCB 34 as a surrogate standard. The mixture was cold column extracted for 1 hr with 50:50 hexane: dichloromethane followed by evaporation to 10 mL. Neutral lipid was determined gravimetrically by removing 1 mL of sample extract and drying for 1 hour at 110 ^{0}C. The remaining extract was concentrated to 2 mL. Sample clean-up was done using florisil columns. After adding the sample to the column, it was eluted with 50 ml of hexane followed by 50 ml of 15% dichloromethane: hexane. The cleaned up sample was concentrated and analyzed using an Agilent 6890, Series Plus

Gas Chromatograph equipped with Agilent-7683 Series autosampler and a 63 Ni-μECD. The method analyzed for 34 PCB congeners using a certified PCB standard (Quebec PCB Congener Mix, Accustandard, New Haven, CT, USA). Samples were extracted in batches of 6, with each batch containing a blank and a reference tissue (homogenized goat liver fortified with Aroclor 1254). Detection limits for individual PCBs averaged 0.056±0.004 ng/g wet weight and ranged from 0.02 – 0.12 ng/g wet wt. Mean recovery of the surrogate standard was 94%.

PCB congeners detected at a frequency of less than 30% (IUPAC #'s 17/18, 33, 74, 70/76, 87, 105/132, 158, 156/171, 191, 195/208, 205, 204 and 209) were excluded from data analyses. For the remaining congeners, non-detected values were substituted with a value equal to 1/3 the congener detection limit. Sum PCBs refers to the sum of frequently detected PCBs (IUPAC # 31/28, 49, 52, 44, 95, 101, 99, 110, 151/82, 149, 118, 153, 138, 187, 183, 128, 177, 180, 170/190, 199 and 194) with non-detected values substituted with 1/3 the detection limit. PCB concentrations were expressed in units of either ng/g wet weight or ng/g lipid equivalent weight. Lipid equivalent PCB concentrations are calculated according to:

$$C_{leq} = \frac{C_{ww}}{(x_{lipid} + 0.05 \cdot x_{ldw})} \quad (1)$$

Where C_{leq} and C_{ww} are the lipid equivalent and wet weight concentration (ng/g), X_{lipid} and X_{ldw} is the fraction by weight of lipids and lean dry weight and 0.05 is a constant relating the partition capacity of lean dry weight relative to lipids (DeBruyn and Gobas, 2007).

Skin-on dorsal muscle (1 g) was dried at 60 ^{0}C for 48 hr and ground to a powder for stable isotopes. Between 400 to 600 μg of powder was lipid extracted by chloroform/methanol and added to a 3 mm x 5.5 mm tin capsule. The samples were analyzed on a Costech Elemental Combustion System coupled to a Delta V Advantage Isotope Ratio Mass Spectrometer. The stable isotope results (‰) were calculated relative to a reference standard as per equation 2:

$$\partial^{15}N \ (or \ \partial^{13}C) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000 \quad (2)$$

Where R is the ratio of heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample relative to the reference. The reference standards were atmospheric nitrogen for nitrogen and Pee Dee Belemnite carbonate for carbon. Ten percent of samples and standards were run in duplicate to assess precision.

Data were analyzed using Statistica 64 statistical software package. Normality and homogeneity of variance was tested by normal probability plot and Levene's test. Linear regressions and analysis of variance (ANOVA) were used to compare lipid, stable isotopes or PCBs as a function of fork length or between fish grouped into size categories. Post-Hoc tests (Tukey's HSD) were used to compare individual size classes. A probability of $p < 0.05$ was considered statistically significant. Measures of central tendency are reported as means ± standard error (SE).

Results

Thirty six sea bream were collected with a body weight range of 8.0-240.5g and fork lengths of 7.3-21.5cm. Although otoliths were collected, the age could not be ascertained with confidence as is common for tropical fish. As such, the samples were also classified into four size categories as a proxy for age classes and to enable categorical detection of non-linear patterns.

The mean±SE (range) lipid content of fish was 2.4 ± 0.3% (0.3 to 6.3%). Lipids demonstrated a significant ($F_{1,34}$=5.2; $p<0.01$; Regression $R^2 = 0.29$) decreasing trend with body length. Post hoc comparisons across size categories indicated lipid differences were significant between size classes 1 and 4 ($p<0.01$; Tukey's HSD; Table 1). For stable isotopes, $\delta^{13}C$ significantly declined with body size ($F_{1, 34} = 4.78$; $p<0.05$; Regression $R^2 = 0.10$) whereas $\delta^{15}N$ was not significantly ($F_{1,34}$=0.38; $p>0.5$) related to length. Post hoc comparisons indicated fish had significantly ($p<0.01$; Tukey's HSD; Table 1) lower carbon isotopes for size classes 3 and 4 relative size classes 1 and 2.

Mean±SE sum PCBs in fish were 4.48±0.51 ng/g wet weight and ranged from 0.5 – 12.8 ng/g wet weight. Wet weight sum PCBs exhibited a significant ($F_{1,34} = 5.0$; $p<0.05$; Regression $R^2 = 0.13$) declining trend with fork length. Post hoc comparisons of wet weight sum PCBs by size category were similar to %lipids, with differences evident between size classes 1 and 4 (Table 1). When sum PCBs were converted to lipid equivalent concentrations, there was no longer a significant relationship with body length ($F_{1,34}$=1.15; $p>0.3$; Regression $R^2 <0.01$).

Table. 1 Summary of fork length, lipids, stable isotopes and sum PCBs in sea bream.

Size Class	Size (cm)	N	Lipid (%)	δ13C (‰)	δ15N (‰)	Sum PCBs wet wt. (ng/g)	Sum PCBs lipid equivalent (ng/g)
1	8.3 ± 0.2	9	3.6±0.2[a]	-14.5±0.1[a]	13.4±0.2[a]	6.5±1.0[a]	129±15[a]
2	11.6 ± 0.5	14	2.4±0.5[a,b]	-14.7±0.3[a]	14.3±0.2[a]	4.2±0.7[a,b]	120±18[a]
3	17.4 ± 0.4	5	2.1±0.7[a,b]	-16.5±0.4[b]	13.6±0.3[a]	5.6±1.5[a,b]	186±24[a]
4	20.4 ± 0.3	8	0.9±0.1[b]	-17.5±0.5[b]	12.9±0.5[a]	1.4±0.4[b]	104±17[a]

Data reported as means ± standard error. Superscripts are significantly different from one another ($p<0.05$; Tukey's HSD).

Linear regressions were then performed on lipid equivalent PCBs as a function of body length for individual congeners. Eleven of the 21 congeners exhibited no significant (regression slopes =0; $p>0.05$; ANOVA) relationship with body length. Ten of the congeners (PCBs 52, 95, 101, 153, 138, 187, 177, 180, 199 and 194) had concentrations that were significantly (regression slopes ≠ 0; $p<0.05$; ANOVA) related to fork length. However, the direction of the slope varied between congeners. PCBs 52 and 95 had significantly (regression slopes <0; $p<0.01$; ANOVA) negative relationships with length whereas PCBs 101, 153, 138, 187, 177, 180, 199 and 194 had significantly (regression slopes >0; p values ranging from <0.001 – $p<0.05$; ANOVA) positive relationships with length. Figure 2 presents bioaccumulation plots for selected congeners (PCBs 52, 110, and 187) across size categories. PCB 52 is representative of a dilution profile with size, PCB 110 reflects apparent steady state (no change) while PCB 187 demonstrates non-steady state net bioaccumulation. Figure 3 provides a plot of the lengthwise bioaccumulation slopes (ng/g lipid equivalent/cm fish) for individual congeners as a function of log K_{OW}. Based on Figure 3, there is a transition in the bioaccumulation slope from negative for low K_{OW} congeners (log K_{OW} < 6.25) to neutral followed by positive slopes for chemicals with log K_{OW} values exceeding 6.75.

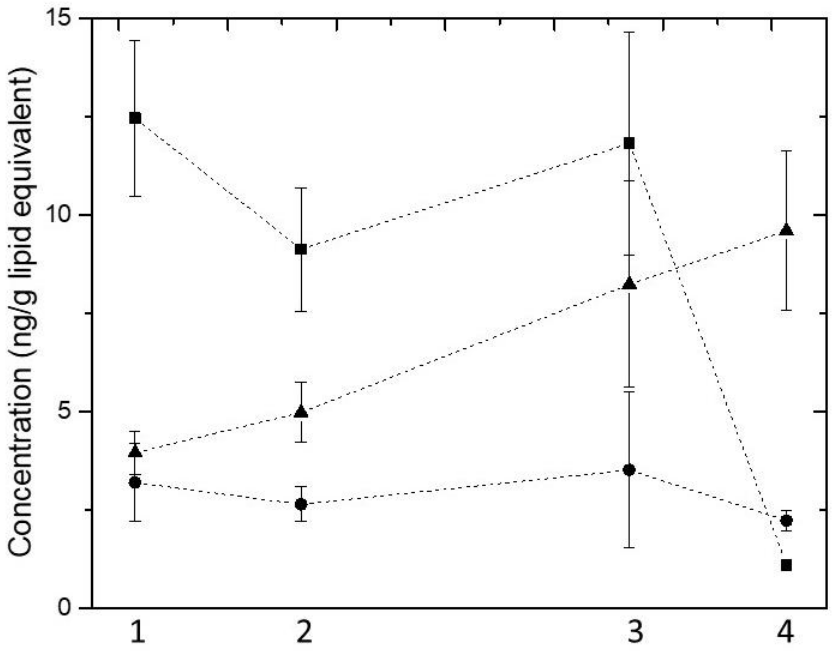

Fig 2. Mean ± SE concentration (ng/g lipid equivalent) of PCBs in fish across size classes for selected congeners, PCBs 52 (■), 110 (●) and 187 (▲). Size classes defined in Table 1.

Discussion

Data on length related changes in tissue lipid and stable isotopes (carbon) provide supporting evidence to indicate altered body condition and feeding ecology of fish over the size range of animals collected. The larger sizes classes of sea bream had lower tissue lipids and lower $\delta^{13}C$ compared to fish from size classes 1 and 2. The change in $\delta^{13}C$ suggests larger fish were more dependent on a pelagic diet while the smaller size classes incorporated either nearshore or benthic signals (Paterson et al., 2007b). However, such changes were not associated with a shift in trophic position given the low variation in $\delta^{15}N$. Stomach contents of fish (data not shown) indicated that mollusks (benthic invertebrates) were more frequently consumed by fish > 20 cm which supports a diet shift but not in the expected direction based on changes in $\delta^{13}C$. Other studies report a diet shift by sea bream from zooplankton to more omnivorous diets that includes algae and vascular plants as fish age (Randall et al., 2004). Indeed, the largest size class of fish from the present study did have the lowest $\delta^{15}N$, although non-significantly so. Vascular plants and algae are expected to have both lower energy density and lower PCBs compared to zooplankton, benthic invertebrates and fish. The low energy density of later aged diets is consistent with the observed decrease in tissue lipids of larger fish. Although diets were not collected separately for measurement of PCB concentrations or isotopes, the patterns in stable isotopes and tissue lipids imply a diet change occurred and the change is likely to have affected the average dietary exposure to PCBs by fish.

With respect to steady state, there was no change in lipid equivalent sum PCBs across fish length which is consistent with a steady state interpretation. However, analysis of individual congener

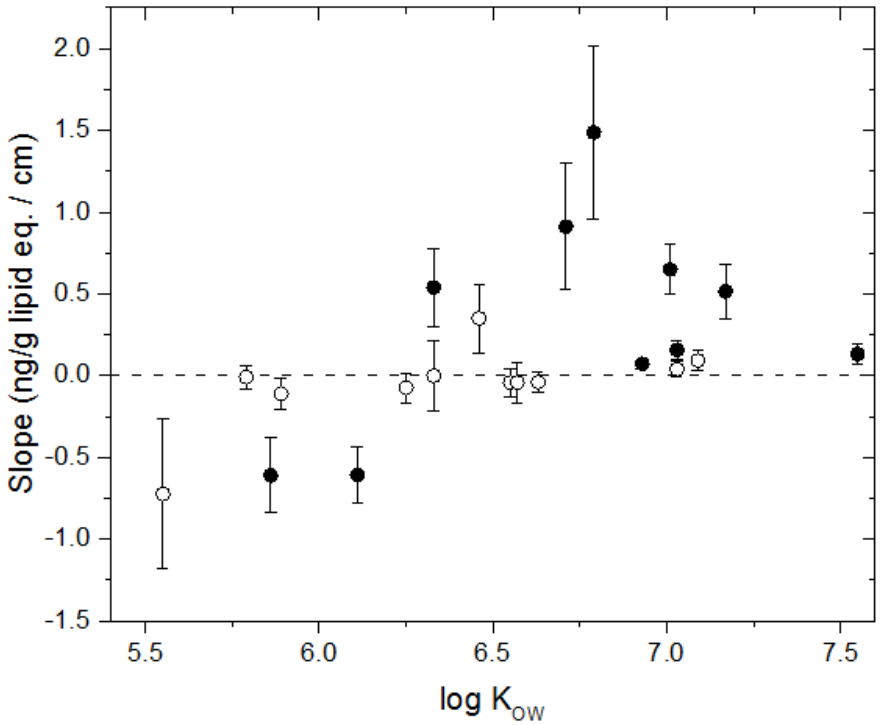

Fig 3. Bioaccumulation slopes (ng/g lipid equivalent/cm fish) of PCBs in sea bream. Solid symbols have slopes significantly ($p<0.05$; ANOVA) different than zero. Open symbols indicate congeners where the slope was not significantly different than zero. Dashed line provides the zero slope reference. Log K_{OW} values from Hansen et al. (1999).

behavior showed differences between contaminants that conformed to a hydrophobicity pattern. Less hydrophobic PCBs exhibited declining trends in concentrations with fish size suggestive of a non-steady state dilution profile. Contaminants of intermediate hydrophobicity presented the predicted steady state profile while the most hydrophobic congeners increased in lipid equivalent concentrations with size indicative of non-steady state net positive bioaccumulation.

These mixed bioaccumulation patterns can be explained as a result of both shifts in diet concentration to lower PCB contaminated diets coupled with a transition from steady state to non-steady state bioaccumulation as a function of chemical hydrophobicity. Thus, if prey PCB concentrations decreased for the larger fish, such changes would be readily tracked by the least hydrophobic chemicals which are most rapidly eliminated from fish (Paterson et al., 2006a, 2007). This non-steady state dilution trend actually represents steady state (or an approach to steady state) between the fish and its diet which are interpreted to have declined with time. PCBs of intermediate hydrophobicity exhibit constant concentrations with fish size and are expected to exhibit intermediate elimination rates from fish. For these congeners, the decline in diet concentrations are only partially compensated by elimination yielding a pseudo-steady state (i.e. apparent non-changing) bioaccumulation pattern even though fish may have become out of steady state with respect to their most current diet for the oldest individuals. For the most hydrophobic congeners, elimination is very slow to negligible and fish have not achieved their full bioaccumulation potential with respect to their early age diet nor with the new diet of older individuals following the diet shift. These congeners continue to accumulate with fish size/age even though prey contamination may have decreased.

The above interpretation assumes that the inferred decrease in PCB concentrations in diet (supported by the lower K_{OW} dilution profiles) occurs similarly for all congeners. It is further assumed that the decrease in diet concentration did not drop to a value of zero PCB content. Finally, it is assumed that fish length provides a valid measure of fish age, although it is recognized that differences in growth between individuals could confound age categories presumed on the basis of fish size categories. In the latter case, biodilution, resulting from an increase in growth rate for larger fish size classes, can be ruled out as a mechanistic interpretation of the overall bioaccumulation pattern because changes in growth rate would influence all PCBs to the same degree and is not compatible with the different bioaccumulation trajectories observed between congeners.

Most food web PCB bioaccumulation models assume steady state kinetics operate (Arnot and Gobas, 2004). This has been challenged, particularly for highly hydrophobic PCBs, in several populations of temperate fish (Olsson et al., 2000; Paterson et al., 2007; Burtnyk et al., 2009; Paterson et al., 2016). It was initially hypothesized that tropical fish are more likely to achieve steady state compared to temperate fish owing to a lack of seasonal temperature cycles experienced by tropical fish which in turn moderates their chemical toxicokinetics. Indeed, steady state (or pseudo-steady state) was observed over a larger range of chemical hydrophobicity's in the present study than reported for temperate fish (Burtnyk et al., 2009). However, the above observations were partly confounded by a diet shift which was interpreted to result in decreased prey contamination for the largest size classes. Despite this, the present research suggests that non-steady state, net bioaccumulation conditions operate for the most hydrophobic PCB congeners in tropical fish as has been described for temperate fish.

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