Experimental study of mercury accumulation in juvenile cyprinid fish *Barbonymus schwanenfeldii* and *Tor tambroides*, exposed through water and feed

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Abstract. This paper reports the experimental study of mercury accumulation in juvenile cyprinid fish of *Barbonymus schwanenfeldii and Tor tambroides*. The juvenile cyprinids were exposed to mercury (Hg) through water (0.5 mg/L and 1.0 mg/L) and feed (0.5 mg/kg and 1.0 mg/kg) for 30 days. The behavioral response during feeding was monitored over the experimental period. The Hg concentration in the juveniles was analyzed after 30 days. The juveniles exposed to water contaminated with 1.0 mg/L Hg was found to disperse upon feeding suggesting an increase in vulnerability towards threats. This however was not observed in other treatments through feed and at 0.5 mg/L Hg. In terms of survival percentage, no statistical difference was deduced between treatments for both species. Among the two species, *T. tambroides* demonstrated greater affinity of accumulation; this is likely due to its biological behavior as a fast swimming species. The uptake of Hg (II) through gills was more pronounce than that through ingestion as the diffusion of Hg(II) was inhibited by the mucosal lining in the latter pathway. No significant correlation was established between size and Hg concentration in juveniles of both species.
Key Words: mercury (II) chloride, behavioral response, survival percentage, bioconcentration factor.

Introduction. Mercury (Hg) is a naturally occurring element. It is found in elemental, inorganic and organic states, of which organic methylmercury (CH_3Hg) is the most toxic and bioavailable species. In the environment, Hg is usually found in the form of elemental and inorganic species. They can be transformed into CH_3Hg that is lipid soluble in the presence of microorganisms and is readily taken up by aquatic organisms. The accumulated Hg would biomagnify as the trophic level increases and eventually transfer to humans *via* food chains.

The accumulation of mercury in fish has been extensively studied allowing inference of the uptake behavior and pattern in relation to numerous factors including species, size, age, sexes, feeding habits, trophic levels and anthropogenic influences (Storelli et al 2007; Kasper et al 2009; Li et al 2009; Drenner et al 2010). Essentially, Hg is taken up through gills and diet with the latter contributing more profoundly. It is learnt that Hg concentration increases with the trophic level, with carnivorous species demonstrating greater accumulation than omnivorous and herbivorous. The data available are largely based on temperate and subtropical species with fewer reported on tropical species.

The cyprinids constitute the most diverse freshwater fish in Borneo. They are found in many river systems of Sarawak (Northwest of Borneo) serving as an important protein source for the indigenous communities (Kottelat et al 1993; Abdullah 2004; Nyanti & Grinang 2007). *Barbonymus schwanenfeldii* and *Tor tambroides* are two major cyprinid species. The State Government has initiated the effort to culture these species reducing the dependence on wild sources (Kottelat et al 1993; Silang & Chai 2004; De Silva et al 2004; Ingram et al 2005; Nguyen et al 2006). *B. schwanenfeldii* is locally called *Tengadak* whilst *T. tambroides* is popularly known as *Empurau* – a highly priced fish with significant economic importance to Sarawak. Under the pressure of escalated development, fish in this region are exposed to the risk of Hg however the accumulation behavior with specific reference to these tropical species is unavailable. Hence in this study, we attempt to assess the uptake and accumulation of Hg in *B. schwanenfeldii* and *T. tambroides*. The juveniles of both species were exposed to Hg through water and feed under the laboratory setting. The findings of this study will provide insights into the tendency of Hg accumulation in these species through different exposure pathways. This information offers fundamental knowledge to support environmental management and health risk assessment pertaining Hg contamination.

Material and Method

Experimental design. The juveniles of *B. schwanenfeldii* and *T. tambroides* reared in plastic tanks were exposed to Hg through water and feed. A total of 150 juveniles of each species were used where 60 were kept under water containing Hg at two-levels (0.5 mg/L and 1.0 mg/L), 60 were fed with Hg tainted feed at 0.5 mg/kg and 1.0 mg/kg whilst the remaining serves as the control. Note that the juveniles of *B. schwanenfeldii* and *T. tambroides* were provided by Tarat Inland Fisheries with average size of 0.59±0.27 g and 0.16±0.06 g, respectively.

Plastic tanks of 40 cm (length) × 18 cm (width) × 28 cm (height) was divided into three compartments using plastic mesh of 1 mm and equipped with filtration system as shown in Figure 1. The tank was filled with 15 L of water and ten juveniles were placed in each compartment. The water was maintained at dissolved oxygen of 7.0 mg/L, pH 7 and temperature of 27°C. The tank was covered with transparent plastic sheets. The loss of Hg was assessed prior to the exposure study. The tank, without juveniles, was spiked with 0.5 mg/L Hg and the Hg concentration was measured every three days for a period of 30 days.

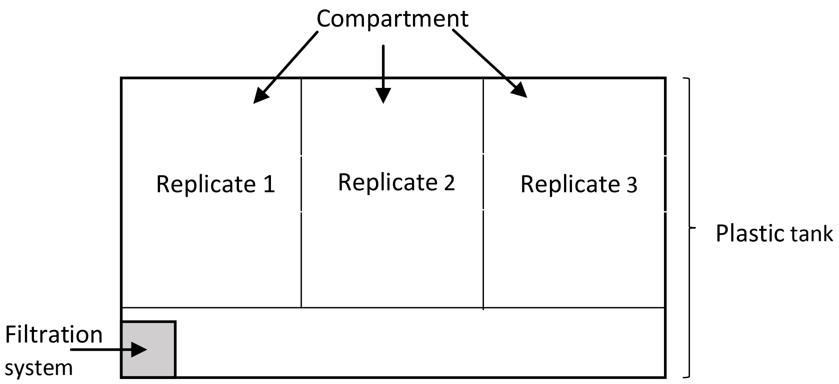

Figure 1. Design of the experimental tank.

Exposure study. The juveniles of *B. schwanenfeldii* and *T. tambroides* were exposed to water containing 0.5 mg/L and 1.0 mg/L Hg. A stock solution of 50 mg/L Hg was prepared with mercury (II) chloride ($HgCl_2$) and added to the tanks to attain the desired concentrations. Prior to Hg addition, the water was aerated for 24 hours to remove chlorine. A total of 30 juveniles were placed in each tank (ten in one compartment serving as one replicate). The juveniles were fed twice a day at 5% body weight. The feed (commercial feed pellets) was analyzed to ensure the Hg content is negligible.

For exposure through ingestion, the tanks were filled with tap water and the juveniles were fed twice a day with Hg containing feed at 0.5 an 1.0 mg/kg. Any uneaten pellets were removed immediately. To prepare the Hg containing feed, a total of 150 g of feed was added to 150 mL of Hg standard at 0.5 mg/L and 1.0 mg/L, respectively. The treated feed was then left in the oven at 65°C overnight. A control was set up along with the exposure experiments through water and feed.

The experiment was maintained for 30 days with the water controlled at dissolved oxygen of 7.0 mg/L, pH 7 and temperature of 27°C. The parameters were chosen according to the optimum conditions recommended. The favorable range of pH for fish is 6.5–8.5 whilst the dissolved oxygen is recommended above 6 mg/L. The temperature is ideally maintained at 24-27°C for tropical fish according to Abowei (2010) and Dmitry (2013). During the experiment, the juveniles were monitored for their behavioral response during feeding and time taken to feed. After 30 days, the juveniles were analyzed for its Hg concentration. Figure 2 shows the photo of the exposure experiments.

Figure 2. Exposure experiment design.

Hg analysis. The juvenile and feed were digested in 1 mL of concentrated HCl and 6 mL of HNO_3 using a microwave digester (CEM MARS6). The digested samples were filtered through 0.45 µm membrane filter and diluted to 50 mL with deionized water. For juvenile, the weight of individual sample was recorded and the entire fish was digested. For each batch of digestion, a blank was prepared. The water samples were filtered through 0.45 µm membrane prior to Hg analysis. The samples were analyzed using a Mercury Analyzer (FIMS 400). The method recovery was evaluated based on the certified reference material of fish muscle (ERM-BB422). The recovery percentage attained was consistently above 85% indicating satisfactory performance. The Hg concentration in fish was reported in mg/kg wet weight (w/w).

All wastewater from the experiment was filtered and left in activated carbon. The activated carbon was replaced every three days until the concentration of Hg was below 0.5 mg/L.

Statistical analysis. Analysis of Variance (ANOVA) was used to compare the mean of Hg concentration and survival percentage between different species and treatments at 95% significant level. Tukey's test was applied for multiple comparisons. Pearson's correlation was used to examine the relationship between Hg concentrations and size of fish. The statistical analysis was performed using Matlab R2013a.

Results. From the volatility assessment experiment, no significant loss was observed in Hg over 30 days. This verifies the consistency of Hg concentration throughout the experiment. Hg was also undetected in the untreated feed. The behavioral responses of juveniles exposed to Hg through water and feed are recorded in Table 1. Both species reared in water at 1.0 mg/L Hg were found to disperse during feeding but this was not observed in the experiments of 0.5 mg/L Hg and those exposed through feed. The dispersion behavior was a response of majority of the juveniles, in some occasion all. The time taken to feed however was rather inconsistent to infer the effect of Hg.

Table 1
Behavioral responses of *Barbonymus schwanenfeldii* and *Tor tambroides* during Hg exposure through water and feed

Species	*Control*	*Water*		*Feed*	
		0.5 mg/L	*1.0 mg/L*	*0.5 mg/kg*	*1.0 mg/kg*
B. schwanenfeldii	No avoidance during feeding and feed taken immediately	No avoidance during feeding and feed taken immediately	Dispersed during feeding and feed taken after 1 min	No avoidance during feeding and feed taken after 1 min	
T. tambroides	No avoidance and feed taken after 1 min	Dispersed during feeding and feed taken after 1 min	Dispersed during feeding and feed taken after 2 min	No avoidance during feeding and feed taken after 1 min	

The survival percentage recorded at the end of the experiment is summarized in Table 2. Overall, the juveniles treated with Hg demonstrate relatively lower survival percentage than the control for both species however no significant difference is identified statistically (p>0.05).

Table 2
The survival percentage after 30 experimental days, under the exposure of Hg through water and feed, for *Barbonymus schwanenfeldii* and *Tor tambroides*

Species	*Water*			*Feed*	
	Control	*0.5 mg/L*	*1.0 mg/L*	*0.5 mg/kg*	*1.0 mg/kg*
B. schwanenfeldii	$67{\pm}25^{a}$	$63{\pm}15^{a}$	$53{\pm}6^{a}$	$57{\pm}5^{a}$	$47{\pm}20^{a}$
T. tambroides	$90{\pm}10^{b}$	$67{\pm}11^{ab}$	$90{\pm}0^{b}$	$60{\pm}10^{ab}$	$57{\pm}20^{a}$

Same letters in row indicate no significant difference (p>0.05). The survival percentage is expressed as the average of triplicates.

The average Hg concentrations accumulated in juveniles of *B. schwanenfeldii* and *T. tambroides* are summarized in Table 3. The results suggest that juveniles reared in Hg-contaminated water consist of higher Hg concentration than those fed with contaminated feed. Comparing between two species, it is found that *T. tambroides* accumulates higher Hg than *B. schwanenfeldii* with significant different (p<0.05).

Table 3
The average Hg concentration in juveniles of *Barbonymus schwanenfeldii* and *Tor tambroides*

Species	*Hg concentration in juveniles (mg/kg)*				
	Water			*Feed*	
	Control	*0.5 mg/L*	*1.0 mg/L*	*0.5 mg/kg*	*1.0 mg/kg*
B. schwanenfeldii	$0.042{\pm}0.015^{a}$	$0.234{\pm}0.063^{b}$	$0.457{\pm}0.246^{b}$	$0.054{\pm}0.037^{abc}$	$0.017{\pm}0.009^{ac}$
T. tambroides	$0.152{\pm}0.100^{a}$	$0.422{\pm}0.248^{b}$	$0.700{\pm}0.475^{b}$	$0.100{\pm}0.040^{ab}$	$0.109{\pm}0.061^{ab}$

Same letters in row indicate no significant difference (p>0.05).

The Hg accumulation in fish may be size-dependent. According to Storelli et al (2007), a significant positive relationship is deduced between fish size and Hg accumulated in seven species studied. Figure 3 shows the Hg concentration in individual juveniles versus size (in weight); statistically, no significant correlation ($p>0.05$) is established between both variables for the two species. The size variation may be too small to elucidate the relationship.

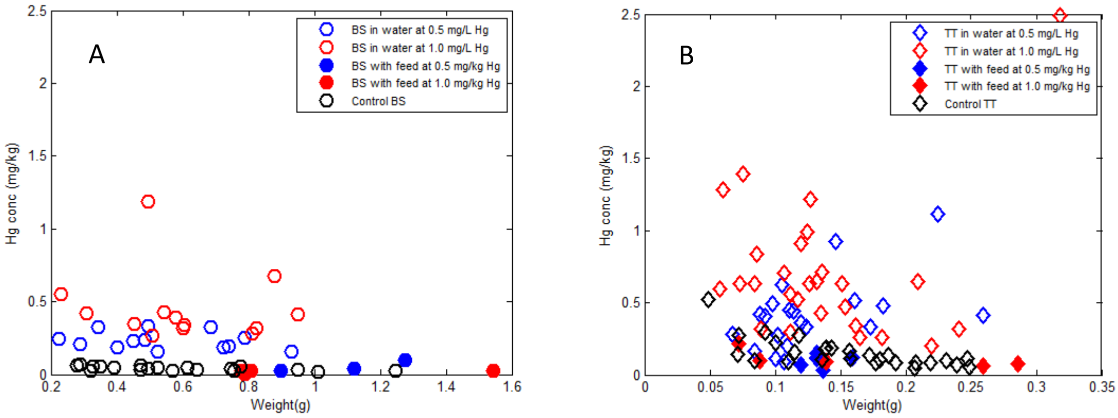

Figure 3. Hg concentration in individual juveniles versus size (in weight) for A) B*arbonymus schwanenfeldii* (BS) and B) *Tor tambroides* (TT).

Discussion. The dispersal behavior was likewise reported by Weber & Haines (2003) in golden shiner (*Notemigonus crysoleucas*) fed with high Hg diet. This response suggests an increase in vulnerability towards threats. Monteiro et al (2010) on the other hand reported hyperactivity and aggressiveness, along with loss of equilibrium and scales in *Brycon amazonicus* exposed to inorganic $HgCl_2$ in water. Those impairments however were unnoticed in this study likely due to differences in species, size, growth stage and other experimental conditions i.e., exposure period and concentrations. In the study of Monteiro et al (2010), the experiment was maintained for 96 h under the concentrations between 0.57 and 1.20 mg/L where the fish were starved for 24 h prior to the experiment and during the experiment.

The survival percentage across all treatments for both species was statistically comparable. According to Monteiro et al (2010), the 96 h-LC_{50} (lethal concentration) of Hg(II) was 0.71 mg/L for *B. amazonicus* whilst Shah (2005) reported 1.0 mg/L for *Tinca tinca*. It is hence anticipated that *B. schwanenfeldii* and *T. tambroides* may be more resilient as the survival percentage is more than 50 after 30-day.

The juveniles exposed to Hg containing water consist of higher Hg concentration than that exposed through dietary uptake. Hg(II) can be taken up through gills and diet with the former governed primarily by the water chemistry (Pickhardt et al 2006). The Hg taken up through gills will be directly transported to the circulation system and subsequently stored in the liver and kidney (Bebianno et al 2007). The Hg in water may be taken into the tissues or merely adsorbed onto the external surfaces. This however is unable to be differentiated in this study. For exposure *via* diet, Hg(II) may enter the cells through diffusion or anionic exchange however the uptake tends to be inhibited by the mucosal membrane lining in the intestines (Part & Lock 1983). The Hg retained in the intestines will eventually be excreted (Lindqvist et al 1995). This offers an explanation to the observation of higher Hg concentration in fish exposed to the contaminated water than that exposed through ingestion. The Hg accumulated in the juveniles increases with the concentration in water; this relationship however is not evidenced in the dietary uptake for the reason that the ingested Hg is largely excreted. If the juveniles are exposed to methylmercury, the compound is expected to assimilate more efficiently as the organic Hg can bind to cysteine thiols strongly facilitating transportation of Hg across the biological membranes (Bradley et al 2017).

The metal accumulation affinity may vary depending on size, age, habitat, feeding habit and growth stage (Raieshkumar & Li 2018). *T. tambroides* demonstrates higher affinity of mercury accumulation than *B. schwanenfeldii*. *T. tambroides* is a fast swimmer that lives in habitats with very strong current (compared to *B. schwanenfeldii* that lives in slow waters). *T. tambroides*, according to Asaduzzaman et al (2016), requires diet of high protein, lipid and unsaturated fatty acids at the early stage of growth for survival and neural development. This species is reported with higher gills ventilation compared to other species, especially when they are subjected to environmental stress (Ahmad et al 2015). This biological behavior may be inherited although the experiment was carried out in rather stagnant environment. Chai et al (2018) compared the Hg concentrations in three species of fish (*T. tambroides* 1.31 mg/kg, *Tor douronensis* 0.70 mg/kg and *Lobocheilos bo* 0.41 mg/kg) from the Baleh River of Sarawak revealing elevated Hg concentration in *Tor* spp. corroborating its profound affinity towards Hg.

The bioconcentration factor (BCF) is expressed as the concentration of Hg in juveniles against the concentration in water. Unlike bioaccumulation factor (BAF), which is site-specific, BCF only considers uptake from the surrounding water with the dietary uptake restricted (Karlsson et al 2002). The calculated BCF of *T. tambroides* (0.772 L/kg) is almost two-fold that of *B. schwanenfeldii* (0.462 L/kg). Comparatively, the BCF values attained in this study is far lower than the reference values reported elsewhere for various aquatic organisms (5–249,000) (IAEA 1994; WHO 1989). The low BCF value in this study is the result of exposure to very high Hg concentration in water.

Conclusions. Hg(II) was taken up through gills and diet with the exposure through water demonstrating a potential of accumulation. The exposure to Hg could result in alteration in behavioral response of juveniles, increasing their vulnerability to threats. Comparatively, *T. tambroides* demonstrated greater affinity of Hg accumulation likely due to its biological behavior as a fast swimming species. In terms of size, no significant correlation is established with the Hg accumulated in juveniles of both species.

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