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Trace metal contents of tropical anguillid eels in Vietnam

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Abstract—In order to examine whether metal accumulation patterns are related to the maturity stages of tropical anguillid eels, two eel species, *Anguilla marmorata* and *A. bicolor pacifica* for both yellow (immature) and silver (mature) stages, were collected in the Ba River in Vietnam. The levels of nine trace metals in liver and muscle tissues were determined. The results indicated that the levels of Zn, which is an essential metal, in the tissues elevate with the gonadal maturation in both species. The other essential metals such as Cr, Co, Mn and Cu tended to be accumulated at higher levels in silver eels than in yellow eels; however, different levels of metal accumulations were observed between the species. Nonessential metals such as Cd and Pb in tissues exhibited no significant difference between the two stages of eels. Although the two species resided in the same river, the Hg levels in *A. marmorata* were found to be higher than that in *A. bicolor*. The results suggest the difference of metal accumulations between the eel species is dependent on the preferred food items and the maturity stages rather than ambient environment.

Key words: anguillid eels, maturation, metal accumulation, energy reserve

Introduction

Life history of diadromous eels is well-known. Being leptocephalus larva in ocean after eggs hatch, they drift toward the continents following ocean currents and recruit to estuaries at elver stage. After migrating upstream at yellow stage (immature eels), they colonize a variety of different habitats (rivers, lakes, marshes, estuaries) for several years to decades depending on the hydrosystem and species. Before eels are able to start sexual maturation, they undergo a metamorphosis called silvering to be onset of downstream migration to the spawning areas (Tesch 2003). Due to fasting during their oceanic migration, maturing silver eels must accumulate energy reserves during their yellow stage in forms of lipid and protein (McKeown 1984). Although silver eels are not fully mature when leaving freshwater system, they must store sufficient energy for the success of gonad development and migration. Hence, there was difference of lipid and protein contents between yellow and silver stages of eel (Boëtius and Boëtius 1980). This can influence the different accumulation levels of organic compounds and trace metals in eels between these stages. Otherwise, eels were considered as bio-indicator to assess the health of aquatic system, especially heavy metals (Barak and Mason 1990, Knight 1997, Usero et al. 2003, Has-Schön et al. 2006). However, these studies mostly examined the metal accumulation in eels at various locations, or size or age-relationship, and have demonstrated large variation in concentration depending upon the fish species and elements studied. Few studies indicate the accumulative differences between yellow and silver stages of anguillid eels (Le et al. 2010a).

Therefore, the aim of this study is to examine whether levels of the metal accumulation are related to maturity stages in the two anguillid eel species, *A. marmorata* and *A. bicolor pacifica*. The results are discussed looking at the use of eels as bio-indicators for environmental monitoring.

Materials and Methods

Study site

Ba River is at a lower part of Da Rang River that is one of the biggest rivers in the central part of Vietnam. The Da Rang River has a length of 374 km, derivers from Ngon Ro Mountain at 1,549 m height in the south west of Kon Tum province. It passes through some provinces before entering Phu Yen province where it named to be the Ba River and ultimately ends in South China Sea. In upstream area of the Ba River, the water flow quickly runs through the Son Hoa highland and its flow is slow down when reaching to Tuy Hoa in downstream area. Although Tuy Hoa is a quite flat area, its geographical situation is sloping eastward to the sea. Thus, the Ba River estuary is less affected by a dynamic tidal range (up to 4–6 km upstream). Due to strong dynamics of water flows in flooding season once a year, therefore, the sediment

is dominated by coarse sand or sandy mud. The Ba River is also natural habitat for anguillid eels and their exploitation constitutes the economic basis of local professional fishermen.

Sampling

A total of 60 wild female eels of *A. marmorata* and *A. bicolor pacifica* were collected by electric shocks or fishing from the upper and lower parts of the Ba River (Fig. 1). In February 2008, 21 specimens of *A. marmorata* were collected, in which 17 yellow eel specimens were collected in the upper part of the river, whereas 4 silver eel specimens were collected in the lower part of the river. In November 2008, a total of 38 specimens of *A. marmorata* and *A. bicolor pacifica* were collected in lower part of the river. Among them, 10 specimens were at silver stage of *A. marmorata*, 29 specimens were at yellow (15) and silver (14) stages of *A. bicolor pacifica*. The total length (to the nearest mm) and body weight (to the nearest gram) were measured (Table 1). Tukey tests indicated that mean length and weight of *A. marmorata* at silver stage were significantly larger than at yellow stage ($p<0.05$). Gonad was weighed in order to calculate gonadsomatic index (GSI), which were defined as the wet weight of the organ divided by the wet weight of whole fish $\times 100$. Liver and dorsal muscle tissues were also dissected for this study, because liver is main site of metal accumulation and muscle is major body burden of metals. All organs were put in clean polyethylene bags and stored at -20°C until chemical analyses.

Chemical analysis

The liver and muscle tissues were dried in oven at 80°C and in freeze-drier (FDU-2200, Japan) at 7.8 Pa and -86.7°C to constant weight, respectively. The dried tissue samples were digested in a Teflon bomb by a microwave oven as described in a previous study of Le et al. (2010a). The digested solutions were diluted with 45 ml Milli-Q water (Milli-Pore Company) into polyethylene and stored at 4°C until metal analysis.

The accuracy of the method was assessed with standard reference materials, SRM1577b (bovine liver, National Institute of Standards and Technology, USA), and SRM2976 (mussel tissue, National Institute of Standards and Technology, USA). Recoveries of all elements ranged from 90.6–113%.

Statistical analysis

The results are expressed as mean±SD. The assumptions of normality of data were verified ($p<0.05$) using the Shapiro-Wilk test. A $\log_{10}$ transformation was used where

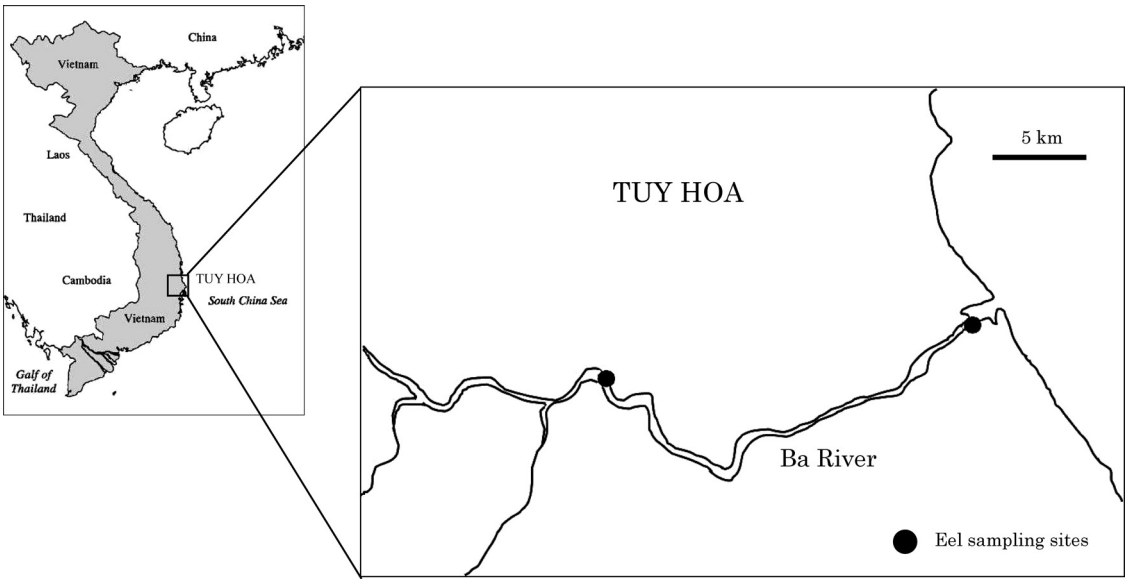

Fig. 1. Sampling sites of anguillid eels from Ba River, Vietnam.

Table 1. Biological information of anguillid eels collected from Ba River Vietnam. All values are presented as mean±SD.

Species	Growth stages	TL (mm)	BW (g)	GSI
A. bicolor pacifica	Yellow (n=15)	677.9±73.3	682.9±263.2	0.6±0.2
	Silver (n=14)	707.4±153.3	930.0±567.2	2.3±0.7
A. marmorata	Yellow (n=17)	864.6±127.8	1818.2±950.0	0.4±0.3
	Silver (n=14)	1129.2±183.3	5222.0±2444.7	3.1±1.2

variances were not homogenous. One way-ANOVA and Tukey tests were performed to reveal any significant differences in metal concentrations between maturity stages of eel species and with total length (TL) or body weight (BW) as covariate. Data for yellow and silver eels were pooled within the sample group to make correlation between metal levels in tissues and biological features. Because TL and BW of the eel samples showed strong correlations, TL, GW (gonad weight) and GSI were selected for correlation study. Linear regression analyses were conducted between the metal concentrations in liver and muscle with the body size of the eels. The statistical analyses were performed using STATISTICA 5.5 for Windows (Statsoft, Inc., USA).

Results

Metal levels residue in organ tissues of anguillid eels

Trace metal concentrations in the liver and muscle of the two eel species are shown in Table 2. In both species, liver was the main target site for most trace metals such as V, Co, Mn, Cu, Zn, Cd, Pb and Hg, and muscle was dominated by Zn, Cu, Mn and Hg. Mn, Cu and Zn were most dominant elements in both tissues and the accumulation trend of these metals was identical in the two eel species.

Comparison of the metal accumulations between two species showed that the levels of V, Cr, Co in liver and muscle of *A. marmorata* were significantly higher than those in *A. bicolor pacifica* (Tukey HSD test for unequal N, $p<0.05$, with BW as covariates). The concentrations of Mn in the muscle of *A. bicolor pacifica* were significantly higher than those in *A. marmorata*, whereas Mn levels in the liver of *A. bicolor pacifica* were found to be lower (Tukey HSD test for unequal N, $p<0.05$, with BW as covariates). Pb levels in liver of *A. bicolor pacifica* were significantly higher than those in *A. marmorata*, while, in muscle, no significant difference of Pb levels was found between the two species. Hg levels in liver and muscle of *A. marmorata* were higher than those in *A. bicolor pacifica*, though only significant differences of Hg levels in liver was found. There were no significant differences of Cd levels in both tissues between two species in the river.

Differences of metal contents in tissues at the maturity stage of eel species

Differences of trace metal levels in tissues between maturity stages of eel species are shown in Table 2. Almost all trace metal levels in tissues tended to be higher in silver eel than those in yellow eels, except for Cd in muscle tissues. In *A. bicolor pacifica*, the levels of Cr and Zn in muscles of silver eels were significantly higher than those of yellow eels, while the levels of Cr, Mn, Cu and Zn in livers of silver eels were significantly higher than those of yellow eels (Tukey

Table 2. Levels of trace metals ($\mu g.\ g^{-1}$) in liver and muscle of anguillid eels from Ba River, Vietnam. All values are presented as mean±SD.

Organs	Species	Maturity stages	V	Cr	Mn	Co	Cu	Zn	Cd	Pb	Hg
Liver	*A. bicolor pacifica*	Yellow	0.49±0.65	0.15±0.08	4.74±1.20	0.49±0.36	51.24±20.10	115.0±27.2	0.55±0.60	0.44±0.49	0.62±0.72
		Silver	0.59 ±0.47	0.22±0.04	6.65±2.17	0.79±0.43	94.94±49.37	198.4±60.2	0.69±0.53	0.46±0.36	0.70±0.44
	A. marmorata	Yellow	0.80±0.72	0.54±0.44	6.61±2.11	1.04±0.50	48.81±22.94	125.8±30.7	0.66±0.52	0.23±0.13	1.51±1.20
		Silver	1.18±0.85	0.60±0.29	8.46±2.94	1.61 ±0.91	80.53±53.06	217.2±96.0	0.73±0.50	0.32±0.32	2.06±1.53
Muscle	*A. bicolor pacifica*	Yellow	0.018±0.014	0.21±0.04	0.87±0.51	0.05±0.03	0.81±0.17	46.5±8.2	0.007±0.004	0.024±0.012	0.63±0.25
		Silver	0.018±0.007	0.18±0.11	0.93±0.47	0.07±0.04	0.94±0.13	55.4±9.1	0.004±0.003	0.028±0.013	0.87±0.40
	A. marmorata	Yellow	0.031±0.024	0.23±0.21	0.66±0.64	0.12±0.07	0.92±0.46	31.2±13.6	0.012±0.022	0.060±0.068	1.43±0.87
		Silver	0.035±0.021	0.47±0.27	0.62±0.24	0.19 ±0.10	1.17±0.60	65.8±25.3	0.008±0.006	0.060±0.086	1.61±0.89

HSD test for unequal N, $p<0.05$). In *Anguilla marmorata*, the levels of Cr, Co and Zn in muscles of silver eels were significantly higher than those in yellow eels. Only Zn levels in livers of silver eels were significantly higher than those in yellow eels (Tukey HSD test for unequal N, $p<0.05$, with BW as covariate). The levels of Hg and Pb in tissues of both species in silver eels were slightly higher than those in yellow eels, but it was not significant.

Relationship between trace metals and biological characteristics

In *A. bicolor pacifica*, Cu and Zn levels in livers showed the positive correlation with both GSI ($r=0.7$ and $r=0.8$, respectively) and GW ($r=0.7$ and $r=0.7$, respectively), while only Mn levels in livers correlated with GSI ($r=0.7$). In muscles, there were correlations between Co levels and TL with $r=0.6$, and Zn levels and GSI with $r=0.6$.

In *A. marmorata*, Cu and Zn levels in livers positively correlated with GSI ($r=0.6$, and $r=0.7$, respectively), while in muscles, Zn level showed correlations with TL, GW and GSI ($r=0.6$, $r=0.7$ and $r=0.7$, respectively) and Cr levels were correlative with GW and GSI ($r=0.6$ and $r=0.6$, respectively). It was not surprising that Hg levels in both liver and muscle tissues in *A. marmorata* were correlated with TL ($r=0.6$, $r=0.7$), however, no significant correlation between Hg levels in tissues and biological features of *A. bicolor pacifica* was found.

Discussion

The differences of metal accumulations such as V, Co, Cr and Mn between species might relate to nutritional requirement of metal intake during metabolic processes, since the metal distribution in environment of the Ba River was identical levels from upper and lower parts and the river was considered as the less polluted area (Le et al. 2010b). In the case of Hg, the higher Hg levels of *A. marmorata* than in *A. bicolor pacifica* is closely related to the biomagnification of Hg via specific local food chain and to preferred preys (Brusle 1990, Hall et al. 1997). Because *A. bicolor pacifica* was dominant in the lower reach of the river and estuary (Chino and Arai 2010), the food content found in the eels' stomach in this study were mostly small crabs whereas *A. marmorata* was abundant in the middle to upper reaches of the river (Shiao et al. 2003, Briones et al. 2007) and the eel fed on a wider range of preys such as shrimps, crabs, and even large freshwater fishes (James and Suzumoto 2006). These food items could be considered as higher potential Hg source, because of biomagnification in trophic level of aquatic organism (Wong et al. 1997, Kehrig et al. 2009). On the other hand, the difference of Hg levels between 2 eel species even might relate to body size and age, because of long half-life of Hg in fish (Brusle 1990) and the positive correlations between Hg levels in muscle and age (Pellegrini and Barghigiani 1989, Brusle 1990, Szefer et al. 2003) or body size (Pellegrini and Barghigiani 1989, Barak and Mason 1990). The body size of *A. marmorata* was significantly larger than that of *A. bicolor pacifica* (Table 1) that can partly explain the difference of Hg levels between two species, however, the otolith rings, ear-stone of fish usually uses to estimate the fish's age of many studies, were not clearly determined the age of tropical eels (Jellyman 1991, Chino and Arai 2010). Because many incomplete rings were observed in both eel species' otolith in this study, it was not possible to validate the annual formation of otolith zones. Thus, it is uncertain whether the difference of Hg levels between eel species was related to age. Otherwise, significant correlations between the body sizes and Hg levels were found in *A. marmorata* but not in *A. bicolor pacifica*. This can be merely explained with regard to the biological mechanisms of these species such as Hg uptake and elimination rate. Unlike Hg, the levels of Cd and Pb in tissues showed no significant difference between species, although the metal levels in *A. marmorata* were slightly higher than those in *A. bicolor pacifica*. This reflected the distribution of these metals at similar levels along river and the slight difference might be related to the feeding habits and habitats of eel species.

Not all trace metal levels in silver eels were significantly higher than those in yellow eels. The significant difference of metal levels between the maturity stages of both species were principally for the essential metals such as Cr, Co, Mn, Cu, and specially Zn. These metals play important roles in metabolic mechanism in animals. However, the remarkably higher levels of Zn, Cu and Mn detected in the tissues of silver eels than those of yellow eels in both species might be related to demands of essential elements for gonad maturation and migration. In fish, the energy use for gonad formation is originated not only directly from food, but also depleted from energy reserve in muscle, mainly as fat, protein and carbohydrate deposits (McKeown 1984, Kamler 1992). In anguillid eels, the energy reserves in muscle are even depleted to provide energy for spawning migration. This could explain the elevation of trace metal levels in muscle of silver rather than yellow eels of both species, especially Zn. Alternatively, liver generally had the higher metal concentrations compared to muscle; this is due to the major roles that liver plays in metabolism and numerous other functions in the body (Heath 1995, Hylland et al. 2003). The liver of mature fish induces the synthesis of egg yolk phosphoproteins, and this mechanism stimulates the accumulation of essential metals such as Mn, Cu, and especially Zn at the onset of sexual maturation (Thompson et al. 2003). Zn is well-known as a constituent of many molecules involved in protein, lipid and carbohydrate metabolism. In mature fish, Zn can be transported from mus-

cle to liver by blood serum to synthesize vitellogenin under the influence of estrogen; it is secreted into plasma, and then taken up by oocytes through receptor-mediated endocytosis during gonadal development (Banaszak et al. 1991, Hogstrand and Wood 1996, Montorzi et al. 1995). This also explains the positive correlations between Zn levels and GSI in both species as observed in this study. Additionally, Danilov and Shevchenko (1973) also found that during prolonged fasting, fish lose Zn from muscle. In the present study maturing silver eels were collected in the estuary and they were at onset of downstream migration. Thus, the muscle depletion of Zn might have not occurred yet. On the other hand, the previous study indicated that the negative correlation between Zn and body size in yellow stage might relate to the metal burden dilution of growth (Le et al. 2009) and the elevation of lipid content in muscle during stored stage (Degani et al. 1986), while positive correlation between Zn and body size was observed in present study. This might relate to the accumulation of protein after lipid storage process in muscle of mature fish (Kamler 1992) and the elevation of the trace metals might relate to increase of metalloproteins in tissues for their biological functions (Garcia et al. 2006, Dudev and Lim 2008). Metalloproteins have many different functions in cells, such as enzymes, transport and storage proteins, and signal transduction proteins. Additionally, Miramand et al. (1991) also reported higher levels of Cu, Zn and Mn in various organs of mature red mullet (*Mullus barbatus*) compared to those in immature fish. They indicated that Cu, Zn, and Mn in liver and gonad were more closely related to the sex and reproductive cycle than to the ambient levels in marine environment.

Therefore, the study suggests the maturity stages of freshwater eels should be determined to avoid misunderstanding of metal monitoring results, especially for the essential metals such as Cr, Co, Mn, Cu and Zn.

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