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Protein and lipid growth rates regulate bioaccumulation of PCBs and Hg in Bighead Carp (*Hypophthalmichthys nobilis*) and Silver Carp (*Hypophthalmichthys molitrix*) from the Three Gorges Reservoir, China☆

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ABSTRACT

This study evaluated the effect of growth of different tissue compartments on the bioaccumulation of mercury (Hg) and polychlorinated biphenyls (PCBs) in Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*Hypophthalmichthys nobilis*) from the Three Gorges Reservoir (TGR), China. A non-steady state bioenergetics/toxicokinetic model was developed to simulate PCB and Hg concentrations in these two species and compared with field data. Simulations using constant whole body growth rate and constant tissue to whole body weight ratios were contrasted against simulations adopting age specific whole body and tissue/age specific growth rates for their goodness of fit to field data. The simulations using age/tissue specific growth rates demonstrated better fit to field data for PCBs compared to the constant growth rate models (22% improved R^2), while both models explained similar variation in Hg concentration data. Both species demonstrated higher growth rates of lipids (on a daily basis) relative to whole body and protein contributing to higher growth dilution of PCBs compared to Hg. Although stable isotope data indicated some degree of diet and/or habitat shift, simulations assuming a constant diet concentration explained between 36 and 40% of the variation in fish concentrations for both contaminants and fish species. This study demonstrates that differences in the bioaccumulation rate of PCBs and Hg by Asian carp can be partially explained by differences in the growth rates of key tissue storage compartments associated with each contaminant. These differences in chemical-specific growth dilution subsequently contribute to differences in chemical retention and bioaccumulation patterns of Hg and PCBs by fish.

1. Introduction

Mercury (Hg) and polychlorinated biphenyl (PCB) contamination of aquatic environments are a global problem (Borgmann and Whittle, 1992; Paterson et al., 2016). Both contaminants are subject to food web biomagnification (Kucklick and Baker, 1998; Morel et al., 1998) and contribute to the majority of fish consumption restrictions issued by government agencies throughout North America and Asia (Ser and Waranabe, 2012). Despite these broad commonalities, Hg and PCB bioaccumulation is rarely co-investigated apart from cursory evaluation of field concentration data used in hazard assessments (McIntyre and Beauchamp, 2007).

Part of the reason for the lack of joint examination of Hg and PCB bioaccumulation is related to differences in analytical requirements used to measure the two contaminants, which historically have divided researchers into those with specialization on persistent

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organic pollutants (POPs) and those with specialization on metal/metalloid contaminants. Another factor has to do with differences in the physiological mechanisms of chemical uptake (Mason et al., 1995; Gobas et al., 1999), tissue distribution (Pickhardt et al., 2006; Guo et al., 2008) and elimination (Van Walleghem et al., 2007; Paterson et al., 2010) between the contaminants making the development of joint physiologically-based bioaccumulation models daunting. Finally, an examination of the literature in each of these fields shows distinct differences in the emphasis of use of process-based modelling versus statistical/empirical approaches to bioaccumulation assessment. For POPs, process-based bioaccumulation models have a long history of application to fish bioaccumulation problems (Mackay and Paterson, 1981; McLeod et al., 2016), while for Hg much more of the aquatic bioaccumulation literature has focused on statistical models to describe patterns of bioaccumulation in the field, e.g. evaluation of trophic magnification factors (Borga et al., 2012) or regression based models used to predict site specific Hg concentrations in fish populations (Qian et al., 2001). Owing to these differences in approaches, it is often difficult to directly compare or distinguish major factors responsible for different chemical behaviors (e.g. bioaccumulation slopes) experienced by fish populations that are simultaneously exposed to both contaminants in their environment.

Despite the aforementioned differences in physiological mechanisms of Hg and PCB bioaccumulation, there are many common features in the toxicokinetics of these contaminants that can be used to develop a common kinetic based bioaccumulation model. For example, chemical uptake by food dominates the overall chemical exposure to fish for organic forms of mercury (methylmercury; MeHg) and hydrophobic PCBs (Hall et al., 1997; Fisk et al., 1998). Furthermore, although different in magnitude (Li et al., 2015), the assimilation efficiencies of Hg and PCBs appear to be highly consistent and have limited dependence on diet composition (Wang and Wong, 2003; Buckman et al., 2004; Pickhardt et al., 2006; Liu et al., 2010). Whole body elimination of Hg and PCBs by fish has been demonstrated to be a pseudo-first order kinetic process (Paterson et al., 2010; Li et al., 2015) and elimination by both contaminants is strongly affected by water temperature and body size, both factors that regulate fish metabolic rate (Trudel and Rasmussen, 1997; Drouillard et al., 2009). Finally growth dilution has been shown to be important for both contaminants (Eichinger et al., 2010; Wang and Wang, 2012).

One notable distinction related to growth dilution effects for the two contaminants is that growth dilution is specific to growth of the major storage compartment for a given chemical. Given that Hg and PCBs have distinctly different tissue associations, the two contaminants differ in what constitutes the major storage compartment. PCBs are associated with neutral lipids and to a lesser extent with non-lipid hydrophobic organic matter in animal tissues (Mackay and Patersen, 1981; deBruynand Gobas, 2007). In contrast, Hg has strong associations with sulfur-rich amino acids and distributes primarily to muscle and other protein rich tissues (Stohs and Bagchi, 1995) while it is largely absent in fat (Rodrigues et al., 2014). Yet, many bioaccumulation studies reference growth dilution to whole body growth rather than growth of tissue storage compartments (Sijm et al., 1992; Wang and Wang, 2012). This assumes that the relevant storage components exhibit a constant and proportional rate of growth relative to whole body weight of fish, something which has already been refuted by previous studies (Dumas et al., 2007; Overturf et al., 2016). Another complication is that growth is interactive with food consumption rates given that a high growth rate necessitates elevated food ingestion, which in turn provides a feedback to chemical uptake flux. Therefore, interpreting the effect of fish growth on contaminant bioaccumulation should consider differences in food intake between fast and slow growing populations. The latter is best achieved by integrating bioenergetic models with empirically derived, or population specific, growth sub-models coupled to toxicokinetic based bioaccumulation models.

The objective of this study was to determine if differences in tissue specific growth can explain differences in the bioaccumulation behavior of Hg and PCBs in two species of Asian carp from the Three Gorges Reservoir, China. In order to address the interdependence of fish feeding rate and growth, a non-steady state, species-optimized bioenergetic model and an empirically derived growth sub-model were coupled with a toxicokinetic bioaccumulation model to predict PCB and Hg bioaccumulation in each fish species. Simulations were performed under two growth scenarios and contrasted for their goodness of fit to field data. Growth scenario 1 assumed whole body growth rate was constant each year across the lifespan of fish and assumed constant proportions of lipid and protein with age. Scenario 2 applied age specific whole body, lipid, and protein growth rates, allowing tissue proportions and whole body growth to vary for each year class as observed in the field populations. Both simulations assumed that diet concentrations of Hg and PCBs remained constant over the lifetime of the fish. The two study species used in the model evaluation included Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*Hypophthalmichthys nobilis*). Both of these species differ with respect to their feeding ecology, Silver Carp feed on phytoplankton and Bighead Carp feed on zooplankton (Jayasinghe et al., 2015), as well as show differences in their overall growth rates in their natural populations (Ke et al., 2008). At the same time, because both species remain planktivorous over their lifespan, changes to the diet concentrations of PCBs and Hg through time were expected to be minimized removing diet variation as a confounding factor to the evaluation of growth.

2. Methods

2.1. Study system

In September 2013, 26 Silver Carp and 17 Bighead Carp were collected from Daning River, Wushan, China (31.1 °N, 109.9 °E) by local fisherman. The Daning River is a large tributary of the Yangtze River, with an annual average temperature of 18 °C. Silver Carp and Bighead Carp are fast growing species and stocked in the TGR as a fisheries resource (He et al., 2015). Total length and body weight were measured for each fish, and 5–10 scales above the lateral line were removed for age determination. Then, fish were individually homogenized using a stainless steel meat grinder within 24 h of their collection. Samples were kept frozen until subsequent laboratory analysis. Total Hg (THg), MeHg, PCBs (17/18, 28/31, 33, 44, 49, 52, 70, 74, 87, 95, 99, 101, 110, 128, 151/82, 149, 118, 153, 105/132, 138, 158, 156/171, 170, 177, 180, 183, 187, 191, 194, 199, 195/208, 205, 206, and 209), stable isotopes ($\delta^{15}N$ and $\delta^{13}C$), moisture, and lipid content were measured for each sample.

2.2. Laboratory analysis

Analysis for MeHg and THg was conducted at Southwest University, Chongqing, China. MeHg was measured by gas chromatography-cold vapor atomic fluorescence spectrometry (GC-CVAFS) as described in Yan et al. (2005). MeHg concentration in the digested sample was determined by aqueous phase ethylation, Tenax trap collection, GC separation, and CVAFS (Brooks Rand Laboratories, Seattle, WA, USA). THg was measured using acid digestion combined with CVAFS as described by Yan et al. (2005). The THg concentration was determined by Tekran 2500 (Tekran®